INTEGRATED DISEASE MANAGEMENT OF BANDED LEAF AND SHEATH BLIGHT OF MAIZE CAUSED BY *Rhizoctonia solani*

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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 **"Integrated Disease Management of Banded Leaf and Sheath Blight of Maize Caused by *Rhizoctonia solani*"** was prepared under the direction of the chairman 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, except where otherwise stated. It has not been submitted previously for a degree at any other university.

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DEDICATED TO MY BELOVED PARENTS
U HLA MYINT AND DAW THAN THAN

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ABSTRACT

The experiments were carried out at the Department of Plant Pathology, Yezin Agricultural University from May 2014 to March 2015. The study was aimed to evaluate the pathogenicity of different isolates of *Rhizoctonia solani*, to determine the antagonistic effect of *Trichoderma* spp. on *Rhizoctonia solani* and to evaluate the effect of integrated disease management practices on banded leaf and sheath blight of maize. The pathogenicity of seven isolates of *Rhizoctonia solani* collected from Tatkone, Pyinmana, Lewe and Kyaukme were evaluated on three hybrid maize varieties, Yezin-10, Yezin-11 and CPDK-888. Out of the seven isolates of *R. solani*, Pyinmana isolate was found to be the most virulent one as it caused the highest disease severity on all test varieties. Among the three test varieties, CPDK-888 was found to be the most susceptible variety as it gave maximum disease severity for all test isolates. Four *Trichoderma* spp. namely, *Trichoderma*-1, *Trichoderma*-2, *Trichoderma*-3 and *Trichoderma*-4 from maize rhizospheric soil and a commercial *Trichoderma harzianum* were tested for their antagonistic effect on high virulent *R. solani*, Pyinmana isolate. In *in vitro* dual culture test, the *Trichoderma* spp., *Trichoderma*-1, *Trichoderma*-3 and *Trichoderma*-4 exhibited excellent inhibitory effects resulting in 91-97% inhibition on mycelial growth of *R. solani* at 4 days after incubation. In *in vivo* test, the highest percent disease control of 76% was obtained from the plants treated with *Trichoderma*-4 at 30 days after sowing of CPDK-888 variety. Out of single and integrated control measures, all were observed to be effective in reducing disease severity and increasing yield over untreated check. All disease control measures were applied up to four times for each treatment except stripping alone which was done up to two times before flowering. Among them, integrated control measures were found to be more effective resulting in 60.7-78.6% disease reduction than single control measure except Carbendazim alone showing 70% in disease reduction. By the economic analysis, higher cost and benefit ratios (C:B) were obtained from the plants treated with *Trichoderma* alone, Carbendazim alone and combination of *Trichoderma* and Carbendazim alternately against the disease. Therefore, based on these findings, alternate sprayings of *Trichoderma* and Carbendazim could be applied as the most suitable method in terms of economic and environmental aspects to control banded leaf and sheath blight of maize.

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CHAPTER I

INTRODUCTION

Maize (*Zea mays* L.) is the third most important cereal crop in the world for agricultural economy and a relevant source of food, feed and industrial products (Singh and Shahi 2012). Among the cereal crops grown in Myanmar, maize is the second most important crop after rice. The bulk of maize grain produced in the country is mainly used in the poultry and livestock feed industries. It is also used as human food and for export, contributing to foreign exchange earning (Thant Lwin Oo *et al*. 2010). It is cultivated on an area of 441,000 ha with the total production of 1,626,000 MT. Average yield of maize is 3.7 MT ha^{-1} in 2013-2014 (MOAI 2014).

In order to meet local demand and also needs of international markets, high yielding hybrid maize research and development is needed as a priority (Thant Lwin Oo *et al.* 2010). However, one of the main constraints to high grain yield in maize is its susceptibility to several diseases. According to some records, 112 diseases of maize were reported from different parts of the world. Among them, 65 are well known disease to occur but only 16 are known to cause a serious threat to crop (Singh and Shahi 2012; Asif 2013). Major maize diseases are seed rot, seedling blight, downy mildew, bacterial stalk rot, banded leaf and sheath blight and smuts. Out of which, banded leaf and sheath blight (BLShB) is economically important (Saxena 2002).

Banded leaf and sheath blight disease is caused by the fungus, *Rhizoctonia solani* (AG 1-IA) and it has soil borne nature. The fungus is one of the most notorious, widespread, destructive and versatile pathogen found in most parts of the world (Divya Rani *et al.* 2013). It can attack very wide host range infecting plant species belonging to 32 families and 20 weed species from 11 families (Gangopadyay and Chakrabarti 1982). Approximately 550 host genera were recorded to be infected by this fungus in the USA alone (Farr *et al.* 1989). It is the most notable as the causal agent of various diseases of crop plants causing seed decay, damping-off, stem canker, root rot, aerial blight and seed or cob decay (Divya Rani *et al.* 2013).

As banded leaf and sheath blight of maize is a chronic disease, it has rapidly gained economic importance in several parts of the world. The disease causes severe loss in several countries of Asia, Nepal, India, Bhutan, Bangladesh, Malaysia, Phillipines, Thailand, Vietnam, Kampuchea, Laos, South China, Taiwan and

Myanmar (Sivakumar *et al.* 2000; Sharma *et al.* 2002a). Depending on the severity level of the disease on susceptible genotypes, grain yield loss may vary between 11- 40% in India (Singh and Sharma 1976), up to 100 % in Indonesia (Sudjono 1995 and Muis 2007) and ranged from 44-66% in Myanmar (Maung Maung Thein 2003).

On the other hand, many research workers have attempted to control *R. solani* using various approaches, such as cultural, biological and chemical controls. The most economical way of controlling this disease is the use of resistant varieties but none of the existing varieties have shown complete resistance to *R. solani* (Naz 2006; Singh and Shahi 2012). Sowing of the disease free seeds cannot manage the disease in the prescence of soil-borne inoculum. Also, the strong pathogen inoculums in soil limit the performance of a new resistant variety (Naz 2006). As one of the cultural control, crop rotation may be ineffective even if long because of very wide host range, or they may be uneconomical or unacceptable to farmers. Deleafing of maize plants proved to be effective in controlling the upward spread of the diseased lesion (Myint Myint San *et al*. 2008). However, Saxena (2002) reported that cultural practice, removal of lower leaves and sheaths alone could not significantly reduce the disease.

The literature on biological control of soil borne pathogens with antagonists is huge and several fungi have been reported to be good antagonists to *R. solani* (Velvis and Jager 1983; Roy 1989; Benyagorub *et al.* 1994; Poromarto *et al*. 1998). The most prominent fungi as biological agents against *R. solani* on various crops are species of *Trichoderma* (Harman *et al*. 1980; Papaviza 1985; Benítez *et al*. 2004). In the most cases, *Trichoderma* sp. required enough time for their activity against pathogens and in severe plant diseases, *Trichoderma* sp. has a little or no effect against pathogens (AL-Kurtany *et al.* 2009) and in this situation chemical fungicides must be applied (Hassan 2011).

