VARIATION IN MORPHOLOGICAL CHARACTERISTICS OF *Sclerotium rolfsii* AND BIOCONTROL EFFICACY OF *Trichoderma harzianum* ON THIS FUNGUS

NAY NAY OO

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A Thesis submitted to the post-graduate committee of the Yezin Agricultural University in the partial fulfillment of the requirements for the degree of Master of Agricultural Science (Plant Pathology)

Department of Plant Pathology
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The thesis attached hereto, entitled "**Variation in Morphological Characteristics of *Sclerotium rolfsii* and Biocontrol Efficacy of *Trichoderma harzianum* on This Fungus**" was prepared under the direction of the chairperson of the candidate supervisory committee and has been approved by all members of that committee and board of examiners as partial fulfillment of the requirements for the degree of **Master of Agricultural Science (Plant Pathology)**.

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DECLARATION OF ORIGINALITY

This thesis represents the original work of the author, expect where otherwise stated. It has not been submitted previously for a degree at any University.

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Date ……

DEDICATED TO MY BELOVED PARENTS

U MYINT NGWE AND DAW THAN HTAY

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ABSTRACT

Three experiments were carried out at the Department of Plant Pathology, Yezin Agricultural University during 2013-2015. *Sclerotium rolfsii* Sacc. isolated from ten host plants, chickpea, chilli, cowpea, eggplant, groundnut, sesame, soybean, sunflower, taro and tomato were studied for their variation based on morphological characters of mycelium, sclerotial characters, and mycelial compatibility grouping. The test was arranged as completely randomized design (CRD) with five replications. It was observed that most of the isolates produced fluffy colonies while the colony of chickpea isolate was less fluffy on potato dextrose agar (PDA) media. All ten isolates were the fast growing fungi since their growth rates ranged from 27 to 30 mm in diameter day^{-1} on PDA media. The maximum number of sclerotia at 28 days after incubation, *i.e.*, 1018, was produced by the sesame isolate while the minimum number, 281, by the chickpea isolate. Based on mycelial compatibility groupings (MCGs) test, only two parings, the cowpea and taro isolates that designated as MCG 3 and the groundnut and sunflower isolates that designated as MCG 5, were compatible. As a result, eight different mycelial compatibility groups (MCG 1 to 8) were identified among them. The second experiment was carried out to study the biocontrol efficacy of *Trichoderma harzianum* on mycelial growth of the eight isolates on PDA media and the experiment was arranged as CRD with five replications. The inhibition efficacy of *T. harzianum* was determined by dual culture technique *in vitro*. The highest inhibition percent (68%) of *T. harzianum* was found against the sesame isolate. The third experiment was carried out to study the cross-pathogenic ability of eight isolates on different hosts and percent disease control of *T. harzianum* on these crops. The experiment was arranged as three factors factorial; Factor A - isolates, Factor B - with or without *T. harzianum*, Factor C - crops, with three replications. The results revealed that all the isolates had cross pathogenesis to all tested crops. However, the chickpea isolate was low pathogenic among them. It was also observed that there was no disease control of *T. harzianum* on the chickpea isolate. The percent disease control of *T. harzianum* on the other seven isolates of *S. rolfsii* ranged from 6% to 30%.

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CHAPTER I

INTRODUCTION

Sclerotium rolfsii Sacc. is a devastating soil borne pathogen and has a very extensive host range including more than 500 plant species of agricultural and horticultural crops (Okabe and Matsumoto 2000). It has wide geographic diversity and commonly found in the tropics, subtropics and other warm temperate regions of the world (Aycock 1966). The fungus induces a variety of symptoms such as seed rots, seedling blight, collar rot, damping-off, stem rot, wilt, crown and root rot, stem canker and sclerotium blight in different host plants (Mullen 2001; Arunasri 2011).

The growth of *S. rolfsii* in nature is almost always prominent at or near the soil surface, and host organs in that zone are commonly attacked by the fungus. Growth of *S. rolfsii* is optimal at 27-30ºC and the optimal pH ranged from 3-6.8 (Aycock 1966; Mehan *et al*. 1995). Disease develops better in loose sandy loam soil than in fine-textured like silt or clay soil. The soil moisture holding capacity (50-75%) favors the disease development than at saturation (Ramarao and Raja 1980). This disease causes severe damage to any growth stage of crop. Yield loss is generally 25%, but sometimes it reaches 80-90% in some cases during wet weather condition (Grichar and Bosweel 1987).

Sclerotium rolfsii does not produce asexual spores and perpetuates as sclerotia on plant debris and in soil (Cilliers *et al.* 2000). The fungus is able to thrive in soil for a long time in the absence of a host as a saprophyte when organic matter is present. Sclerotia are considered to be extremely hard and relatively resistant survival structures (Singh *et al*. 2003). They disseminate by means of cultural practices, water, wind and contaminated seeds and infested soils. The wide host range, profuse mycelial growth and ability to produce persistent sclerotia contribute to the large economic losses associated with this pathogen (Kokub *et al*. 2007).

The variation of *S. rolfsii* in morphological characteristics has been noticed in worldwide collections of the pathogen from different hosts and the same host as well. The culture of *S. rolfsii* originating from various plant species and different geographical regions presented wide variations in growth rate, morphological and cultural characteristics, mycelial compatibility and also exhibited genetic variability. However, the cultures of *S. rolfsii* can be identified by the size, colour and structure of their sclerotia (Kokub *et al*. 2007). The variation of *S. rolfsii* can be investigated by

mycelial and sclerotial characters, pathogenicity and mycelial compatibility groupings (MCGs). Moreover, the differences in sclerotial forming capacity among isolates could be a useful parameter for characterizing isolates. A number of genetic diversity studies have been performed on *S. rolfsii* to characterize the population structure or to monitor the distribution and spread of isolates over time based on mycelial compatibility grouping (Le *et al*. 2012). Introduction of resistant host varieties and environmental changes could contribute to the development of new strains while recombination and mutations within the isolates and also leads to new MCGs (Xie 2012).

The diseases caused by *S. rolfsii* can be controlled by using a variety of control measures including cultural, physical, chemical, biological control methods and resistant cultivars. Cultivation of resistant varieties is the ideal and feasible management of the disease and resistant sources against this disease had been identified in various countries (Sugha *et al*. 1991). The use of soil fumigants, such as chloropicrin and metam-sodium, are the most practical means to treat seed beds and fields for a number of soil-borne pathogens, including *S. rolfsii*. They must be applied days to weeks before planting (Mullen 2001).

However, biological control can be expected to be one of the most alternative methods complementary to the sustainable agriculture program and a promising approach for the management of soil borne diseases. Fungi in the Genus *Trichoderma* have been known since 1920s for the ability to act as biocontrol agent against plant pathogens (Harman 2006). *Trichoderma harzianum* may act as mycoparasites of *S. rolfsii*, produced extracellular β (1, 3) glucanase and chitinase compound (Chet and Baker 1980). Deepthi (2013) reported that *Trichoderma* spp. inhibited the growth of *S. rolfsii* ranged from 44.44% to 75.55% in dual culture.

In Myanmar, many of crops were grown almost every season throughout the country. The diseases caused by *S. rolfsii* had been noticed in cultivated and uncultivated soils for a long time. Since *S. rolfsii* causes soil-borne diseases and the damages caused by these diseases generally do not recorded at economic level, there is little awareness on these diseases. Therefore, repeated planting of host plants and the build up of *S. rolfsii* inoculums may be gradually enhanced in that soil (Xie 2012). In addition, there are many hosts of *S. rolfsii* in different locations in Myanmar. Therefore, there would be many isolates and they would be varied in their morphological, sclerotial characters and pathogenicity. But, the information

concerned with morphology and pathogenicity aspects of *S. rolfsii* in Myanmar was still limited. Studies on variability within the population in a geographical region are important because the changes occurred in the population since the discovery of pentachloronitrobenzene (PCNB)-tolerant strains of *S. rolfsii* isolated from groundnut in Texas (Nelin 1992). Studying the phenotypic and genetic variability among isolates is important for a better understanding of disease epidemics and forecasting of disease development (Huff *et al*. 1994).

Moreover, as the pathogen is soil-borne and causes diseases on several kinds of economic important crops, it is necessary to find out effective control measures for sustainable agriculture. Integrated disease management including application of *T. harzianum* could be effective in controlling these diseases to reduce yield losses. Therefore, this study was conducted with the following objectives.

(1) To evaluate the variability of *Sclerotium rolfsii* isolates from different hosts plants

(2) To determine the inhibition efficacy of *Trichoderma harzianum* on different *Sclerotium rolfsii* isolates

CHAPTER II

LITERATURE REVIEW

2.1 Causal Fungus, *Sclerotium rolfsii* Sacc.

2.1.1 Taxonomy and nomenclature

The fungus (anamorphic or asexual stage) was reported for the first time from USA by Rolfs (1892) as a cause of tomato blight. Saccardo (1911) gave the binomical nomenclature of the fungus *Sclerotium rolfsii* (Le 2011).

The teleomorph (sexual or perfect stage) of *S. rolfsii* was described as *Athelia rolfsii* (Curzi) Tu and Kimbrough (Tu and Kimbrough 1978).

Anamorphic stage	**Teleomorphic stage**
Sclerotium rolfsii Sacc.	*Athelia rolfsii* (Curzi) C.C. Tu & Kimbr
Kingdom: Fungi	Kingdom: Fungi
Division: Deuteromycota	Division: Basidiomycota
Subdivision: Deuteromycotina	Subdivision: Agaricomycotina
Form-Class: Agonomycetes	Class: Agaricomycetes
Form-Order: Agonomycetales	Order: Atheliales
Form-Family: Agonomycetaceae	Family: Atheliaceae
Form-Genus: *Sclerotium*	Genus: *Athelia*
Form-Species*: rolfsii*	Species: *rolfsii*

2.1.2 Morphology

Hyphae of *S. rolfsii* (anamorphic or asexual stage) are hyaline with thin cell walls and sparse cross walls. At least two types of hyphae are produced. Coarse, straight and large hyphae (2-9 µm x 150-250 µm) have two clamp connections at each septation, but exhibit branching in place of one of the clamps. In another type, branching is common in the slender hyphae (1.5-2.5 µm in diameter) which tend to grow irregularly and lack clamp connections. Slender hyphae are often observed penetrating the substrate (Aycock 1966).

Sclerotia (0.3-3.0 mm diameter) begin to develop after 4-7 days of growth (Punja and Rahe 1993) when hyphae cluster together as a compact mass. The sclerotial bodies become smooth and change in color from white to light tan to brown and possibly to black (Mullen 2001). After an initial white appearance, the sclerotia

quickly become dark brown (Aycock 1966). Young sclerotia often exude droplets of clear to pale yellowish fluids. Mature sclerotia are hard, slightly pitted, and have a distinct rind. Although most sclerotia are spherical, some are slightly flattened or coalesce with others to form irregular sclerotia. An outer melanized rind, an underlying cortical layer, and an innermost medulla comprise the mature scleotia. *S. rolfsii* does not form asexual fruiting structures or spores (Aycock 1966).

The teleomorphic stage is rarely observed on hosts or in culture. *A. rolfsii* produces basidia on an exposed hymenium and basidia produce four haploid basidiospores. The appressed hymenium develops in small, thin and irregular patches. The clavate basidia are 4-6 µm x 7-14 µm; basidiospores are hyaline and 1.0-5 µm x 5-12 µm (Aycock 1966).