Traditionally, control of soil-borne populations of *R. solani* has been attempted through application of fungicide (Du Plessis 1999; Stevenson 2000). Several chemicals have been successfully used to suppress *R. solani* (Naz 2006). However, *R. solani* has also acquired resistance to both protectant organic fungicides such as Captan, Maneb and Thiram and systemic fungicides such as Benomyl, Carboxin, Thiophenate methyl (Van Bruggen and Arneson 1984). Also, the indiscriminate use of chemicals resulted in existence of resistance of pathogen to some chemicals, non-target effects on microbial population present in the ecosystems and hazardous to nature (Prasad and Kumar 2011; Singh and Shahi 2012). Therefore,

this has diverted the attention of plant pathologist towards alternate methods for the control of plant diseases.

Due to dynamically changing climate, the disease has come up to the most noticeable. Combination of competitive saprophytic ability and high pathogenic potential of *R. solani* that makes its persistent and destructive plant pathogen (Saxena 1997). Moreover, its soil borne nature and scarcity of resistance germplasm makes it's management a challenge for pathologists across the world (Asif 2013). Therefore, effective control measures for banded leaf and sheath blight is extremely needed to minimize destruction of crop and to prevent economically crop losses (Singh and Shahi 2012).

Because of the above considerations and the unattainability of real varietal resistance against *R. solani*, control of the disease should be taken under serious consideration. However, management of this disease is difficult by single control measure because of soil-borne nature and very high degree of survival of this pathogen (Hide *et al*. 1973; Kumar 1976; Frank and Leach 1980; Naz 2006). Also, prevention of the disease using single control method cannot be reliable for long term eradication of pathogen. Once the pathogen is established in a field, its sclerotia can persist in soil almost indefinitely in the absence of host (Wicks *et al.* 1996; Du Plessis 1999; Agrios 2005). So, for practical solution of this problem, it is indispensable to conduct a comprehensive study of various control measures and integrated all of the best one, out of chemicals, biological and cultural practices (Naz 2006).

Now, banded leaf and sheath blight disease is one of the major diseases in Myanmar and has become a serious threat to its cultivation in hot humid areas especially in Mandalay region and northern Shan State (Myint Myint San *et al*. 2008). In spite of several years of research, there are very limited of high level of tolerant germplasm available over locations under different environments in Myanmar (Win Win Nwe *et al*. 2008). Therefore, integrated disease management (IDM) strategies are the only solution to control such highly destructive disease. However, in Myanmar, there is only a little information of IDM research for this disease. Therefore, this research was carried out with the following objectives.

1. To evaluate the pathogenicity of different isolates of *Rhizoctonia solani*
2. To determine the antagonistic effect of *Trichoderma* spp. on *Rhizoctonia solani*
3. To evaluate the effect of integrated disease management practices for banded leaf and sheath blight of maize

CHAPTER II

LITERATURE REVIEW

2.1 Banded Leaf and Sheath Blight of Maize

2.1.1 Background history and occurrence

The disease was first recorded on maize as sclerotial disease in Srilanka (Bertus 1927). Further, it was reported from different parts of the world; Siera Leone (Deighton 1932), Philippines (Reyes 1941), Malaysia (Wiltshire 1956), Nigeria (Van Eijanathen 1961), England (Kinght and Burril 1964), Japan (Kujware 1968), India (Parak and Renfro 1966), China (Zhu 1982), Indonesia (Rahamma *et al.* 1984), Mexico (Payak 1988), USA (Hirrel *et al*. 1988), Korea (Lee *et al*. 1989) and Venezuela (Cardona *et al.* 1999). Now, the disease is of common occurrence in China, South Asia and Southeast Asia including Myanmar (Sivakumar *et al.* 2000).

2.1.2 Economic importance and losses

The importance of the disease was only realized in early 1970s when an epidemic occurred in warm and humid foot hill areas (Thakur *et al.* 1973). The disease results in the direct loss exhibiting premature death, stalk breakage, and ear rot. Losses in grain yield showed a high positive correlation with premature death of plants and disease index that caused drastic reduction in grain yield to 97% (Butchaiah 1977). At disease score levels ranging from 3 to 5, a direct correlation with other yield parameters was exhibited in a yield loss of 5 to 97% (Lal *et al.* 1980; Liang *et al.* 1997).

The disease reaches the ear shoot in favourable weather within 15-20 days of sheath infection (Saxena 1997). Crop damage is caused by loss of photosynthetic leaf area due to foliar infection and stalk rot which lead to crop lodging (Lu *et al.* 2012). Ahuja and Payak (1982) found that the disease caused maximum damage when ears are infected. In addition to ear rots, kernels are often wrinkled, dry, chaffy and light in weight (Saxena 1997).

It can cause considerable grain yield loss in susceptible genotypes. Lal *et al.* (1985) reported that the losses in grain yield may vary to the extent of over 90%. Surprisingly, in China, yield losses close to 100% have been attributed to this disease (Singh and Shahi 2012). Dela Vega and Silvestre (2003) reported that as the disease

intensities increase, the yield loss and yield reduction also increase with a directly proportional relationship.

2.1.3 Disease symptoms

The symptoms of banded leaf and sheath blight were observed on all aerial parts of the maize plant except tassel (Bertus 1927). Under natural conditions, disease appears at pre-flowering stage on 30 to 40 days old plants (Saxena 2002) but infection can also occur on young plants which may subsequently result in severe blighting and death of apical region of growing plants (Divya Rani *et al.* 2013).

Ahuja and Payak (1982) recorded the disease symptoms on leaves as irregularly globular to elongated lesions (1-3 mm diameter) which appear as water-soaked areas. Later, they become straw colored and necrotic lesions with alternating narrow brown zones which become more prominent resulting in the characteristic symptoms of banded leaf and sheath blight like a shape of snake skin. Divya Rani *et al.* (2013) reported that the pathogen also causes spots or lesions on the rind of the stalk under the affected sheaths. Dark brown to black depressed lesions extend on the lower four or five internodes. Sometimes, these lesions are transformed into cankers (Saxena 1997). Maiti (1978) reported that the disease develops on leaves, sheaths, and stalks and can spread to the ears. When infection reaches ear, light brown cottony mycelial growth and small round mustard seed sized small round black sclerotia are observed. Premature drying and caking of ear sheath is also observed (Lu *et al.* 2012).

2.1.4 Causal organism

2.1.4.1 Taxonomy and nomenclature

Anamorph : *Rhizoctonia solani* f. sp. *sasakii*

Teleomorph : *Corticium sasakii,* syn. *Thanantephorus cucumeris*

Kingdom: Fungi

Division: Basidiomycota

Sub division: Agaricomycotina

Class: Agaricomycetes

Order: Cantharellales

Family: Ceratobasidiaceae

Genus: *Rhizoctonia*

species: *solani*

Imperfect stage of causal organism of banded leaf and sheath blight of maize is *Rhizoctonia solani* Kuhn. The fungus is a soil inhabitant basidiomycete (Agrios 2005) and it was first reported by Kuhn in 1858. He observed a fungus on diseased potato tuber and named it *R. solani*. It has long been recognized as a destructive pathogen on a wide variety of plants throughout the world. Identification, taxonomy and nomenclature of *R. solani* have been studied precisely by many workers (Duggar 1915; Bertus 1927; Whitney and Parmeter 1964).

Talbot (1970) mentioned the perfect stage of the fungus as *Thanantephorus cucumeris.* However, it has not been recorded on maize and hence its description is not provided here (Tu and Kimbrough 1978).