2.1.3 Physiology

Sclerotium rolfsii can survive at temperatures ranging from 8-40°C, but its ability to infect is greatly reduced at temperatures below 20°C and above 35°C (Aycock 1966; Mehan *et al*. 1995). In most cropping systems, the optimal temperature for infection was 30°C (Aycock 1966). Growth of *S. rolfsii* is optimal at 27-30ºC and sclerotia do not survive at temperatures below 0ºC (Singleton *et al.* 1992).

Sclerotium rolfsii produces asexual reproductive propagules, sclerotia. During sclerotium development, drops of clear exudates are produced at the surfaces which contain proteins, cations, amino acids, carbohydrates, enzymes and oxalic acid (Christias 1980; Punja and Grogan 1981). An outer melanized rind, an underlying cortical layer and innermost medulla comprise the mature sclerotia. Nutrient composition of mature sclerotia consists of amino acids, fatty acids, sugars and lipids (Liu and Wu 1971).

Sclerotia contain viable hyphae and serve as the primary inoculum source in the disease cycle. When conditions are favourable, white mycelial growth and sclerotia can be observed at the soil line on infected plants (Mc Carter 1991). Host tissues death can be found in areas of host and mycelial contact (Aycock 1966). Tissue death is the result of oxalic acid produced by *S. rolfsii*, which is toxic to plant tissue (Punja and Jenkins 1984). Oxalic acid plays an important role in the virulence of *S. rolfsii* (Kritzman *et al.* 1977; Punja 1985). By producing oxalic acid as well as pectinolytic and cellulolytic enzymes, *S. rolfsii* kills and disintegrates host tissues

before it penetrates (Prasad and Naik 2008). In the host, oxalic acid binds to calcium resulting in calcium oxalate (Punja and Jenkins 1984). The formation of calcium oxalate lowers the pH of the plant tissue and allows for cellulases and polygalacturonases to degrade the host tissue (Bateman 1972).

Mycelial growth can occur on artificial media between pH values of 1.4 to 8.8, with an optimal pH range of 3.0 to 6.4 (Mehan *et al*. 1995). Both mycelial growth and sclerotial germination are severely reduced when pH is greater than 7.0 (Punja and Grogan1982). Sometimes, stem rot can be severe in soil of pH > 7 (Shim and Starr 1997).

2.1.4 Variability

Field isolates of *S. rolfsii* from various hosts and geographical areas differ in growth rate, numbers and size of sclerotia produced (Punja and Grogan 1983). Most isolates tend to be highly virulent (Punja *et al.* 1985). Collections made within a small geographical area tend to be similar morphologically and it suggested that the dissemination can be occurred by vegetatively and not from dissemination of basidiospores. Extensive variation among isolates from a small geographical area could be used as indirect evidence of the involvement of basidiospores in dissemination since colonies arising from basidiospores would be highly variable (Punja 1988).

The genetic diversity of *S. rolfsii* has been studied by a variety of techniques, including mycelial compatibility, restriction fragment length polymorphism (RFLP) analysis of ITS-rDNA, and by ITS-rDNA or Lanuch Sequence Unit (LSU) sequencing (Okabe *et al.* 2001; Okabe and Matsumoto 2003; Xu *et al.* 2010). Harlton *et al.* (1995) found 49 mycelial compatibility groups (MCGs) and 12 RFLP-ITS groups in a worldwide collection of isolates, but could not establish correlations between MCGs and pathogenicity. Some RFLP-ITS grouping patterns were correlated with MCGs, but isolates belonging to one MCG sometimes showed different RFLP-ITS patterns and certain patterns were dispersed among different MCGs (Harlton *et al.* 1995).

2.1.5 Mycelial or vegetative compatibility grouping

In recent years, mycelial or vegetative compatibility was used as a technique for the identification of genetic diversity within fungal species. The term mycelial compatibility refers to the ability of individual strains of the same fungal species to

undergo mutual asexual hyphal anastomosis, which results in viable fused cells containing nuclei of both parental strains in a common cytoplasm (Singleton *et al.* 1992; Katan 1998). If pairings are compatible, the hyphae intermingle, anastomosis bridges are formed from growth of lateral branches followed by fusions, the individual mycelia cannot be discerned, and therefore, belong to the same mycelial compatibility group (MCG) (Punja 1988). The occurrence of the same MCG on different host species or from widely different geographic areas reflects the wide host range and/or spread of the pathogen (Cilliers *et al.* 2000). Also, the fact that isolates from within a given geographical area are diverse suggests that genetic changes have occurred within subpopulations (Cilliers *et al*. 2000), or basidiospores may be involved in spread since. In certain cases, isolates from the same geographic area and host plant group together (Cilliers *et al.* 2000).

Nalim *et al*. (1995) described 25 MCGs among 366 peanut isolates in Texas. There were observed that 39% of MCG was found among 12 isolates from chickpea in Pakistan (Akram *et al*. 2007). Prasad *et al.* (2012) reported that nine mycelial compatibility groups were observed among 22 isolates of *S. rolfsii* from sunflower. Xie (2012) stated that 23 MCGs were found among 84 isolates of *S. rolfsii* from different hosts including groundnut and vegetables and ornamental crops.

2.1.6 Host range

Sclerotium rolfsii has a wide host range with more than 500 plant species (Aycock 1966) including monocotyledons and dicotyledons (Farr *et al.* 1989). More than 270 host genera have been reported in the USA (Le 2011). These include agricultural crops such as sweet potato (*Ipomoea batatas*), pumpkin (*Cucurbita pepo*), corn (*Zea mays*), wheat (*Triticum vulgare*), groundnut (*Arachis hypogaeae*), and some horticultural crops such as Narcissus (*Narcissus* spp.), Iris (*Iris* spp.), Lilium (*Lilium* spp.), Zinnia (*Zinnia* spp.), and Chrysanthemum (*Chrysanthemum* spp.) (Farr *et al.* 1989). In Vietnam, many crops are infected by *S. rolfsii* including groundnut (*Arachis hypogaea*), mungbean (*Vigna radiata*), soybean (*Glycine max*), tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*), eggplant (*Solanum melongena*), pepper (*Capsicum annuum*), cabbage (*Brassica oleracea*), cucumber (*Cucumis sativus*) and taro (*Colocasia esculenta*) (Do 2001). Some of the common hosts include legumes, crucifers, tomato, chrysanthemum, peanuts and tobacco in which the pathogen causes foot rot or root rot (Anahosur 2001).

2.1.7 Symptoms caused by *Sclerotium rolfsii*

Sclerotium rolfsii induces a variety of symptoms such as seed rots, seedling blight, collar rot, damping-off, stem rot, wilt, crown and root rot, stem canker and sclerotium blight in different host plants. *S. rolfsii* infects seedlings, fleshy roots, bulbs or fruits of herbaceous plants and woody plants. Most frequently, this fungus infects lower stems near or at the soil surface, but it also may infect any part of a susceptible plant as long as favorable environmental conditions exist. The first symptom is usually wilt. Wilted plants often decline and die rapidly as a result of an extensive lower stem rot (Mullen 2001).

Wilt

On tomato, peanut, pepper, and many other herbaceous and woody hosts, disease begins with a small, water-soaked lesion on the lower stem at or near the soil surface. The lesion spreads rapidly to girdle the stem. On many herbaceous plants and seedlings, the girdling lesion will quickly cause the plant to wilt and fall over (Mullen 2001).

Crown and stem rot

On mature pepper and tomato, the stem cortex several centimeters above and below the soil surface will decay, but the stem central cylinder does not decay. As the lower stem decay develops, plants usually remain erect and foliage wilts. On many host plants, wilted leaves gradually become brown and remain hanging on the plant. On peanut, one or more branches may wilt and leaves on these branches appear slightly faded and then turn brown. When stems are partly girdled, leaves are often small with a mild brown coloration, but wilt does not occur. When peanuts are infected in wet, hot weather, stems become totally rotted except for the xylem (Mullen 2001).

As lower stems of herbaceous plants decay, a white mat of mycelium develops at the lesion site. This white mat will often spread out onto the nearby soil surface. Shortly after the mycelial mat develops, small (0.5-1 mm), white, round, fuzzy mycelial bodies begin to appear. These mustard-seed-sized structures, called sclerotia, soon become smooth and light tan, brown or black in color. Sclerotia serve as overwintering bodies and may be seen in the mycelium, on diseased tissues above or below ground, on soil surfaces, or in soil crevices (Mullen 2001).

Root rot

On some plants, such as tomato, pepper, and sweet potato, root infection may follow crown infection. On apples, roots are the primary infection site and crown rot develops subsequently. Usually the characteristic white mycelial mat and sclerotia develop near and on infected crown tissues or in and around roots close to the soil surface. The leaves eventually die, and branch dieback develops. The lower stem (trunk) above the girdling lesion usually appears normal for a long time and will be the last part of the plant to die (Mullen 2001).

Tuber, bulb and fleshy organ rot

Sclerotium rolfsii causes a soft rot of fleshy organs. On potato tubers, small slightly sunken lesions (2-3 mm) at lenticels will develop into yellow-tan colored lesions. Tissues become soft and collapse. Mycelium and sclerotia develop abundantly on rotting tissues (Mullen 2001).

Fruit rot

Tomato fruit and other fruit at or near the soil surface may become infected with *S. rolfsii.* Soft, water-soaked, sunken, slightly yellowish lesions develop. These lesions quickly spread throughout most or all of the fruit, which will eventually collapse. Coarse white mycelium develops with sclerotia (Mullen 2001).

Leaves (Sclerotium blight)

On low growing plants such as strawberries, statice, and peanut, leaves touching the soil may be infected with *S. rolfsii*. Necrotic leaf spots have been described on many plants. Usually the high humidity near the soil surface is an important factor in disease development. There have been some reports of leaf spots at locations considerably above the soil. The spots are circular, medium brown to light brown or straw-colored with narrow borders and are usually one cm or less in diameter. Many of the lesions have concentric rings, and small clumps of mycelium on them often develop into sclerotia (Mullen 2001).

2.1.8 Distribution and economic importance

Sclerotium rolfsii was a destructive soil-borne pathogen causing diseases on many crop plants especially in the tropics and subtropics (Mukherjee and Raghu 1997). The large number of sclerotia produced by *S. rolfsii* and their ability to persist in the soil for several years as facultative parasite and a pathogen of major importance throughout the world (Punja 1988).

The first report of losses due to this pathogen in the USA was made by Rolfs in 1892 on tomato (*Lycopersicon esculentum* Miller) in Florida (Aycock 1966). Later, *S. rolfsii* diseases have been reported on cowpea in Nigeria, Brazil, USA and Asia (Singh and Rachie 1985; Singh *et al.* 1997). *S. rolfsii* was recorded in South Africa as causal agent of stem rot or southern blight of groundnut (Cilliers *et al.* 2000), in Benin on cowpea (Adandonon 2000; Kossou *et al*. 2001).

The pathogen causes a great economic loss in various crops. In groundnut, it caused 25% of seedling mortality in the cultivar JL-24 at Parbhani (Ingale and Mayee 1986). Thiribhuvanamala *et al.* (1999) observed that 30% of crop loss in tomato was due to *S. rolfsii*. Harinath Naidu (2000) reported that *S. rolfsii* caused 40-50% mortality in crossandra in Chittoor district of Andhra Pradesh.

2.1.9 Life cycle

Sclerotium rolfsii overwinters as sclerotia and mycelium in or on infected plant tissues and debris. As sclerotia have a high demand for oxygen, they commonly germinate when they are in the upper regions of the soil (less than 5 cm depth). Sclerotia can be easily disseminated through transplant seedlings, water, wind or any cultural practice that moves infested soil or plant debris (Punja 1985).