2.1.4.2 Morphology of *Rhizoctonia solani* f. sp. *sasakii*

R. solani is generally identified by characteristics of the mycelium and sclerotia. The mycelium was colorless when young, but becomes brown color as it matures. On agar media, *R. solani* produces white to deep brown, cottony mycelium. Under microscopic examination, hyphae are multinucleate, septate and branching at right angles and acute angles approaching 45˚. Hyphae are septate and typically constricted at the point of branching and contain dough nut shaped pore that enables nuclei, mitochondria to migrate between cells. Vegetative diameter of the hyphae are 3 to 17µm wide (Akhtar *et al.* 2009; Singh and Shahi 2012; Divya Rani *et al.* 2013).

Sclerotia are produced abundantly in culture and on infected plant parts. Mostly, sclerotia are 1 to 5 mm in diameter with spherical shape, and dark brown to black in colour (Akhtar *et al.* 2009; Singh and Shahi 2012; Divya Rani *et al.* 2013).

2.1.4.3 Physiology of *Rhizoctonia solani* f. sp. *sasakii*

The fungus is attracted to the plant by chemical stimulants released by actively growing plant cells and decomposing plant residues. As the attraction process proceeds, the fungal hypha will come in contact with the plant and become attached to its external surface. After attachment, the fungus continues to grow on the external surface of the plant and produces a specialized infection structure (either an appresorium or infection cushion) that penetrates the plant cell and releases nutrients for continuing fungal growth and development. The infection process is promoted by the production of many different extracellular enzymes that degrade various components of plant cell walls (e.g. cellulose, cutin and pectin). As the fungus kills

the plant cells, the hyphae continue to grow and colonize dead tissue, often forming sclerotia (Ceresini 1999).

2.1.4.4 Anastomosis group

The *Rhizoctonia* strains are distinguished from one another based on fusion of touching hyphae which occurs only between isolates of the same anastomosis group. The anastamosis group AG1-IA is the causal agent of BLShB (Pascual and Raymundo 1993). The isolates fall in anastomosis group AG1-IA which also cause sheath blight of rice. Rice and maize isolates are indentical with respect to host range, nuclear number in hyphae, influence of temperature and relative humidity on growth and other characters (Ahuja and Payak 1985). Currently, 14 different AGs of *R. solani* have been reported on a variety of different host plants (Zhao *et al*. 2006).

Isolates within AG1 have been divided into three subgroups based on host origin, symptoms and cultural characteristics: AG1-IA (sheath blight), AG1-IB (web blight) and AG1-IC (damping-off). However, the host ranges of AG1-IA and AG1-IB overlap (Ogoshi 1987; Sneh *et al*. 1991; Liu and Sinclair *et al.* 1993). The isolates of AG1-IA collected from various host species growing on or near maize fields in different geographic regions were the most strongly pathogenic to leaf sheaths and stalks of maize and they caused typical sheath blight symptoms on maize (Chen *et al*. 1997; Li *et al.* 1998; Pascual *et al*. 2000)

2.1.5 Environmental factors affecting the causal fungus

On potato dextrose agar (PDA) media, colonies were fast growing and formed silky white at 28±1˚C (Divya Rani *et al.* 2013). Optium temperature for fungal growth was at 25˚-30˚C and growth rate was 45 mm per 24 hours (Asif 2013). Saprophytic survival of *R. solani* may be influenced by soil environment (Davey and Papavizas 1963). Moreover, growth of *R. solani* in soil is dependent on the presence of a food base. It was observed that *R. solani* remained dormant as thick wall mycelia and sclerotia in the exhausted food base (Boosalis and Scharen 1959).

R. solani was more active saprophytically at 26-30˚C in soil and significantly less active above and below these temperatures. In humid weather with a temperature range of 25-32˚C, infection spreads very rapidly from the infected field to the healthy areas (Saikia 1976). Optimum growth and saprophytism of *R. solani* occurred at a neutral or slightly alkaline reaction of soil (Papavizas and Davey 1961). Good growth

of *R. solani* was observed over a reaction range of soil pH 5.8-8.0 (Blair 1943). It was found that saprophytism was significantly higher when the soil moisture was maintained at 20-60% of the moisture-holding capacity (Papavizas and Davey 1961).

The disease severity is often closely associated with prevailing climatic conditions and farming practices. High relative humidity and rain fall significantly favours development and spread of the disease. An optimum temperature about 28°C and high relative humidity (88-90%) in the first week of infection favor rapid disease progress. If the relative humidity goes below 70%, disease development and spread becomes slow (Sharma 2005).

2.1.6 Host range

The pathogen has wide host range and infects plant belonging to over 32 families in 188 genera (Saxena 1997). *R. solani* f. sp. *sasakii* infects a number of crop plants belonging to families Gramineae, Papilionaceae and Solanaceae: *Oryza sativa* (rice), *Zea mays* (maize), *Sorghum bicolor* (sorghum), *Arachis hypogea* (groundnut), *Glycine max* (soybean), *Saccharum officinarum* (sugarcane), *Vigna radiata* (mungbean), *Vigna unguiculata* (bean), *Lycopersicum esculentum* (Tomato), *Solanum tuberosum* (potato), *Capsicum annuum* (chilli), etc. However, *R. solani* isolates of rice and maize are indistinguishable on the basis of cross inoculation tests, host range, virulence, number of nuclei per hyphal cell, and other morphological characters including pathogenicity. Comparison studies of rice, maize, sugarcane and sorghum isolates revealed that *R. solani* of maize and rice isolates are similar than those of sugarcane and sorghum (Saxena 1997).

This fungus causes various diseases depending on host, banded leaf and sheath blight in maize, rice and sorghum, damping-off in cotton, aerial blight and stem rot in mungbean and soybean, sheath rot in sugarcane, heart rot in cabbage, black scurf and sprout canker in potato and foliar blights of fruits in plantation crops (Tangonan and Quebral 1992; Pascual *et al*. 2000).

2.1.7 Disease management of banded leaf and sheath blight

2.1.7.1 Use of resistant varieties

Varietal resistance of maize against *R. solani*, has been reported by many workers. Among the inbred lines, CM 104 and CM 300 were determined to be the resistant (Sharma *et al.* 2002a). In Korea, maize varieties Jinjuok, Suweon 83,

Suweon 87, Suweon 89, P 3055, P 3160, DK 689 and XCG 51 showed high tolerance level to banded leaf and sheath blight (Lee *et al.* 1989).

A number of CML lines and other materials have been evaluated in India, China and Indonesia and many lines have been indentified having reasonable level of resistance (Balla *et al*. 2000). Singh and Sharma (1976) reported that inbreds (CM 104, CM 105, CM 200), hybrids (CM 107 x CM 108), (RN_6Ht_1 x GE 440) and composites (JML 32, JML 36, JML 306 and JML 403) showed resistant type of reaction in field trial. CM 104 was recommended as a source of resistance (Vimla *et al*. 1988) and moderately resistant to test isolate (Srinivas 2002). Although CM 104 which introduced from Colombia in 1970s, offered resistance to banded leaf and sheath blight, it was susceptible to Delhi isolate over two years.

In Myanmar, Maung Maung Thein (2003) evaluated inbred lines and hybrid varieties for resistance to banded leaf and sheath blight under artificial inoculation. However, none of the test entries were found to be resistant to the disease.