Sclerotia germinate and hyphal growth occurs once favorable environmental conditions are present. Susceptible plant tissues, lower stems, roots, and fruit can be directly penetrated by contacting hyphae under ideal conditions. During the infection process, the fungus secretes oxalic acid and endopolygalacturonase, which degrade plant tissues and cells, and eventually lead to decay (Punja 1985).

Diseases caused by *S. rolfsii* are favored by warm, wet weather and keep spreading when hyphae and sclerotia reach new susceptible plant tissues while these favorable conditions persist. These conditions not only favor rapid disease development but also plant-to-plant spread of the pathogen. Disease develops better in loose sandy loam soil than in fine-textured like silt or clay soil and better when the soil moisture holding capacity is 50-75% than at saturation (Ramarao and Raja 1980).

The exact role of the teleomorphic stage (sexual or perfect stage) in the disease epidemiology of *S. rolfsii* remains unclear. This perfect stage and basidiospore production can be induced under special laboratory conditions. Greenhouse trials have clearly demonstrated that basidiospores can infect host tissues (Punja 1985).

Usually the disease is a single cycle within the growing season, with limited spread to neighboring plants under conductive conditions. Any potential of a secondary cycle caused by basidiospores is not clear. However, aerial leaf spots caused by *S. rolfsii* were reported in India and may support basidiospores as a source of inoculum in the field (Ramaiah and Jayarajan 1976).

2.1.10 Disease management

The disease can be controlled by using cultural and physical methods, resistant cultivars, chemical control and biological control strategies (Vargas Gil *et al*. 2008).

Deep ploughing to bury sclerotia or diseased tissues under 6-20 cm, was reduced the viability of sclerotia or killed hyphae of the pathogen in the fields (Mihail and Alcorn 1984). Rotation with non-host crops improved the soil nutritional status and might adversely affect pathogen inoculum densities. Taylor and Rodriguez-Kabana (1999) found that stem rot of groundnut can be suppressed by rotation with cotton. Also paddy rice was recommended as a rotation crop with groundnut in order to reduce stem rot in Vietnam.

Because it is not easy to breed to produce highly resistant cultivars against *S. rolfsii*, disease tolerant cultivars may be used as a component of an integrated control method (Punja 1985). In the mid- 90s, two groundnut cultivars, *i.e.,* Toalson and Southern Runner, were developed which are less susceptible or partially resistant to *S. rolfsii* in USA (Mehan *et al.* 1995; Branch and Brenneman 1999). Three peanut cultivars, Georgia - 03L, Georgia - 02C and AP-3, were described to have a moderate level of resistance (Woodward *et al*. 2008). Two other peanut cultivars, DP-1 and Georgia-07W were registered resistant to *S. rolfsii* in 2008 (Gorbet and Tillman 2008).

In many cases, fungicides such as tebuconazole, pentachloronitrobenzene (PCNB) and flutolanil are used to control *S. rolfsii*. *Sclerotium* rots are controlled by chloroneb and prothiocarb, which are used as a soil drench or as an in-furrow spray near the roots of crops (Jones 1987). Other fungicides that are used to control stem rot disease include difenoconazole, carbendazim, flusilazole and chlorothalonil (Cilliers *et al*. 2003). Manu *et al*. (2012) reported that mancozeb inhibited *S. rolfsii* about 55%, 73% and 83% at 500, 750 and 100 ppm respectively *in vitro*.

Fungal biocontrol agents may directly or indirectly kill sclerotia or mycelium of *S. rolfsii*. Lectins produced by *S. rolfsii* were proposed to serve as recognition

factors for fungal biocontrol agents. Among the fungal biocontrol agents, *Trichoderma* species are the most widely studied. In a direct interaction, hyphae of *Trichoderma* penetrate the rind and the cortex of sclerotia and lyse the medullar tissue. Degraded sclerotia become dark, soft and disintegrate under slight pressure and it was shown that chitinase and β-1, 3-glucanase play a role in the interaction between *Trichoderma harzianum* and *S. rolfsii* (Prasad and Naik 2008). *Trichoderma* spp. successfully controlled *S. rolfsii* both in culture and in the greenhouse (Singh *et al.* 1997). Several other fungal genera such as *Gliocladium virens, G. roseum, Glomus fascicatum, Penicillium pinophilum,* have been tested for their ability to control diseases caused by *S. rolfsii* on bean, carrot, chilli, ginger, wheat, lentil, sesame, soybean, sugar beet, sunflower, tomato, or groundnut (Le 2011). *Pseudomonas* strains can restrict *in vitro* hyphal growth or reduce germination of sclerotia of *S. rolfsii* (Kishore *et al.* 2005; de Curtis *et al.* 2010; Pastor *et al.* 2010; Tonelli *et al.* 2010). Several *Bacillus* species and strains have been studied for their efficacy to control stem rot disease of groundnut. Pretreatment of groundnut seeds with *B. subtilis* protected groundnut seeds against *S. rolfsii* and significantly increased the number of pods (Abd-Allah 2005).

Muthamilan and Jeyarajan (1996) reported that integration of *T. harzianum, Rhizobium* and carbendazim remarkably reduced the root rot of groundnut caused by *S. rolfsii*. Seed treatment with Vitavax 200 (carboxin 37.5 + thiram 37.5) 75 WP (0.2%) + *T. harzianum* or *G. virens* effectively controlled seed and seedling rot of soybean caused by *S. rolfsii* (Singh and Thapliyal 1998). Seed and soil treatment with *T. harzianum* plus neem cake significantly reduced stem rot of groundnut caused by *S. rolfsii* with increased dry shoot and root weight (Kumaran 2000). Integration of *G. virens* and *T. harzianum* with carboxin (0.1%) effectively controlled seed and seedling rot of soybean incited by *S. rolfsii* (Rajeev and Mukhopadhyay 2001). Gogoi *et al.* (2002) reported that corm treatment with captan (0.2%) integrated with soil application of *T. harzianum* or *B. subtilis* reduced collar rot of elephant's foot yam caused by *S. rolfsii*. Damping-off of eggplant and tomato was effectively controlled by combined seed treatment with *P. fluorescens* and *T. harzianum* (Vishwakarma *et al.* 2002).

2.2 Biocontrol Agent, *Trichoderma* spp.

The genus *Trichoderma* is a soil-borne and cellulose decomposing fungi. *Trichoderma* species are green-spored ascomycetes present in nearly all types of temperate and tropical soils. They can often be found in decaying plant material and in the rhizosphere of plants (Schuster and Schmoll 2010). Their diverse metabolic capability and aggressively competitive nature made them as the successful colonizers of their habitats (Gams and Bissett 2002). They are frequently dominant components of the soil microflora in widely varying habitats. This may indicate that *Trichoderma* spp. could be aggressively competitive nature. The genus *Trichoderma* includes nine different species aggregates: *T. harzianum, T. hamatum, T. viride, T. koningii, T. polysporum, T. longibranchiatum, T. reesei T. piluliferum* and *T. saturnisporum* (Rifai 1969).

2.2.1 Control of soil borne plant diseases

Trichoderma are biolcontrol agent occurring in the world. They were used against diseases in a wide variety of economically important crops, e.g. apples, carrots, cotton, grapes, lettuce, onion, peas, plum, sweet corn, barley, durian and tomato (Chamswarng *et al*. 1992; Hjelgord and Tronsmo 1998). They have been used with success against soil-borne diseases, seed-borne diseases, diseases in the phyllosphere and storage rots (Papavizas 1985; Tronsmo 1986; Chet 1987). *Trichoderma* spp. have many advantages as a biocontrol agents. *Trichoderma* could control various plant diseases caused by *Pythium* spp., *Phytophthora* spp., *Rhizoctonia* spp., *Sclerotium* spp., *Botrytis cinerea*, *Fusarium* spp., *Cryptosporiopsis curvispora* and *Colletotrichum faltatum*, (Chamswarng 1992; Elad 1994; Singh 1994; Chamswarng 1995; Datnoff *et al*. 1995).

2.2.2 *Trichoderma*-pathogen interactions

Trichoderma species possess several control mechanisms to combat against phytopathogenic organisms. These biocontrol mechanisms include competition with plant pathogens, mycoparasitism, antibiosis, production of lytic enzymes and secretion of secondary metabolites (Vinale *et al*. 2008).

Trichoderma species are relatively good antagonists against pathogenic fungi. They are able to survive under extreme competitive conditions. They are able to overcome fungistatic effects (Benítez *et al*. 2004). Moreover, they are resistant against many toxic compounds, including metabolites produced by soil microflora and plants,

fungicides, herbicides and antibiotics. These abilities might be due to the presence of ATP-binding cassette (ABC) transporter. The increased expression of these ABC-transporter genes reduces toxicant accumulation in the cells (Harman *et al.* 2004). Thus, allowing them to survive under extreme conditions and become more competitive compared to other soil fungi.

Other than that, *Trichoderma* species are good in mobilizing and uptaking of nutrients compared to other organisms (Benítez *et al.* 2004). They compete for nutrients, growth factors and space with plant pathogens (Vinale *et al*. 2008). Lack of easily accessible nutrients in the soil starved the pathogens and thus controls the growth of pathogens. For example, biological control strains of *Trichoderma* are able to make highly efficient siderophores that chelate iron from other filamentous fungi. Those fungi such as *Pythium*, need iron for survival will be killed (Benítez *et al*. 2004).

Besides that, *Trichoderma* species can parasitize many other fungi. Under normal conditions, *Trichoderma* species always secrete low level of cell wall degrading enzymes (CWDEs) such as chitinases and glucanases. When pathogenic fungi are present, CWDEs lyses the cell wall of pathogens and release cell wall oligomers from pathogens. The degradation products from pathogens further induce the expression of mycoparasitic gene expression (Vinale *et al*. 2008). After that, *Trichoderma* species grow towards pathogens. When *Trichoderma* species come into contact with pathogenic fungi, they attach and coil around the pathogens, and a specialized pressing organ known as appressoria will be formed to infect pathogens. Holes can be produced at the site of appressoria, and *Trichoderma* hyphae enter into the lumen of target fungi. As a result, the pathogenic fungi can be killed (Harman *et al*. 2004).

Furthermore, *Trichoderma* species can be the active colonizers of their habitats because they can produce a wide variety of secondary metabolites, including antibiotics and other natural compounds (Vinale *et al*. 2008).

According to Ghisalberti and Sivasithamparam (1991), secondary metabolites produced by *Trichoderma* can be classified into three categories: (i) volatile antibiotics such as 6-pentyl-α-pyrone (6PP), (ii) water soluble compounds such as heptelidic acid and (iii) peptaibols which are classified under a class of linear oligopeptides, and shown to inhibit β-glucan synthase in pathogenic fungi (Benítez *et al*. 2004). As a result of the inhibition, pathogens are prevented from reconstructing

their cell walls which are degraded by β-glucanase produced by *Trichoderma*. This also allows the β-glucanase to act more effectively (Vinale *et al*. 2008).

Thangavelu *et al*. (2004) who tested the potential of *Trichoderma* species in controlling the Fusarium wilt of banana reported that *T. harzianum* isolate Th-10 was most effective in inhibiting the mycelial growth of *Fusarium in vitro*. Soil application of *T. harzianum* Th-10 in dried formulation was shown to be effective in suppressing the disease. The efficacy was comparable to that of the fungicide carbendazim.

Several papers reported that *Trichoderma* spp. could induce resistance in plants. Yedidia *et al*. (2000) said that *T. harzianum* strain T-203 succeeded to induce resistance of cucumber and cucumber produce pathogenesis related (PR) proteins and enzymes after it was colonized by *T. harzianum.*

2.2.3 *Trichoderma*-plant interactions

Trichoderma species are usually found colonizing plant root ecosystems, establishing symbiotic relationship with plants. However, colonization of the root tissues are only limited at the root cortex due to the deposition of callose which restrict the penetration of hyphae. The callose barriers made *Trichoderma* become harmless to the plants (Vinale *et al*. 2008). However, elicitors produced by *Trichoderma* species during penetration stimulate the activation of plant defence system, causing an increase in the production of defence-related plant enzymes, such as chitinase, glucanase, and enzymes associated with the biosynthesis of phytoalexins. This has been shown in the plants treated with *Trichoderma* (Benítez *et al.* 2004; Vinale *et al*. 2008). Some of the induced resistances in plants are localized, while most of them are systemic, where the control of plant disease happens at a site distant from *Trichoderma* (Harman *et al*. 2004).

Furthermore, presence of *Trichoderma* species at the root ecosystems had shown to enhance plant root development (Harman *et al*. 2004; Benítez *et al*. 2004; Vinale *et al.* 2008). This in turn increase drought tolerance of the plants, and may improve the resistance of plants towards compacted soils. Besides that, *Trichoderma* species are capable in controlling deleterious microbes that reduce root development. *Trichoderma* species are resistant to the cyanide produced by these deleterious microbes. They are even able to remove these microbes from the root zone through mycoparasitic effects. Therefore, the *Trichoderma*-plant interactions are always associated with improvements in plant yield and biomass. For example, maize treated

with *Trichoderma* strain T-22 had shown to increase about 5% in average yield (Harman *et al*. 2004).

2.2.4 *Trichoderma harzianum*

2.2.4.1 Morphology

Colony morphology of *Trichoderma harzianum* on potato dextrose agar medium formed one-two concentric rings with green conidial production. The conidia production was denser in center then towards the margin. On corn meal dextrose agar, *T. harzianum* shows the colony with one-two concentric rings. Green or whitish conidia are more or less restricted in the concentric rings. On Czapek's dox agar media, it shows single green and yellowish concentric ring with a cluster of yellow conidia around the point of inoculum. Some white conidia appear to grow scarcely toward the edges. Conidia are light green colour on PDA media. The size of conidia is 2.8 x 2.6 µm and they are globose to subglobose. Phialides were flask-shaped in *T. harzianum* (Shah *et al*. 2012).

2.2.4.2 Antagonistic activity of *Trichoderma harzianum* on *Sclerotium rolfsii*

Antagonisms of *T. harzianum* may operate by using the pathogen as a food source. The cell wall of *S. rolfsii* is composed of β-1-3 glucan and chitin (Chet *et al.* 1967). *T. harzianum* attacks *S. rolfsii* as a mycoparasite and produces chitinase to break down the walls of the fungus. The hyphae of *Trichoderma* may penetrate resting structures such as sclerotia or may parasitize growing hyphae. In the later case, the hyphae grows alongside the pathogen and send out side branches that coil around the hyphae and cause death sometimes without obvious evidence of holes in the attacked hyphae (Sunil *et al.* 2007).

T. harzianum also softened and killed the sclerotia. It heavily colonized the sclerotia covering the entire surface area. The hyphae penetrated through the rind of the sclerotium and multiplied inside the sclerotium producing spores. Such penetration sites on the rind of the infected sclerotia were frequently found. In case of hard sclerotia, the degree of colonization was relatively less and the penetration sites were also rarely observed. There was very scantly mycelial multiplication inside the sclerotium leading to its normal germination (Desai and Schlosser 1999).

CHAPTER III

MATERIALS AND METHODS

3.1 Evaluation on Variability of Different Isolates of *Sclerotium rolfsii*

The experiment was conducted at the Department of Plant Pathology, Yezin Agricultural University (YAU), Nay Pyi Taw during September to November, 2014.

3.1.1 Collection of *Sclerotium rolfsii* infected specimens

The disease specimens were collected from naturally infected plants growing in the fields of Yezin area. The name of collected plants, their disease symptoms and collected areas were presented in Table 3.1.

3.1.2 Isolation and identification of *Sclerotium rolfsii*

The fungus was isolated from the infected plants and cultured on potato dextrose agar medium (PDA: 200 g potato, 20 g Dextrose, 20 g Agar, 1 l H_2O). Small pieces of infected tissue (about 5 mm) including the advancing margins of infection were cut and surface sterilized with 0.5% sodium hypochloride solution for 5 minutes. Then, these pieces were subsequently washed in three changes of sterilized water. After drying the pieces between sterilized filter papers, the pieces were placed on water agar media (WA: 20 g Agar, 1 l H_2O). The cultures were incubated at room temperature, 28°C for four days. The growing mycelia were checked under the microscope and subcultured on the PDA medium. The pure cultures of each isolates were maintained on PDA slants throughout the present study.

The pathogen was identified as *S. rolfsii* based on its mycelial and sclerotial characters (Barnett and Hunter 1960). The designated name for each *S. rolfsii* isolates was described in Table 3.1.

3.1.3 Morphological and cultural characteristics of *Sclerotium rolfsii*

Sclerotium rolfsii isolates from ten host plants were evaluated for their variations based on mycelial and sclerotial characters on PDA medium. Mycelial discs (5 mm in diameter) taken from the advancing margins of 4 day-old cultures of each isolate by the aid of a cork borer were placed at the centre of the petridish containing PDA medium and incubated at 28°C for 28 days. The diameters of the colonies were measured daily and the growth rates were calculated. Appearance of the colony and sclerotial character such as number, size and colour of sclerotia were recorded.

Table 3.1 List of collected host plants, names of isolates, their disease symptoms and collected areas

No.	Host plants	Designated names of isolates	Disease symptoms	Collected areas
1.	Chickpea (*Cicer arietinum*)	CK	Collar rot	DAR, Yezin
2.	Chilli (*Capsicum annum*)	CL	Stem rot	YAU, Yezin
3.	Cowpea (*Vigna unguiculata*)	CP	Collar rot	DAR, Yezin
4.	Eggplant (*Solanum melongena*)	EP	Stem rot	DAR, Yezin
5.	Groundnut (*Arachis hypogaea*)	GN	Stem rot	DAR, Yezin
6.	Soybean (*Glycine max*)	SB	Collar rot	DAR, Yezin
7.	Sesame (*Sesamum indicum*)	SS	Stem rot	DAR, Yezin
8.	Sunflower (*Helianthus annus*)	SF	Foot rot	DAR, Yezin
9.	Taro (*Colocasia esculenta*)	TR	Sclerotium blight	YAU, Yezin
10.	Tomato (*Lycopersicon esculentum*)	TT	Stem rot	DAR, Yezin

DAR - Department of Agricultural Research

YAU - Yezin Agricultural University

3.1.4 Mycelial compatibility grouping (MCG) test

Mycelial compatibility of ten isolates was studied on PDA media. Each *S. rolfsii* isolate was cultured on PDA media. At 4 days after incubation, about 5 mm diameter mycelial discs of each isolate were cut from the edge of actively growing colony by using cork borer. Then the mycelial discs of two isolates were placed approximately 15 mm apart on opposite sides of petridishes. The two isolates were paired on one petridish and these petridishes were incubated at 28^{o}C for eight days. and the test was repeated twice. The pairings were visually examined after 5 to 8 days for the presence of an antagonistic (barrage or aversion) zone in the region of mycelia contact as described by Punja and Grogan (1983).

3.1.5 Experimental design and data collection

The experiment was laid out in a Completely Randomized Design (CRD) with five replications. Data on the morphological and cultural characteristics such as, mycelia growth rate (mm day^{-1}), colony morphology (fluffy or less fluffy) at seven days after inoculation (DAI), number of sclerotia $petridish^{-1}$ produced by each isolate, sclerotia size (mm) and colours were evaluated at 28 DAI. For colony diameter, the linear growth was assessed by measuring two marked diameters which were perpendicular each other and the mean colony diameter was calculated. At 28 DAI, the sclerotia from each petridish were harvested and counted. Among them, ten sclerotia were randomly selected for each replication of each isolate. The diameter of each sclerotium was measured as mm to determine the size using USB digital microscope.

For mycelial compatibility grouping test, normal intermingling and barrage or clearing zone between the colonies of two isolates were visually examined after eight days incubation. Pairings were marked as incompatible when antagonistic barrage zone was observed between two paired isolates and put into different groups. When mycelia of two isolates intermingled without a barrage zone between them, they were compatible and they were placed into the same group.

3.1.6 Data analysis

The data were statistically analyzed by using Statistix version 8.0 program and mean comparisons were performed using Least Significant Difference (LSD) at 5 % level.

3.1 Inhibition Effect of *Trichoderma harzianum* on Different Isolates of *Sclerotium rolfsii*

The experiment was conducted at the Department of Plant Pathology, YAU, Nay Pyi Taw during December, 2014 to February, 2015.

3.2.1 *In vitro* test

Trichoderma harzianum was obtained from Department of Agricultural Research and subcultured on the Martin's media slants for further use.

The effect of *T. harzianum* on eight *S. rolfsii* isolates namely CK, CL, CP, EP, GN, SS, SF and TT studied by dual culture technique on PDA medium as followed by Rangeswaran and Prasad (2000). *T. harzianum* and each of *S. rolfsii* isolates to be tested were cultured separately on PDA medium for 4 days. After 4 days, 5 mm mycelial discs of each fungal colony to be tested were cut using cork borer and transferred to the petridish containing PDA media. The mycelial discs of *T. harzianum* and each isolate of *S. rolfsii* were placed at opposite side and 60 mm apart from each other on a PDA surface. Only one disc of each *S. rolfsii* isolate was inoculated on PDA medium and they were kept as controls. All culture petridishes were incubated at 28°C for 5 days.

3.2.1.1 Experimental design and data collection

The experiments were laid out in a Completely Randomized Design (CRD) with five replications. Mycelial growth of *T. harzianum* and *S. rolfsii* were measured when the controls were observed as full growth in culture. The inhibition percentage "I%" was calculated using the following formula (Datta *et al.* 2004).

The inhibition %,

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

where, C is radial growth in the control, and

T is radial growth in the treatment.

3.2.1.2 Data analysis

The data were statistically analyzed by using Statistix version 8.0 program and mean comparisons were performed using Least Significant Difference (LSD) at 5 % level.

3.2.2 *In vivo* test (Cross-inoculation study)

Inhibition effect of *T. harzianum* on the diseases caused by eight *S. rolfsii* isolates, namely CK, CL, CP, EP, GN, SS, SF and TT was investigated on eight different crops by cross inoculation in nursery trays. The crops were chickpea, chilli, cowpea, eggplant, groundnut, sesame, sunflower and tomato. The varieties of these crops were shown in Appendix 7.

3.2.2.1 Preparation of mass culture of *Sclerotium rolfsii*

Sorghum grains were soaked overnight in 2% sucrose solution, drained and boiled in fresh water for about 1 hours. About 250 g of sorghum grains were placed in cellophane bags. They were autoclaved at 121ºC for 20 minutes and cooled down for one day. The sterilized sorghum grains were inoculated with 5 mm diameter mycelial disc of each *S. rolfsii* isolate, taken from the margin of actively growing cultures using a cork borer and were incubated at room temperature for 15 days (Ganesan *et al.* 2007).