2.1.7.2 Cultural control

Application of cattle compost before planting decreased the disease level and its subsequent spread in field (Lee *et al*. 1989). Plants in pot with added potassium (112.5-262.5 kg ha^{-1}) had a significantly lower disease severity and consequently a significantly higher yield. Field investigation showed that disease severity was lower when rotation, intercropping and rational plant density were used. The selection of a well drained field and planting on raised beds are also important aspects to avoid contact of water with seeds and faster growth of seedlings (Liang *et al*. 1997).

Mechanical stripping of the second and third leaf sheath from the ground level at the age of 35 to 40 days old crop is effective in checking further disease development (Sharma and Hembram 1990). In Japan, resistance to this disease has been observed after the fall of the lower sheath (Kato and Inove 1995; Qing *et al.* 1994), thus, providing additional scientific basis for the leaf stripping method. Dalmacio *et al*. (1990) described that mechanical control by deleafing of maize plants was proved to be effective in controlling the upward spread of lesion.

2.1.7.3 Biological control

Evaluation of biocontrol agents against *R. solani* was conducted by many workers. These are various species of *Trichoderma* (Roy 1977; Roy and Sayre 1984;

Nagmani and Mew 1987), *Gliocladium* spp., *Streptoverticillium* (Baby and Manibhushan Rao 1993) and *Glomus* spp., *Pseudomonas fluorescence* and *Aphelenchus avenae* (Meena *et al.* 2003a; Singh and Shahi 2012).

Peat based formulation of *P. fluorescence* which applied to seed (20 g kg^{-1}), soil (2.5 kg ha^{-1}) and foliage (6 g L^{-1}water) significantly controlled the disease and increased maize grain yield in field trial (Sivakumar *et al*. 2000; Meena *et al.* 2003a)). Peat based formulation of *P. fluorescence* at 16 and 20 g $kg^{-1}$of seed was found to be most effective in reducing BLShB by 51% and 53% respectively (Sivakumar *et al.* 2000). This antagonist not only reduce the disease but also increase in grain yield approximately 1.4 times of the yield of control (Sharma *et al.* 2002b).

Application of *T. viride* and *P. fluorescens* resulted in a significant reduction of rice sheath blight disease caused by *R. solani* and increased yield when compared with control (Wang *et al*. 2000; Tang *et al*. 2002; Kazempour *et al*. 2003 and Mathivanan *et al*. 2005). In wheat, seed treatment with *Bacillus subtilis* significantly reduced the *Rhizoctonia* disease and increased grain yield (Merriman *et al*. 1974). According to McMullen and Lamey (2000), *B. subtilis* used as seed treatment colonize the developing root system, suppressing *Rhizoctonia*.

Effectiveness of biological disease control depends not only on suitable biocontrol organisms but also on methods for introducing and maintaining population levels and activities of these organisms in associations with crops and plants (Stack *et al.* 1988; Jin *et al.* 1991). Regardless of the qualities of the biocontrol agents, the methods used to produce, formulate, and deliver these organisms may profoundly influence their efficacy (Cook and Baker 1983; Jin *et al.* 1991). The ability to survive on the phylloplane is also a desirable trait for strains of *Trichoderma* used as biocontrol agents against foliar diseases (Nelson and Harman 1997).

2.1.7.4 Use of phytoextracts

Many attempts have been made to control banded leaf and sheath blight of maize by botanicals for eco-friendly management. Several botanicals *viz.*, Lantana, Neem, Marigold, Turmeric, Garlic leaf extracts gave a promising result when tested against *R. solani* and enhanced seed germination (Asif 2013). Sharma *et al.* (2005) evaluated eight plant extracts against BLShB pathogen. Among all, garlic extract significantly controlled the disease in the field when applied before inoculation (52% efficacy of disease control-PEDC), during inoculation (58% PEDC) and after

inoculation (42% PEDC) on maize plants. Maximum inhibition followed by *Allium cepa* (92%) and *Eucalyptus globules* (85%).

2.1.7.5 Chemical control

In the absence of host resistance, many farmers are reliant on the use of systemic fungicides for management of banded leaf and sheath blight of maize. Chemical controls of BLShB has been reported that Bavistin 50 WP was the most effective in controlling the disease by 87%, followed by Benlate 50 WP (Benomyl) which controlled by 82%, respectively (Sinha 1992). The systemic fungicides, such as Orthocide 50 WP (Captan) 3 g L^{-1}, Dithane M 45 (Mancozeb) 2 g L^{-1} reduced disease intensity of 42% and yield loss of 7.8% of maize (Sinha 1992; Beti and Iriani 1996). Thiophenate at 0.7 g L^{-1} were found as effective fungicides in controlling *R. solani* on maize in field and laboratory tests by Sharma and Rai (1999). In the field efficacy of 9 chemicals against *R. solani* in maize, the best disease control was achieved by Carbendazim (0.1%) (Puzari *et al.* 1998; Kumar and Jha 1999). Saxena (2002) tested efficacy of chemicals (*viz.*, Propaconazole, 0.1% and Carbendazim, 0.05%), by applying as foliar sprays at 30, 40 and 50th day of planting, alone or in combinations. Effectiveness of Propaconazole was markedly observed. Under field conditions, a high level of control of BLShB could be achieved using Bavistin (Carbendazim), Rhizolex and Thiophenate (Sharma *et al*. 2002b).

Meena *et al*. (2003b) evaluated fungicides against banded leaf and sheath blight of maize. Soil drenching of Carbendazim (0.1%) 500 mL pot^{-1} resulted in 51.3% disease reduction over control. Kitazin (0.05%) also showed effectiveness, resulting in disease reduction of 34.1% and 43.5% over control. Rakesh *et al*. (2011) evaluated some seed dressing fungicides *viz.,* Bavistin (2.5 g kg^{-1} of seed), Vitavax Power (2.5 g kg^{-1} of seed) and Thiram (2.5 g kg^{-1} of seed) for the management of BLShB. Myint Myint San *et al*. (2008) reported that Benomyl (0.2%) and Bavistin (0.1%) were more effective fungicides against BLShB of maize than the other two fungicides, Topsin (0.2%) and Daconil (0.2%). However, use of fungicides for the control of soil borne diseases is costly and also produces environment and health hazards to men and also adversely affects the beneficial microorganisms in soil (Dłużniewska 2003).

2.1.7.6 Integrated disease management

Integrated disease management (IDM) which combines cultural, chemical, biological and host genetic factors to control targeted plant pathogens is more effective and sustainable ranther than single control (Asif 2013). Also, IDM calls for minimal use of pesticides and only if deemed necessary, giving preference to other control methods such as host-plant resistance, cultural practices and biological control (Thomas and Waage 1996). Integrated disease management was late popularized in California (Stern *et al*. 1959). Effectiveness of *Trichoderma viride* can be increased by 20% by combining biocontrol with fungicides treatment (Dumitras 1984). Baicu *et al*. (1989) reported the effectiveness of some *T. viride* mutant tolerants to fungicides like Thiram and Thiophenate methyl and found Td-K mutants as most effective against *R. solani*. Ali and Pathak (1997) reported lack of effect of Hinosan on *T. harzianum* and suggested that this fungicide be scheduled in integrated disease management strategies. Zang (1994) suggested some cultural and chemical methods for integrated disease control of *R. solani.* Dalmacio *et al*. (1990) were conducted three experiments on the mechanical, biological, and chemical control of the disease. In case of mechanical control, deleafing of maize plants proved to be effective in controlling the upward spread of lesion.