3.2.2.2 Preparation of mass culture of *Trichoderma harzianum*

Rice husks were soaked in water for about 2 hours and seived to remove excess water. The rice husks were mixed with rice bran (8:1) wt/wt and 5 g of sugar. After that, the mixture was filled in cellophane bags and autoclaved at 121ºC for 20 min and cooled down for one day. Then, a mycelium disc of 5 mm diameter of 3 days old culture of *T. harzianum* was added into the rice husk substrate in cellophane bags. The substrates were incubated at room temperature for 15 days.

3.2.2.3 Soil inoculation

Cross inoculation study was carried out in nusery trays (150 cm x 75 cm x 2.5 cm). The sterilized soil was mixed with the inocula of different *S. rolfsii* isolates at the rate of 50 g kg^{-1} soil. For *Trichoderma* application treatment, *T. harzianum* substrates were added in the mixture of sterilized soil and each of *S. rolfsii* isolates at the rate of 35 g kg^{-1}. About 4 kg of the mixture were placed in each tray and incubated at 29 °C for 3 days in wire house.

3.2.2.4 Seeds preparation

The tested crops were chickpea, chilli, cowpea, eggplant, groundnut, sesame, sunflower and tomato. The seeds were obtained from Food Legume Section, Oil Seed Crop Section and Horticulture Section, Department of Agricultural Research. The

seeds were surface sterilized with 5% sodium hypochloride solution for 5 minutes. Then, the surface sterilized seeds were washed with sterilized water for three times. Ten pregerminated seeds of each of eight crops were sown in each tray with the spacing of 2.5 cm between the plants and 5 cm between the rows. The total plants in each tray were eighty plants.

3.2.2.5 Experimental design and data collection

The experiment was laid out in a 3 factors CRD factorial arrangement with three replications. Factor A was *S. rolfsii* isolates, Factor B was levels of *T. harzianum* application and Factor C was different crops. Number of pre-emergence dead plants, post-emergence dead plants and the infected plants were recorded starting at 3 days after sowing with 3 days interval until 3 weeks and calculated as disease incidence percent (DI %).

The disease incidence was calculated using the following formula (Cooke 2006).

$$\text{DI \%} = \frac{\text{No. of infected plant unit}}{\text{Total no. of plant units assessed}} \times 100$$

Then, percent disease control of *T. harzianum* was calculated by the following equation described by Engelhard (1997).

$$\text{\% disease control} = \frac{\text{DI(C)} - \text{DI(T)}}{\text{DI(C)}} \times 100$$

DI (C) = Mean disease incidence in control

DI (T) = Mean disease incidence in treatment

3.2.2.6 Data analysis

The data were analyzed by using Statistix version 8.0 program. Mean comparisons were performed using Least Significant Difference (LSD) at 5 % level. Percentage data were transformed into arc sine angles before carrying out the ANOVA to produce approximately constant variance (Gomez and Gomez 1984).

CHAPTER IV

RESULTS

4.1 Evaluation of Variability of Different Isolates of *Sclerotium rolfsii*

4.1.1 Isolation and identification of *Sclerotium rolfsii*

The mycelium of the fungus isolated from the diseased tissues was formed at two days after isolation on PDA medium. The fungus was transferred on the petridish containing PDA medium and checked for the characters of the fungus under the microscope. The hyphae were hyaline with thin cell and cross walls are sparse. Main branch hyphae had clamp connections on each side of the septum (Plate 4.1). Advencing mycelium and colonies grew as distinctive fan-shaped appearance and the hyphal strands had a somewhat ropy appearance. Sclerotia were darken as they matured, becoming tan to dark brown in colour (Plate 4.2). Mature sclerotia at 28 days after incubation were hard and have a distinct rind. Based on these characters, this fungus was identified as *S. rolfsii* (Barnet 1960). The pure cultures of each isolate were maintained on PDA throughout the persent study.

4.1.2 Morphological and cultural characteristics of *Sclerotium rolfsii*

Variability in cultural morphology, mycelial growth rate, sclerotia formation, sclerotial size and colour of the isolates of *S. rolfsii* were observed on PDA media and the results were presented in Table 4.1.

The fungus was first silky white in colour and later turned to dull white with radial spreading given fan-shaped appearance. Sclerotia were formed sparsely in five to seven days on colony at room temperature of 28°C. They began to develop at 4 to 7 days after incubation as small tufts of white mycelium then that became spherical sclerotia. Young sclerotia often exude droplets of clear to pale yellowish fluids.

There were two types of colony among the ten isolates of *S. rolfsii*. Out of the ten isolates, the less fluffy colony was produced by only one isolate namely the chickpea (CK) (Plate 4.3a) and that of other isolates were fluffy (Plate 4.3b). The mycelial growth rates were statistically different among the isolates. The growth rates of *S. rolfsii* ranged from 27 to 30 mm diameter day^{-1}. The slowest growth rate, 27 mm diameter day^{-1} was found in the CK and the tomato (TT) isolates.

There were also significantly differences in sclerotial production of the ten isolates (Table 4.1). The number of sclerotia produced by the ten isolates was ranged from 281-1018 $petridish^{-1}$ at 28 days after incubation (DAI). The maximum number

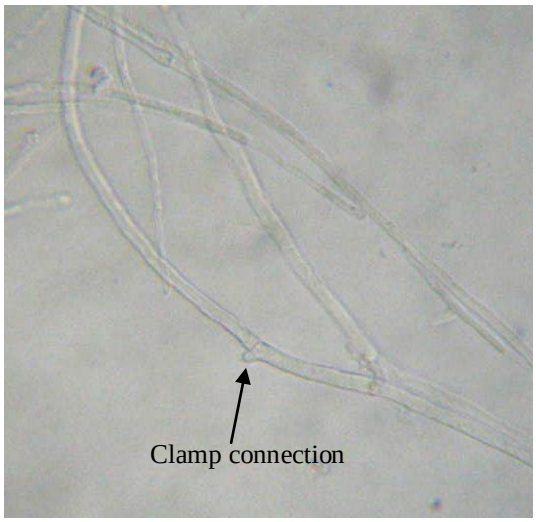

Plate 4.1 Mycelium with clamp connection of *Sclerotium rolfsii* (40x)

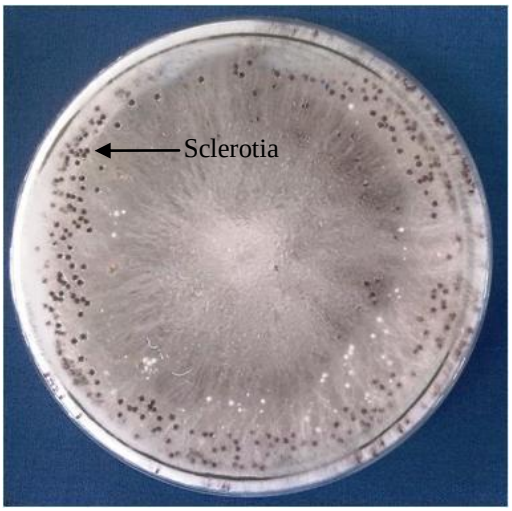

Plate 4.2 Dark brown sclerotia of *Sclerotium rolfsii* on PDA medium at 7 days after incubation

Table 4.1 Variation in morphological and cultural characteristics of different isolates of *Sclerotium rolfsii*

Isolates	Hosts	Mycelial character		Sclerotial characters		
		Type*	Mycelial growth rate (mm day^{-1})*	No. of sclerotia plate^{-1}**	Sclerotial size (mm)**	Sclerotial colour**
CK	Chickpea	Less Fluffy	26.9 ± 0.9	281 ± 58.1	0.63 ± 0.04	Dark brown
CL	Chilli	Fluffy	30.0 ± 0.0	743 ± 38.0	0.48 ± 0.01	Dark brown
CP	Cowpea	Fluffy	30.0 ± 0.0	352 ± 57.9	0.47 ± 0.01	Dark brown
EP	Eggplant	Fluffy	29.8 ± 0.2	381 ± 45.2	0.49 ± 0.01	Dark brown
GN	Groundnut	Fluffy	30.0 ± 0.0	383 ± 95.3	0.61 ± 0.02	Dark brown
SB	Soybean	Fluffy	30 .0 ± 0.0	407 ± 17.5	0.46 ± 0.02	Dark brown
SS	Sesame	Fluffy	30.0 ± 0.0	1018 ± 109.2	0.37 ± 0.02	Reddish brown
SF	Sunflower	Fluffy	30.0 ± 0.0	345 ± 89.2	0.49 ± 0.03	Dark brown
TR	Taro	Fluffy	29.9 ± 0.2	389 ± 51.4	0.52 ± 0.01	Dark brown
TT	Tomato	Fluffy	27.1 ± 0.5	575 ± 92.0	0.45 ± 0.01	Brown
$LSD_{(0.05)}$			**0.94**	**203.31**	**0.05**	
Pr ≥ F			**0.0000**	**0.0000**	**0.0000**	
CV %			**2.52**	**32.61**	**8.59**	

* Recorded after 3 days of inoculation,

** Recorded after 28 days of inoculation

± standard error

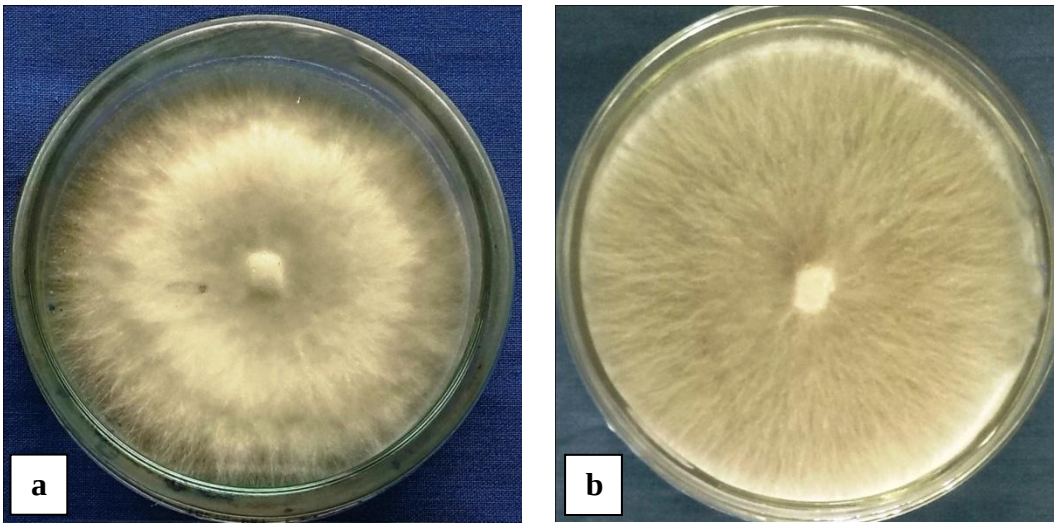

Plate 4.3 Colony morphology of *Sclerotium rolfsii* at 7 days after incubation; less fluffy colony of the chickpea isolate (a), and fluffy colony of the sunflower isolate (b) on potato dextrose agar media

of sclerotia, 1018, produced by the sesame (SS) isolate (Plate 4.4a). The minimum number of sclerotia (281 petridish^{-1}) was observed in the CK isolate (Plate 4.4b).

The sizes of sclerotia were also statistically different from each other. The average size ranged from 0.37 to 0.63 mm in diameter (Table 4.1). The two isolates, CK and groundnut (GN) produced the largest sclerotial diameter of 0.63 mm and 0.61 mm respectively. The smallest sclerotia were produced by the sesame isolate and its diameter was 0.37 mm.