Bora *et al.* (1999) studied the integrated management of sheath blight of rice with *T. harzianum* and chemicals. Suruliranjan (2003) studied the effect of *T. viride* ITCC-3235, Carbendazim, farm yard manure (FYM) and sawdust on *R. solan* in rice. It was observed that Carbendazim at 100 ppm concentration completely inhibited the pathogens and *T. viride* could tolerate the same concentrations of fungicides to a greater extent. Foliar application of Carbendazim (0.1%) followed by *Trichoderma* spray in saw dust and FYM amended field significantly reduced the disease to a range of 7.79 to 40.09% at different growth stage of rice. The integrated treatment (Carbendazim + *Trichoderma* + Soil amendments) gave higher percent increase in grain and straw yields than the single component treatment alone. This treatment achieved as high as 85.48% more than grain yield than the control.

Singh Akhilesh and Singh Dhanbir (2011) conducted a field trial using cultural practices, bioagents and fungicides. Out of 11 treatments, minimum disease intensity and maximum yield was found in case of foliar spray of Validamycin (0.25%) followed by Tilt (0.1%) and Bavistin (0.1%).

Bunker *et al*. (2012) reported that Carbendazim, neem oil and *T. harzianum* were evaluated as seed treatment (ST) and also as ST plus spray in various combinations for managing the disease. Treatments with combination of ST and spray application were more effective than ST alone. Significantly reduced disease (46.8%) and increased grain yield (51.6%) were recorded in the ST + spray of Carbendazim (0.1%), followed by treatment with neem oil (0.2%), over other treatments and control. Use of neem oil as seed treatment and spray could be a cost effective and eco-friendly strategy in managing the BLSB.

2.2 *Trichoderma*

Trichoderma is the most common saprophytic fungus that was described in 1794. They are within the subdivision Deuteromycotina (Persoon 1794) and generally found in all soils (Chet 1987; Samuels *et al.* 1996). *Trichoderma* species are green-spored ascomycetes present in nearly all types of temperate and tropical soils. They are highly interactive in root, soil and foliar environments (Taylor and Francis 1998; Ranasingh *et al.* 2006). 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).

2.2.1 Taxonomy of *Trichoderma*

The taxonomy of *Trichoderma* was first described by Persoon in his classification of fungi in 1794. Bisby (1939) proposed that *Trichoderma* consists of a single species, *T. viride*. This concept led to nearly all strains of *Trichoderma* was identified as "*T. viride*" in literatures before 1969 (Hui 2013). Therefore, most of the taxa determined before 1969 are probably misidentified since *T. viride* is a relatively rare species (Druzhinina and Kubicek 2004).

In 1969, Rifai proposed the concept of "aggregate" species, where *Trichoderma* species are divided into nine "species aggregates", namely *T. aureoviride* Rifai, *T. hamatum* Bain, *T. harzianum* Rifai, *T. koningii* Oudem, *T. longibrachiatum* Rifai, *T. piluliferum* Rifai, *T. polysporum* Rifai, *T. pseudokoningii* Rifai and *T. viride*. However, Rifai admitted that each species aggregate was likely to contain more than one morphologically indistinguishable species (Chaverri and Samuels 2004).

2.2.2 Biodiversity of *Trichoderma*

Most of the *Trichoderma* species are morphologically very similar and were considered for many years as a single species: *T. viride* (Bisby 1939). Since new species were discovered, a consolidated taxonomical scheme was needed and Rifai (1969) proposed and defined nine morphological species aggregates. DNA methods brought additional valuable criteria to the taxonomy of *Trichoderma* which are being used today for studies that include identification (Lübeck *et al.* 2000; Hermosa *et al.* 2001) and phylogenetic classification (Lieckfeldt and Seifert 2000; Kullnig-Gradinger *et al.* 2002). Most isolates of the genus *Trichoderma* that were found to act as mycoparasites of many economically important aerial and soil-borne plant pathogens, have been classified as *T. harzianum* Rifai (Gams and Meyer 1998).

Due to the fact that the species *"harzianum"* are generally considered as mycoparasitic and biocontrol strains and there is large morphological plasticity that results in character overlaps with other species, the identification of the species may be difficult. Several authors have reported a large genetic variability among *T. harzianum* isolates (Muthumeenakshi *et al.* 1994; Bowen *et al.* 1996; Gomez *et al.* 1997; Grondona *et al.* 1997). In fact, it has been demonstrated that at least four distinct species are present within the biocontrol *T. harzianum* aggregate: *T. harzianum, T. atroviride, T. longibrachiatum* and *T. asperellum* (Hermosa *et al*. 2000).

2.2.3 Morphology of *Trichoderma*

Most identification was based on the morphological characters. Since 1969, morphological characteristics have been used to characterize and distinguish *Trichoderma* species (Gams and Bissett 2002). Besides that, Samuels *et al.* (2002a) also provided detailed observations on the morphological characters of defined species in *Trichoderma*. However, the identification of the isolates to species level is difficult and confusing due to the complexity and closely related character of the species (Samuels *et al.* 1996).

2.2.3.1 Macroscopic features

Certain colony characters like growth rate, pigmentation, pustules formation and odours can be characteristics of a species. However, colony appearance does not

provide sufficient information for characterization due to the difficulty to establish a precise description (Gams and Bissett 2002).

According to Samuels *et al.* (2002a), majority of the *Trichoderma* cultures grow rapidly at 25°C to 30°C and typically not growing at 35°C. Yet, some species grow well at 35°C. This served as an important distinguishing criterion between morphologically similar species. For example, *T. harzianum* can be distinguished from morphologically similar species such as *T. aggressivum* and *T. atroviride* by growing them at 35°C. After 96 hours, neither *T. aggressivum* nor *T. atroviride* can have colony radius more than 5 mm while *T. harzianum* grows well and sporulates at 35°C (Samuels 2004; Hui 2013).

Characteristics of mycelia development and pigmentation can be better observed in rich medium like potato dextrose agar (PDA). The colonies are white on rich media such as PDA and transparent on cornmeal dextrose agar (CMD) (Samuels *et al.* 2002b). Scattered blue-green or yellow-green patches become observable when conidia are formed. Occasionally, concentric rings made by these patches can be observed. Reverse of the colonies are pale, tan or yellowish (Rex 2001; Hui 2013). Furthermore, some species of *Trichoderma* such as *T. viride*, will produce a characteristic sweet smell resembling 'coconut' odour (Gams and Bissett 2002).

2.2.3.2 Microscopic features

Trichoderma species usually form vegetative hyphae which are septated, hyaline and smooth-walled (Gams and Bissett 2002). Conidiophores are highly branched. Lateral side branches produced from main branches may or may not be paired, and sometimes may rebranch. Normally, the branches will form at or near right angle (90°) with respect to the main branch. Paired branches will assume a pyramidal structure. The typical conidiophore terminates with one or a few phialides that usually arising directly from the axis near the tip. In some species, however, the main branches are terminated with sterile or fertile elongations (Samuels *et al.* 2002a).

Phialides, also known as conidiogenous cells, are typically enlarged in the middle like a flask-shape, and may be cylindrical or nearly subglobose. They are held in divergent verticils at the end of the conidiophores, or in whorls beneath septa along the conidiophores and branches. They may be held irregularly, paired, or in solitary (Samuels *et al.* 2002a; Gams and Bissett 2002). Conidia are one-celled, and either ellipsoidal or globose. They are typically green, or sometimes colourless, grayish or

brownish. Their surfaces are typically smooth, but roughened conidia can be found in a few species, such as *T. viride* (Samuels *et al.* 2002a; Gams and Bissett 2002).

Chlamydospores play important role in survival. They are normally found as thick-walled, enlarged vegetative cells with condensed cytoplasm (Lin and Heitman 2005). These unicellular, globose to subglobose chlamydospores are either formed within hyphae or at the hyphal tips. Typically, they are colourless, pale yellowish or greenish (Samuels *et al*. 2002a; Gams and Bissett 2002).

2.2.4 Roles of *Trichoderma* as biological control agent (BCA)

2.2.4.1 Mechanisms of control by *Trichoderma*

Trichoderma spp. have evolved numerous mechanisms that are involved in attacking other fungi and enhancing plant and root growth. These mechanisms include competition for space and nutrients (Elad *et al*. 1999), mycoparasitism (Dennis and Webster 1971a; Haran *et al*. 1996; Lorito *et al*. 1996), production of inhibitory compounds (Sivasithamparam and Ghisalberti 1998), inactivation of the pathogen's enzymes (Roco and Perez 2001) and induced resistance (Kapulnik and Chet 2000)

Competition: *Trichoderma* spp. has a great ability to compete the nutrients and space and this ability plays a main role against pathogenic fungi (Naar and Kecskes 1998). Through rapid growth and reproduction, *Trichoderma* spp. can get water, nutrients, space and oxygen so that it can destroy, even get rid of some pathogens in the same environment (Vizcaino *et al.* 2005). *Trichoderma* species are generally considered to be aggressive competitors, grow very fast and rapidly colonize substrates to exclude pathogens such as *Fusarium* spp. (Papavizas 1985). Rhizosphere competence, following seed treatment is an important strategy to create a zone of protection against pathogens (Howell 2003). *Trichoderma* species, either added to the soil or applied as seed treatments, grow readily along with the developing root system of the treated plants (Ahmad and Baker 1987).

Antagonism: Lots of strains of *Trichoderma* spp. have antagonism to plant pathogen, such as *T. harzianum, T. viride, T. hamatum* and so on. Antagonistic microorganism excretes antifungal metabolites to inhibit pathogens directly, which is common phenomenon in their metabolic process. Usually these metabolites are grouped into two categories: one is amylose with low molecular weight, which point to antibiosis; another is antagonistic proteases and cell wall degrading enzymes. *Trichoderma* spp. can degrade cell wall and toxin (for example *Rhizoctoniatoxin)* of

pathogens by excreting chitinases, ß-1,3-glucanases, proteases and cellulase, also these metabolites can inhibit activation ofpathogens (Tong *et al.* 2003). Harman (2000) thought that these cell wall degrading enzymes can cooperate with chemical fungicides or bacterial biocontrol agents.

Mycoparasitism: Mycoparasitism is a phenomenon that other micro-organisms parasitize plant pathogens. *Trichoderma* species are economically important, in part because of their mycoparasitic ability, which makes them suitable for application as biocontrol agents against soil borne plant-pathogenic fungi (Benitez *et al.*1998; Manczinger *et al.* 2000).

The mode of hyphal interaction and parasitism of *Trichoderma* spp. with several soilborne pathogenic fungi has been documented (Chet *et al.* 1981). *Trichoderma* grows tropically toward hyphae of other fungi, coil around them in a lectinmediated reaction, and degrade cell walls of the target fungi by the secretion of different lytic enzymes. This process (mycoparasitism) limits growth and activity of plant pathogenic fungi.

Trichoderma attaches to the host hyphae via coiling, hooks and appressorium like bodies, and penetrate the host cell wall by secreting lytic enzymes. The interaction is specific and not merely a contact response. *Trichoderma* recognizes signals from the host fungus, triggering coiling and host penetration. The cell wall degrading enzymes of *Trichoderma* such as ß-1,3-glucanases and different chitinolytic enzymes have been suggested as the key enzymes in mycoparasitism (Schirmbock *et al.* 1994).

Induced resistance: In addition to control pathogens, many microorganisms can promote the growth of host plant. Specific strains of fungi in the genus *Trichoderma* colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense response, which subsequently leads to induced systemic resistance (Bailey and Lumsden 1998; Saba *et al.* 2012).

2.2.5 Problems in using *Trichoderma* spp.

Although *Trichoderma* spp. are an effective biocontrol agents against several soilborne fungal pathogens, possible adverse effects of this fungus on arbuscular mycorrhizal (AM) fungi might be a drawback in its use in plant protection. AM fungi are obligate biotrophic endosymbionts in roots of most herbaceous plants. These fungi

grow from the roots out into the surrounding soil, forming an external hyphal network, which increases uptake of mineral nutrients and consequently promotes plant growth. The presence of *T. harzianum* in soil reduced root colonization by *Glomus intraradices*. The external hyphal length and density of *G. intraradices* was reduced by the presence of *T. harzianum.* Another problem has been low field performance of *Trichoderma* as biocontrol agent. Understanding the mechanisms by which *Trichoderma* controls fungal diseases assume importance. Further recent molecular biological techniques can be used to exploit these mechanisms, so that, fungal diseases can be effectively controlled in the field (Sharma *et al.* 2012).

CHAPTER III

MATERIALS AND METHODS

3.1 Evaluation of Pathogenicity of *Rhizoctonia solani* Isolates

The experiment was conducted at Department of Plant Pathology, Yezin Agricultural University (YAU) from May to September 2014 (Plate 3.1).

3.1.1 Collection of diseased leaf sheaths and stems

The diseased specimens were collected from infected maize plants in the fields of Pyinmana, Lewe and Tatkone townships, Nay Pyi Taw Council Area and Kyaukme township, northern Shan State.

3.1.2 Isolation of *Rhizoctonia solani* from different infected maize fields

The small sections from an advancing lesion of collected diseased leaf sheaths and stems were cut using sterilized steel scissors. The pieces were surface sterilized with 5% sodium hypochloride (NaOCl) solution for 1 minute followed by rinsing three times in sterilize distilled water. After that, the pieces were placed between two layers of sterilized blotting papers (9 cm in diameter) to absorb moisture. Then, they were transferred asceptically and plated on 2% water agar media (Appendix 1). These plates were incubated at room temperature (28-30˚C) for 3 days. The fungi that grew in the form of thin hyphal strands were examined by inverting the plates under the microscope. The hyphal tips were marked with a marking pen and then carefully subcultured onto potato dextrose agar (PDA) medium (Appendix 2) slants. Then, the slants were kept at room temperature (28-30˚C) for 7 days.

3.1.3 Identification of *Rhizoctonia solani* isolates

The isolates of *R. solani* were grown individually on PDA medium and identified based on their mycelial and sclerotial characters according to Barnett and Hunter (1972).