Three colours of sclerotia were observed among the isolates as dark brown, reddish brown and brown. The sclerotia of most isolates were dark brown in colour. The sclerotial colour of the SS isolate was reddish brown and the TT isolate produced brown-coloured sclerotia.

4.1.3 Mycelial compatibility grouping (MCG)

There were 45 parings of ten isolates and out of all, only two combinations showed compatible reaction and 43 combinations were incompatible (Table 4.2). It was observed that the two parings, the CP and the TR isolates, and the GN and the SF isolates showed compatible reactions. In these parings, mycelia of the two different isolates intermingled without the development of clearing zone between them and the sclerotia were found at the mycelial contact (Plate 4.5). The other combinations, such as the GN and the SB isolates paring, and the SS and the TT isolates paring, showed incompatible reactions. In these combinations, the clearing or barrage zone was formed at the region of interaction between the two isolates (Plate 4.6). Based on the mycelial compatibility, the ten isolates were grouped into eight mycelial compatibility groups (MCGs) (Table 4.2). In MCG 3, there were two isolates of the CP and the TR and similarly, MCG 5 had the GN and the SF isolates.

4.2 Inhibition Effect of *Trichoderma harzianum* on Different Isolates of *Sclerotium rolfsii*

4.2.1 Inhibition effect of *Trichoderma harzianum* on mycelial growth of different isolates of *Sclerotium rolfsii in vitro*

In *in vitro* experiment, eight *S. rolfsii* isolates namely chickpea (CK), chilli (CL), cowpea (CP), eggplant (EP), groundnut (GN), sesame (SS), sunflower (SF) and

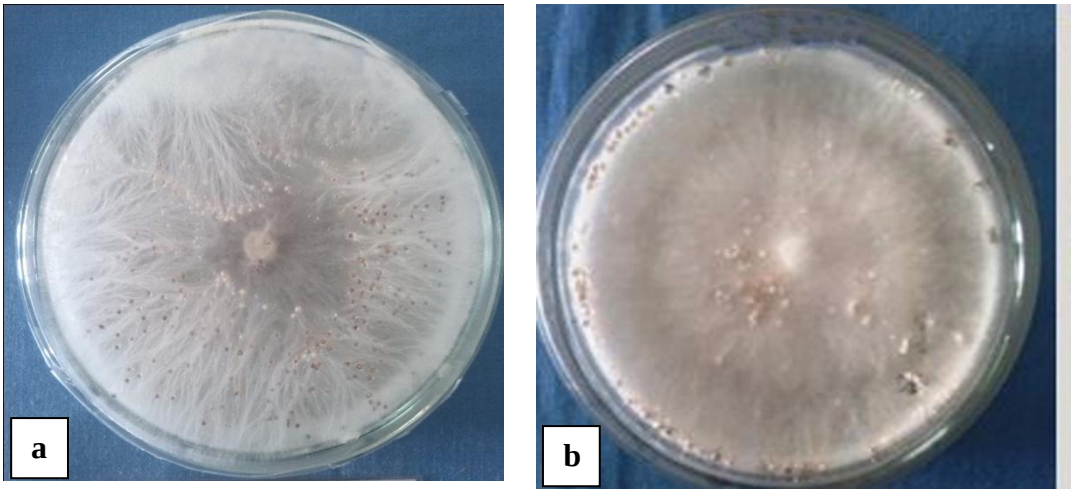

Plate 4.4 Sclerotial production of *Sclerotium rolfsii* at 7 days after incubation; maximum sclerotia by the sesame isolate (a), and minimum sclerotia by the chickpea isolate (b) on potato dextrose agar media

Table 4.2 Mycelial compatibility and groupings (MCGS) of ten isolates of *Sclerotium rolfsii*

Isolates	Hosts	CK	CL	CP	EP	GN	SB	SS	SF	TR	TT	MCG
CK	Chickpea	+	-	-	-	-	-	-	-	-	-	1
CL	Chilli		+	-	-	-	-	-	-	-	-	2
CP	Cowpea			+	-	-	-	-	-	+	-	3
EP	Eggplant				+	-	-	-	-	-	-	4
GN	Groundnut					+	-	-	+	-	-	5
SB	Soybean						+	-	-	-	-	6
SS	Sesame							+	-	-	-	7
SF	Sunflower								+	-	-	5
TR	Taro									+	-	3
TT	Tomato										+	8

+ = compatible reaction

- = incompatible reaction

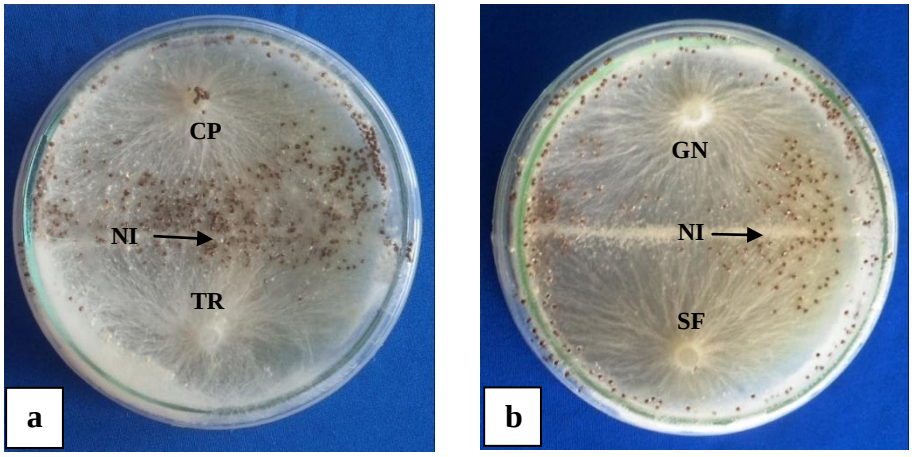

Plate 4.5 Mycelial compatible reactions between cowpea (CP) and taro (TR) isolates (a), and groundnut (GN) and sunflower (SF) isolates (b) with normal intermingling (NI) on potato dextrose agar media

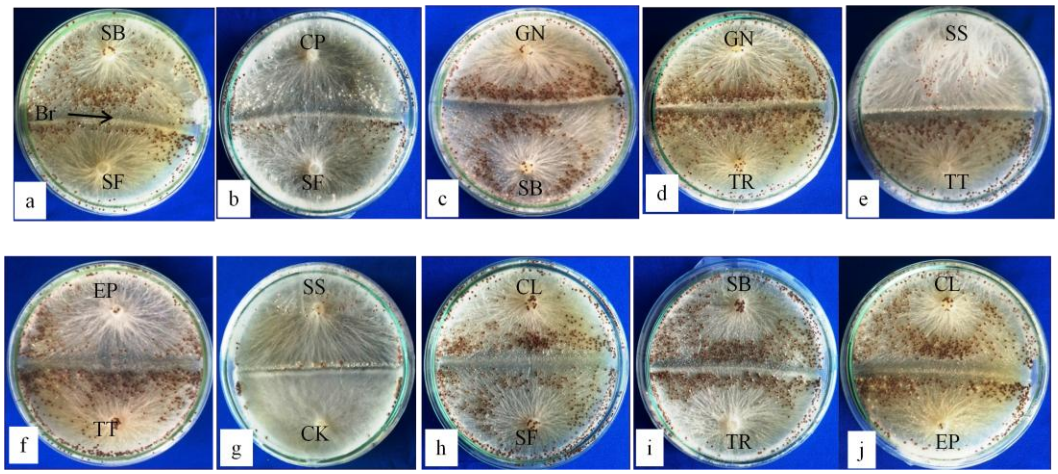

Plate 4.6 Mycelial incompatible reactions of some isolates of *Sclerotium rolfsii* with a presence of barrage or clearing zone (Br) where mycelium meet; soybean (SB) and sunflower (SF) (a); cowpea (CP) and sunflower (SF) (b); groundnut (GN) and soybean (SB) (c); groundnut (GN) and taro (TR) (d); sesame (SS) and tomato (TT) (e); eggplant (EP) and tomato (TT) (f); sesame (SS) and chickpea (CK) (g); chilli (CL) and sunflower (SF) (h); soybean (SB) and taro(TR) (i); chilli (CL) and eggplant (EP) (j) respectively

tomato (TT) were used. The effectiveness of *T. harzianum* on the mycelial growth of eight *S. rolfsii* isolates was evaluated on PDA medium at 5 days after inoculation. It was observed that the inhibition percent of *T. harzianum* against the mycelial growth of *S. rolfsii* isolates were significantly different ranging from 58.4% to 68.3% (Table 4.3 and Plate 4.7). *T. harzianum* was found to have the most suppressive effect on the mycelial growth of the sesame (SS) isolate showing the lowest radial growth and the highest inhibition percent (68.3%). However, *T. harzianum* was observed to have the least suppressive effect against the mycelial growth of the groundnut (GN) resulting the largest radial growth and the lowest inhibition percent (58.4%).

4.2.2 Effect of *Trichoderma harzianum* on diseases caused by different isolates of *Sclerotium rolfsii* on different host crops

The disease incidence was significantly different among the eight different *S. rolfsii* isolates, among two levels of *T. harzianum* application and also among different host plants at 21 days after sowing (Table 4.4). The disease incidence of *S. rolfsii* isolate ranged from 31% to 86%. It was observed that the lowest disease incidence (31%) was found in the chickpea (CK) isolate inoculated soil and the highest percent disease incidence (86%) was observed in the eggplant (EP) isolate inoculated soil.

The disease incidence in *S. rolfsii* infested soil was significantly reduced due to *T. harzianum* application. It was 70% in *S. rolfsii* infested soil with *T. harzianum* and 81% in the infested soil without *T. harzianum*.

The responses of the eight crops to disease caused by *S. rolfsii* isolate were also significantly different from each other. The highest disease incidence (80%) was found on the eggplant and the lowest disease incidence (68%) was found on the chilli.

There were interactions between *S. rolfsii* isolates and the crops, between *S. rolfsii* isolates and effect of *T. harzianum* and also between the crops and effect of *T. harzianum* (Table 4.4).