3.1.4 Preparation of test plants

Three hybrid maize varieties, Yezin-10, Yezin-11 and CPDK-888 were used as test varieties for this experiment. The seeds were surface sterilized for 10 minutes in 1% sodium hypochloride (NaOCl) solution, washed three times with sterilized water and sown in plastic bags containing sterilized sandy soil. Then, 7 days old maize

Plate 3.1 Experimental layout for evaluation of pathogenicity of *Rhizoctonia solani* isolates on three hybrid maize varieties in screen house at Department of Plant Pathology, YAU

seedlings were transplanted into plastic pot (30 cm in diameter x 28 cm in height) containing 10 kg of sterilized sandy loam soil. Urea at the rate of 100 kg N ha^{-1} (0.45 g pot^{-1}), Triple super phosphate at 100 kg P_2O_5 ha^{-1} (0.45 g pot^{-1}) and Muriate of potash at 75 kg K_2O ha^{-1} (0.33 g pot^{-1}) were applied as basal dressing. Urea at the rate of 100 kg N ha^{-1} (0.45 g pot^{-1}) was top dressed again at 30 days after sowing.

3.1.5 Preparation of inoculum for each *Rhizoctonia solani* isolate

The pure fungal isolates of *R. solani* from slant culture were transferred individually onto the PDA media and incubated at room temperature for 3 days. Then, 5 mm in diameter of mycelial discs were cut from actively growing mycelial point and used as inoculums (Plate 3.2.A).

3.1.6 Pathogenicity Test

The mycelial discs of each of seven *R. solani* isolates were individually inoculated between the second lower most leaf sheath and stem of each test variety at 30 days after sowing by using mycelial insertion technique (Maung Maung Thein 2003) (Plate 3.2.B).

3.1.7 Experimental design

The experiment was conducted in Randomized Complete Block Design with factorial arrangement consisting of two factors, the seven isolates (Tatkone-1, Tatkone-2, Lewe-1, Lewe-2, Lewe-3, Pyinmana and Kyaukme) and the three hybrid maize varieties (Yezin-10, Yezin-11 and CPDK-888). Seven pots (one plant pot^{-1}) were arranged for each treatment.

3.1.8 Data recording

Parameters including disease score, lesion length and relative lesion length of each variety were recorded at 45 days after inoculation. Disease scores of inoculated plants were assessed by using 1-5 scoring scale of BLShB disease of maize (Shekhar and Kumar 2012) as described in Table 3.1 and Plate 3.3. Relative lesion length (disease severity) was computed by using the following equation (Pascual *et al.* 2000).

$$\text{Relative lesion length (\%)} = \frac{\text{Length of lesion}}{\text{Plant height}} \times 100$$

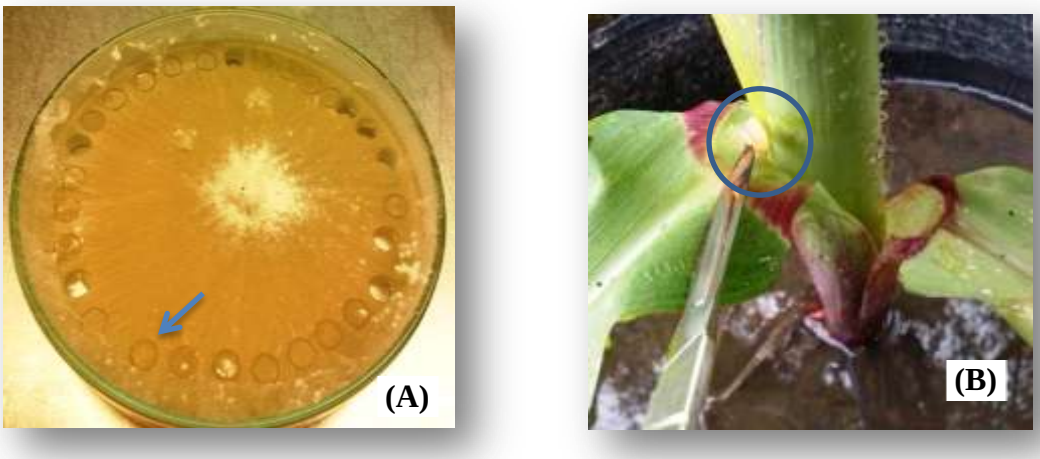


Plate 3.2 (A) Preparation of inoculum by plugging 5 mm in diameter mycelial discs of 3 days old culture of *Rhizoctonia solani* and (B) Inoculation of *Rhizoctonia solani* by inserting mycelial disc between second lower most leaf sheath and stem at 30 days after sowing

Table 3.1 Scoring scale of banded leaf and sheath blight of maize

Score	Description
1	Infection is on one leaf sheath, lesions are one or few, non-coalescent.
2	Infection is on two to three leaf sheaths, lesions are few and non-coalescent on third leaf sheath from ground level.
3	Infection is not up to ear shoot but heavy on more than two leaf-sheaths.
4	Infection is on all leaf sheaths up to ear shoot but shank is not infected.
5	Infection presents beyond the ear shoot; reduced ear size, husk leaves bleached and caked with or without sclerotial development kernel formation absent or rudimentary.

(Shekhar and Kumar 2012)

Disease score 1

Disease score 2

Disease score 3

Disease score 4

Disease score 5

Plate 3.3 Representative symptoms for 1-5 scoring scale of banded leaf and sheath blight of maize (Shekhar and Kumar 2012)

3.2 Effect of *Trichoderma* spp. on *Rhizoctonia solani* (*in vitro* and *in vivo*)

The expriments were done in the laboratory and screen house at Department of Plant Pathology, Yezin Agricultural University from July to October 2014.

3.2.1 Collection of soil samples

Soil samples were taken from a depth of 10 to 15 cm around rhizosphere of healthy maize plant from five different fields at Tatkone. The collected soil samples were sieved (2 mm mesh) to remove gravel and plant debris, and then, made air dried for 24 hours at room temperature.

3.2.2 Isolation and identification of *Trichoderma* spp.

Isolation of indigenous *Trichoderma* spp. from soil was done by serial dilution technique. The *Trichoderma* spp. were cultured from soil suspensions according to Anil Kumar and Raj Kumar (2013). Ten gram of each soil sample was taken and added to 90 mL of sterilized distilled water and then, homogenized by using a rotary shaking machine (OS-340C) at 180 rpm for 30 minutes.

Each soil suspension was serially diluted to obtained dilution factor from 10^{-1} to 10^{-5}. From each of dilutions, 1mL of the suspension was taken with the help of a micropipette and transferred into sterilized plates. Then, sterilized Martin's media (Appendix 3) as described by Martin 1950 was poured into the plate seeded with 1 mL of soil suspension. The plates were incubated at room temperature for 7 days. After incubation, each single colony of *Trichoderma* spp. was screened and purified on PDA media. Then, morphological characteristics of mycelia and spores of *Trichoderma* spp. were checked under microscope as described by Rifai (1969).

T. harzianum from commercial substrate was also cultured on PDA medium and used as check for comparison with the effect of indigenous *Trichoderma* spp. on *R. solani* in both *in vitro* and *in vivo* experiments.

3.2.3 Test isolate of *Rhizoctonia solani*

Among the seven isolates, the most virulent *R. solani* isolate was selected from the previous experiment.

3.2.4 Effect of *Trichoderma* spp. on mycelial growth of *Rhizoctonia solani* (*in vitro*)

The inhibitory effect of *Trichoderma* spp. was evaluated on *R. solani* by dual culture technique as described by Dennis and Webster (1971b). The five isolates of *Trichoderma* spp. (four indigenous isolates from soil and *T. harzianum* from commercial inoculant) were used for this study. In order to get fresh active culture, each of the fungal isolates (*R. solani* and five *Trichoderma* spp.) were subcultured on PDA media and incubated at room temperature for 3 days. After that, mycelial discs (5 mm in diameter) from the edge of mycelium of each fungal colony were cut by using sterilized cork borers.