The interaction effect was observed between *S. rolfsii* isolates and the crops (Table 4.5). Almost all the isolates except the CK caused high disease incidence on all tested crops, ranging from 62% to 88%. The disease incidence on the tested crops

Table 4.3 Inhibition effect of *Trichoderma harzianum* on mycelial growth of eight isolates of *Sclerotium rolfsii* at 5 days after inoculation *in vitro*

Isolates	Hosts	Radial growth (mm)*	Inhibition %*
CK	Chickpea	28.6abc	61.9bc
CL	Chilli	27.6bc	63.2b
CP	Cowpea	27.4c	63.5b
EP	Eggplant	30.4ab	59.5bc
GN	Groundnut	31.2a	58.4c
SS	Sesame	23.8d	68.3a
SF	Sunflower	30.0abc	60.0bc
TT	Tomato	29.2abc	61.1bc
$LSD_{(0.05)}$		**2.99**	**1.96**
Pr ≥ F		**0.0007**	**< 0.0001**
CV %		**8.16**	**5.01**

* Means of five replications in the same column followed by the same letters are not significantly different from each other

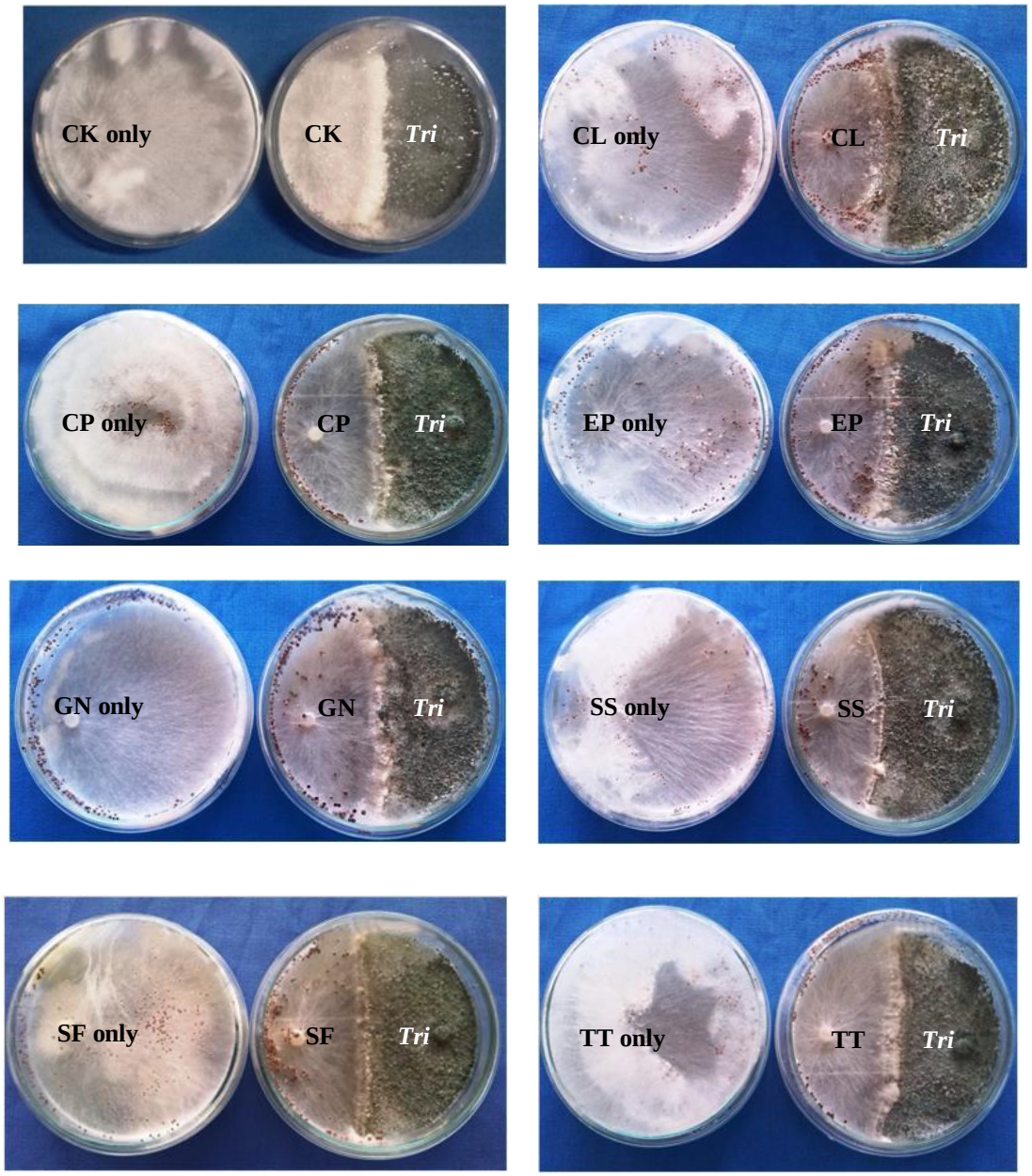

Plate 4.7 Inhibition effect of *Trichoderma harzianum* on mycelial growth of *Sclerotium rolfsii* isolates at 5 days after incubation

CK - Chickpea isolate
CL - Chilli isolate
CP - Cowpea isolate
EP - Eggplant isolate
Tri - *Trichoderma harzianum*
GN - Groundnut isolate
SS - Sesame isolate
SF - Sunflower isolate
TT - Tomato isolate

Table 4.4 Mean values of disease incidence (%) of the eight crops as affected by *Trichoderma harzianum* and the eight isolates of *Sclerotium rolfsii*

Treatment	Disease incidence(%)*
Isolates of *S. rolfsii* (I)	
CK	30.9e
CL	83.6 abc
CP	79.5cd
EP	86.2a
GN	84.4ab
SS	75.5d
SF	84.8ab
TT	81.1bc
$LSD_{(0.05)}$	**4.24**
***Trichoderma harzianum*(*TRI*)**	
Without *TRI*	81.2a
With *TRI*	70.3b
$LSD_{(0.05)}$	**2.12**
Crops (C)	
Chickpea	77.4abc
Chilli	67.9d
Cowpea	73.8c
Eggplant	80.6a
Groundnut	73.5c
Sesame	74.9bc
Sunflower	79.1ab
Tomato	78.9ab
$LSD_{(0.05)}$	**4.24**
Pr ≥ F	
Isolates of *S. rolfsii* (I)	< 0.0001
T. harzianum (*TRI*)	< 0.0001
Crops (C)	< 0.0001
Interaction	
I x C	< 0.0001
I x *TRI*	< 0.0001
C x *TRI*	0.017
I x C x *TRI*	0.865
C.V %	**13.92**

* Means in the same column followed by the same letter are not significantly different from each other at 5% level.

Table 4.5 Disease incidence (%) in eight crops caused by eight *Sclerotium rolfsii* isolates

***S. rolfsii* isolates**	**Disease incidence (%)***							
	Crops							
	Chickpea	**Chilli**	**Cowpea**	**Eggplant**	**Groundnut**	**Sesame**	**Sunflower**	**Tomato**
CK	26.7	4.7	19.8	51.5	5.8	42.8	41.6	54.9
CL	80.4	82.2	83.8	85.7	85.7	82.9	85.9	82.2
CP	83.3	76.6	75.5	82.4	80.9	80.2	80.2	77.0
EP	86.0	88.6	80.6	85.7	85.7	84.5	89.0	88.0
GN	86.3	86.3	76.6	85.7	82.4	85.9	82.6	88.6
SS	80.9	81.7	75.1	81.7	79.3	61.9	78.3	78.7
SF	89.2	80.8	83.3	89.0	82.4	82.9	89.0	81.8
TT	86.3	65.5	86.3	82.6	85.7	78.3	85.9	78.3
$LSD_{(0.05)}$	**11.9**							

CK - chickpea, CL - chilli, CP - cowpea, EP - eggplant, GN - groundnut, SS - sesame, SF - sunflower, TT - tomato

* Means of three replication

caused by the CK isolate ranged from 4.7% to 55% on the tested crops. The disease incidence on the sesame infected by the SS isolate was 62% and on the chilli infested by the tomato (TT) isolate was about 65%.

The effect of *T. harzianum* on *S. rolfsii* isolates was shown in Table 4.6. *T. harzianum* could not control the disease caused by the CK isolate. The percent disease control of *T. harzianum* on other seven *S. rolfsii* isolates ranged from 6 % to 30 %. The effect of *T. harzianum* on the sesame (SS) was 30%.

The effect of *T. harzianum* on the disease incidence of the eight different crops was presented in Table 4.7. The highest effect of *T. harzianum* was shown on the chilli and the lowest effect was observed on the eggplant.

Table 4.6 Effect of *Trichoderma harzianum* on disease incidence (%) of eight isolates and percent disease control at 21 days after sowing

Treatments	Disease incidence (%)*	% Disease control
Without *T. harzianum*		
CK	29.9g	-
CL	89.0a	-
CP	89.0a	-
EP	89.0a	-
GN	89.0a	-
SS	89.0a	-
SF	89.0a	-
TT	85.7ab	-
With *T. harzianum*		
CK	32.1g	-7.4
CL	78.2cd	12.1
CP	70.0e	21.3
EP	83.4abc	6.3
GN	79.7bcd	10.4
SS	62.1f	30.2
SF	82.4bcd	7.4
TT	76.5d	10.7
$LSD_{(0.05)}$	**5.99**	

CK - chickpea, CL - chilli, CP - cowpea, EP - eggplant, GN - groundnut, SS - sesame, SF - sunflower, TT - tomato

* Means of three replications,

* Means in the same column followed by the same letters are not significantly different from each other

Table 4.7 Effect of *Trichoderma harzianum* on disease incidence (%) caused by eight *Sclerotium rolfsii* isolates on eight crops at 21 days after sowing

Treatments	Disease incidence (%)*
Without *T. harzianum*	
Chickpea	81.5ab
Chilli	77.9abcd
Cowpea	79.7abc
Eggplant	83.7a
Groundnut	78.0abcd
Sesame	89.0ab
Sunflower	83.4ab
Tomato	82.4ab
With *T. harzianum*	
Chickpea	73.3def
Chilli	57.9h
Cowpea	67.9fg
Eggplant	77.4bcd
Groundnut	69.0efg
Sesame	66.9g
Sunflower	74.9cde
Tomato	75.3cd
$LSD_{(0.05)}$	**5.99**

* Means of three replications

* Means in the same column followed by the same letters are not significantly different from each other

CHAPTER V

DISCUSSION AND CONCLUSION

Variability in cultural morphology, mycelial growth rate, sclerotium formation, sclerotial size and color among *S. rolfsii* isolates from different geographical areas and also in different hosts were reported by many researchers (Almeida *et al*. 2001; Sarma *et al* 2002; Okereke and Wokocha 2007; Akram *et al*. 2008). Variation of isolates is very important to better understand disease outbreaks and predict future disease development (Stakman and Christensen 1953). In the present study, the isolates from the ten different host plants were varied in their morphological and sclerotial characters. Similar findings were recently reported by many researchers (Prasad *et al*. 2012; Rasu *et al*. 2013; Gawande *et al*. 2014).

In India, Pakistan and Vietnam, fluffy, compact and filamentous mycelial growth types were reported among isolates and in Georgia, flat and compact colony were observed among groundnut isolates (Sarma *et al*. 2002; Xie 2012; Rasu *et al*. 2013; Gawande *et al*. 2014). However, only two types were observed among the isolates from different hosts in this study. Most of the isolates have the same colony morphology and the colony of only one isolate was different from others. This result indicates that this might be a wide host range of *S. rolfsii*. Rasu *et al*. (2013) reported the fluffy and compact types of colony among 17 isolates of *S. rolfsii* and Sarma *et al*. (2002) also observed these two types of colonies among 26 isolates from different hosts.

In the present study, although most of *S. rolfsii* isolates from different hosts may be morphologically similar, their growth rates were varied ranging from 27-30 mm diameter day^{-1}. It might be due to differences in ecology, genetic differences or the nutrient level of the soil (Okereke and Wokocha 2007). However, most of the isolates covered the whole petridish within 3 days and the diameter of the colonies ranged from 80.9 to 90 mm. The colony diameter of 81-90 mm of *S. rolfsii* was the fast growing, 51-80 mm was medium growth and 30.6-50 was slow growing (Prasad *et al*. 2012). Therefore, all isolates seemed to be fast growing ones. Akram *et al*. (2007) also reported that *S. rolfsii* isolates with the colony diameter of 76.7-90 mm were recorded as fast growing isolates, that of 40.8-61.7 mm as medium growing and that of 16-30.6 mm as slow growing isolates.

The size, number and colours of sclerotia were also varied greatly among the isolates. Several workers had been reported variation in size, number and colours of sclerotia (Sarma *et al*. 2002; Kokub *et al*. 2007; Xie 2012; Rasu *et al*. 2013). The number of sclerotia produced by ten isolates was ranged from 281-1018 petridish^{-1} (9 cm diameter) at 28 days after inoculation and the sclerotial size ranged from 0.37-0.63 mm in diameter. In this study, it was observed that the sesame isolate was not only fast growing but also produced the maximum number of sclerotia. On the other hand, the chickpea isolate was also the fast growing isolate but it produced the minimum number of sclerotia. Therefore, it could be assumed that the growth rate and the sclerotial production were not correlated on PDA medium and the sclerotial production seems to be correlated with the colony morphology in this study. However, Kokub *et al*. (2007) reported that the fast growing isolates produced the maximum number of sclerotia and the slow growing isolate produced the minimum number of sclerotia on the agar plate. They also mentioned that the number of sclerotia produced by different isolates were ranged from 91-1210 petridish^{-1} with average diameter of 0.5-2.0 mm in diameter. Rasu *et al*. (2013) also found the average sclerotial size of 1-1.2 mm in diameter, whereas the largest sclerotia were 1.5-1.6 mm in diameter. Xie (2012) also observed that average sclerotial size ranged from 0.56 mm to 1.91 mm in diameter, while the sclerotia number ranged from 49-1427 petridish^{-1} among 84 isolates from different hosts. In other country, the largest sclerotium was about 2 mm in diameter and the lowest number was about 49-91. However, in this study, the largest sclerotial size was about 0.6 mm and the lowest number was 281. This may be also due to the differences in geographical areas and different hosts. There were three sclerotial colours such as brown, reddish brown and dark brown among *S. rolfsii* isolates. Sarma *et al*. (2002) reported that the colour of sclerotia was mostly dark and very light brown among different isolates.

The mycelial compatibility grouping (MCGs) are valuable tools to evaluate genetic variability in fungal plant pathogens and to understand the epidemiology of diseases caused by them (Mahmoud *et al*. 2007). The higher percentage of incompatible reactions (96%) in the mycelial compatibility test revealed the extent of the diversity among the ten isolates of *S. rolfsii* in Yezin area. In this study, eight mycelial compatibility groups (MCGs) were observed among the ten *S. rolfsii* isolates. The composition and distribution of *S. rolfsii* MCGs can vary greatly depending on the host plants and geographic regions (Xie 2012). Mycelial

compatibility or incompatibility reaction could be used to distinguish different strains belonging to the same species (Kokub *et al*. 2007). MCG 3 had two isolates of the cowpea and the taro. However, these isolates were collected from different crop fields. This result indicates that the spread of isolates can be found in different areas. The spread may be related to human activities such as the movement of infected plant materials, contaminated soil, or equipment that help to spread the isolates over long distance. In MCG 5, there were the two isolates, the groundnut and the sunflower and they were collected from the same area. It also indicates that some MCGs are more predominant in certain areas. In this study, the cowpea and the taro isolates and the groundnut and the sunflower isolates seem to be genetically similar because they were found in the same MCGs, respectively. Isolates in the same MCG were to be genetically more similar than isolates from different MCGs (Khon *et al*. 1991; Leslie 1993). This MCGs results indicate that it might be the presence of significant diversity in *S. rolfsii* population. Mehri *et al*. (2013) reported that 6 MCGs were found among 92 isolates of *S. rolfsii* from different 12 hosts. Sarma *et al*. (2002) also observed that 13 MCGs were identified among 26 isolates from different hosts. Xie (2012) also reported that the composition and distribution of MCGs within a population of *S. rolfsii* can vary greatly depending on the host plant and geographic region. This study might be able to provide the valuable information for further study that determines the genetic similarities and differences among the isolates of *S. rolfsii* by advanced molecular techniques.

All the isolates of *S. rolfsii* were cross-pathogenic because every isolates from different hosts could attack all tested crops including their main hosts. These results were agreement with the findings of Mahato and Mondal in 2014. They studied that five isolates of *S. rolfsii* were cross inoculated to each other and they observed that pathogenicity through cross-inoculation technique did not show any host specificity but the isolates showed different degrees of virulence. In this study, the chickpea isolate showed low pathogenicity on tested crops compared with other isolates.

Punja (1985) reported that there might be a positive correlation between sclerotial size and virulence since larger sclerotia could secrete a larger dose of oxalic acid and other cell wall degrading enzymes which are necessary for disease development. However, in this study, there was no correlation between the sclerotial size and virulence because the sesame isolate produced the smallest size of sclerotia but the pathogenicity was as high as other virulent isolates. On the other hand, the

largest sclerotia were produced by the chickpea isolate but it infected the tested crops with the lowest disease incidence.

The responses of crops to *S. rolfsii* isolates were also significantly different from each other. The disease incidence on the eight crops ranged from 68% to 81%. The lowest disease incidence was found on chilli and the highest disease incidence was found on eggplant. However, the disease incidence was very high on the tested crops. Therefore, it could be assumed that these crops were highly infected by *S. rolfsii* isolates. Yaqub and Shahzad (2005) tested the responses of the crops such as sunflower, mungbean, sugar beet, tomato, lentil, sweet pumpkin, cabbage and cauliflower against *S. rolfsii*. They observed that *S. rolfsii* was highly pathogenic on sunflower, mungbean and sugar beet, mildly pathogenic to tomato, lentil, sweet pumpkin and cabbage and non-pathogenic on cauliflower in pot experiments.

In this study, the inhibition percent of *T. harzianum* ranged from 58% to 68% against the *S. rolfsii* isolates *in vitro*. This result was in agreement with the findings of many researchers (Ganesan *et al*. 2006; Bhuiyan *et al*. 2012; Yasmin *et al*. 2014; Biasumatary *et al*. 2015). They reported that *T. harzianum* showed 57%-77% reduction of radial growth of *S. rolfsii in vitro*. Ganesan *et al*. (2007) used *T. harzianum* which showed 57 % inhibition *in vitro* in pot experiment.

The percent disease control of the commercial product of *T. harzianum* on different isolates of *S. rolfsii* except the chickpea isolate ranged from 6%-30%. It was observed that there was no effect of *T. harzianum* on the chickpea isolate. It might be due to some extent of *T. harzianum* effect on the chickpea isolate. In this experiment, the effectiveness of *T. harzianum* on *S. rolfsii* isolates was shown as a little effect and only 30% of disease control was found on the sesame isolate. It might be due to the variation of *S. rolfsii* isolates and the high pathogenic ability of the most isolates. Because of the wide host range and fast growth rate of *S. rolfsii* isolates and the production of large number of persistent sclerotia, the stem rot disease caused by *S. rolfsii* is very difficult to control (Punja 1985; Lakpale *et al*. 2007). Moreover, because of the highest inhibition percent of *T. harzianum* on *S. rolfsii* isolates was only 68% *in vitro*, it seems that *T. harzianum* could not control the diseases effectively *in vivo*. Foliar application of *T. harzianum* (10^8 spore ml^{-1}) reduced 15-25% plant mortality caused by *S. rolfsii* (Maurya *et al*. 2008). However, Ganesan *et al*. (2007) reported that *T. harzianum* successfully decreased the stem rot incidence caused by *S. rolfsii* and increased the growth of the groundnut plants.

Trichoderma harzianum is a biological control organism against a wide range of soil borne pathogens and also has plant growth promoting capacity (Chet 1990; Mc Govern *et al*.1992; Datnoff and Pernezny 1998). In this study, the effect of *T. harzianum* was also observed on the tested crops. The highest effect was found on the chilli and the lowest effect was found on the eggplant. It could be assumed that it depends on the infection of *S. rolfsii* isolates on the crops.

Based on this experiment, it can be concluded that the isolates of *S. rolfsii* from 10 different hosts varied in their morphological and sclerotial characters and the MCG groups. All the isolates of *S. rolfsii* were found to be cross-pathogenic to each other. *T. harzianum* inhibited the mycelial growth of *S. rolfsii* isolates about 58%-68% *in vitro* and could not control the diseases effectively *in vivo.*

Since *S. rolfsii* is a polyphagus pathogen and can overwinter in the soil for as long as 3 years, selection of non-host crops should be done carefully for crop rotation. Besides, due to weak effectiveness of *T. harzianum* used in this study, other control practices should be combined with *T. harzianum* to control the diseases caused by these isolates effectively. Moreover, alternative ways of control measures should be investigated to control *S. rolfsii* for the further study.

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APPENDICES

Appendix 1 Analysis of varience for mycelial growth rate (mm day^{-1}) of the ten *Sclerotium rolfsii* isolates

Source of varience	df	Sum of squares	Mean squares	Probability
Treatments	9	499.62	55.5133	< 0.0001
Residual	40	53.2	1.3300	
Total	**49**	**552.82**		

CV (%) = 2.52

Appendix 2 Analysis of varience for number of sclerotia produced by the ten *Sclerotium rolfsii* isolates

Source of varience	df	Sum of squares	Mean squares	Probability
Treatments	9	236956	263285	< 0.0001
Residual	40	101192	25298	
Total	**49**	**338194**		

CV (%) = 32.61

Appendix 3 Analysis of varience for scleroital size of the ten *Sclerotium rolfsii* isolates

Source of varience	df	Sum of squares	Mean squares	Probability
Treatments	9	0.2628	0.02920	< 0.0001
Residual	40	0.0726	0.00182	
Total	**49**	**0.3354**		

CV (%) = 8.59

Appendix 4 Analysis of varience for radial growth of the eight *Sclerotium rolfsii* isolates in dual culture

Source of varience	df	Sum of squares	Mean squares	Probability
Treatments	7	188.775	26.9679	0.0007
Residual	32	173.200	5.4129	
Total	**39**	**361.975**		

CV (%) = 8.16

Appendix 5 Analysis of varience for inhibition percent of *Trichoderma harzianum* on the eight *Sclerotium rolfsii* isolates *in vitro*

Source of varience	df	Sum of squares	Mean squares	Probability
Treatments	7	335.384	47.9119	< 0.0001
Residual	32	307.854	9.6204	
Total	**39**	**643.238**		

CV (%) = 5.01

Appendix 6 Analysis of varience for disease incidence (%) as affected by the eight *Sclerotium rolfsii* isolates, *Trichoderma harzianum* and the eight crops *in vivo*

Source of varience	df	Sum of squares	Mean squares	Probability
Isolates (I)	7	113908	16272.6	< 0.0001
T. harzianum (TRI)	1	11302	11302.2	< 0.0001
Crops (C)	7	5674	810.6	< 0.0001
I x *TRI*	7	6331	904.5	< 0.0001
I x C	49	16883	344.5	< 0.0001
TRI x C	7	1943	277.6	0.0170
I x *TRI* x C	49	4191	85.5	0.8653
Residual	256	28480	111.2	
Total	**383**	**188712**		

CV (%) = 13.92

Appendix 7 Variety names of the eight crops used in the experiment and their sources

Names of the crops	Cultivar names	Sources
Chickpea	Yezin-3	DAR
Chilli	Demon	DAR
Cowpea	Yezin-6	DAR
Eggplant	Padathar	DAR
Groundnut	Sinpadathar-11	DAR
Sesame	Sinyadanar-12	DAR
Sunflower	Sinshwekyar-2	DAR
Tomato	Kyarchayar	DAR

Appendix 8 Composition of media

8.1 Potato Dextrose Agar (PDA)

Potato	200 g
Dextrose	20 g
Agar	20 g
Water	1 L

8.2 Water Agar (WA)

Agar	20 g
Water	1 L

8.3 Martin's media

KH_2PO_4	1 g
$MgSO_4$	0.5 g
Peptone	5 g
Rosebengal	3 ml (1% Rosebengal)
Agar	20 g
Water	1 L