Each mycelial disc of *R. solani* and five *Trichoderma* spp. was transferred individually into the plates containing PDA media. Each plate received two discs, one with individual *Trichoderma* spp. and another with *R. solani* mycelium, was placed at a distance of 6 cm away from each other on PDA media. The mycelial disc of *R. solani* alone on PDA media was used as control. The plates were incubated at room temperature for 7 days.

3.2.4.1 Experimental design

The experiment was arranged in a completely randomized design (CRD) with five replications.

3.2.4.2 Data recording

When the mycelium of *R. solani* grew on the entire surface of control plate, radial growth of its mycelium was measured. Inhibition percentage of mycelial growth of *R. solani* was calculated by the following formula (Anil Kumar and Raj Kumar 2013).

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

Where, I = Percent inhibition (%) in growth of test pathogen, C = Radial growth of pathogen (cm) in untreated plate, T = Radial growth of pathogen (cm) in the presence of *Trichoderma* isolates.

3.2.5 Effect of *Trichoderma* spp. on banded leaf and sheath blight disease of maize caused by *Rhizoctonia solani* (*in vivo*)

3.2.5.1 Preparation of *Rhizoctonia solani* inoculum

The growth substrate for culturing *R. solani* inoculum was prepared by using rice hull rice grian substrate (Appendix 4) according to Muis and Quimio (2006). The mixture of substrate was packed in autoclavalbe plastic bags (10 cm in diameter x 15 cm in height) sterilized for 1 hour at 15 psi and then, cooled down for 1 day. The sterilized substrate bags were inoculated with 5 mm diameter mycelium disc of 3 days old pure culture of *R. solani* and then, incubated for 14 days at room temperature.

3.2.5.2 Preparation of *Trichoderma* inoculum

The rice hull rice grain substrate was also used for culturing *Trichoderma* inoculum and similarly prepared as *R. solani* substrate as described in the section 3.2.5.1. The sterilized substrate bags were inoculated with 5 mm diameter mycelium disc of 3 days old pure culture of each *Trichoderma* spp. and then incubated for 7 days at room temperature.

3.2.5.3 Inoculation of *Rhizoctonia solani* to the soil

Inoculation of *R. solani* was done at 7 days before sowing of maize. Sterilized sandy soil was mixed with Urea at the rate of 0.09 g kg^{-1} soil (200 kg N ha^{-1}), Triple super phosphate at 0.04 g kg^{-1} soil (100 kg P_2O_5 ha^{-1}) and Muriate of potash at 0.03 g kg^{-1} soil (75 kg K_2O ha^{-1}) respectively. Then, 14 days old of *R. solani* substrate was thoroughly mixed with the sterilized sandy soil at the rate of 100 g kg^{-1} of soil (1:10 w/w) according to Muis and Quimio (2006) and allowed to stand for six hours.

3.2.5.4 Inoculation of *Trichoderma* spp. to the soil

Seven days old of each *Trichoderma* substrate was individually mixed again with *R. solani* inoculated soil at the rate of 35 g kg^{-1} of soil. Then, the inoculated soil mixture was placed onto plastic pot (9 cm in diameter x 10 cm in height) and incubated for 7 days before sowing. The sterilized soil inoculated with *R. solani* alone was used as control.

3.2.5.5 Preparation of test plant

The most susceptible variety from experiment-1 was used as test plant. The seeds were surface sterilized for 10 minutes in 1% sodium hypochloride solution (NaOCL), washed three times with sterilized water, pregerminated for 2 days and sown in plastic pots containing sterilized sandy soil inoculated with mixture of *R. solani* and each *Trichoderma* substrate seperately.

3.2.5.6 Experimental design

The treatments were laid out in a completely randomized design (CRD) with five replications.

3.2.5.7 Data recording

The plant height and lesion length (cm) were recorded at 30 days after sowing of the test variety. Relative lesion length (%) was calculated by the formula as described in section 3.1.8.

Percent disease control was computed by using the following formula (Pascual *et al*. 2000).

$$\text{Percent disease control (\%)} = \frac{\text{A-B}}{\text{A}} \times 100$$

In which A= relative lesion length of maize plant without treatment and B = relative lesion length of maize plant with treatment.

3.3 Evaluation of Effectiveness of Different Disease Control Measures on Banded Leaf and Sheath Blight of Maize

This experiment was carried out at the Department of Plant Pathology, YAU from November 2014 to March 2015 (Plate 3.4).

3.3.1 Preparation of test plants

The most susceptible variety showing the highest disease severity against all isolates of *R. solani* was used as test variety according to the first experiment described in section 3.1. Then, 7 days old maize seedlings were prepared and transplanted into polythylene pot (36 cm in diameter x 30 cm in height) containing 13 kg of sterilized sandy loam soil as described in section 3.1.4.

Plate 3.4 Experimental layout for evaluation of effectiveness of integrated disease control measures on banded leaf and sheath blight of maize at Department of Plant Pathology, YAU

3.3.2 Preparation of *Rhizoctonia solani* inoculum

The most virulent *R. solani* isolate resulting in the highest disease severity on all test varieties was used as test pathogen according to the first experiment described in section 3.1. The inoculum was prepared as described in section 3.1.5.

3.3.3 Inoculation of *Rhizoctonia solani*

Inoculation of the most virulent *R. solani* isolate was done by using mycelial insertion method at 30 days after sowing of tested variety as described in section 3.1.6. Twelve plants were inoculated for each treatment. Four times of sprinklings per day were done to encourage disease development.

3.3.4 Preparation of *Trichoderma* inoculum

According to the result of the second experiment described in section 3.2.4 and 3.2.5, a highly antagonistic isolate of *Trichoderma* sp. was used as bio-control agent for this study. The pure *Trichoderma* sp. from slant culture was transferred onto PDA media and incubated at room temperature for 7 days. Then, the mycelia containing conidia suspended in sterilized water were scraped with sterilized tooth brush and filtered through two layers of water filter. The conidial suspension was adjusted to a final spore concentration 10^8 spore mL^{-1} by using hemacytometer.

3.3.5 Application of chemical, biological and mechanical controls

At the time of symptom appearance, different control practices were applied for 12 plants $treatment^{-1}$. Carbendazim (Dazine 50 SC) at the rate of 0.2% and the spore suspension (10^8 spores mL^{-1}) of the test *Trichoderma* sp. were sprayed on the inoculated plants for each treatment. Total of four times of application with different managements except stripping method, was done at 10 days intervals. For the treatment of mechanical control, stripping of diseased leaves and sheaths was done up to 2 weeks before flowering. The inoculated plants without application of disease control methods were maintained as untreated check. Eight treatments were applied at 37 DAS (days after sowing) until 67 DAS (Table 3.2).

3.3.6 Experimental design

The eight treatments were laid out in a randomized complete block design (RCB) with three replications.

Table 3.2 Treatments and their application time of schedule

	Treatments	Application time			
		1st (37 DAS)	**2nd (47 DAS)**	**3rd (57 DAS)**	**4th (67 DAS)**
T_1	Carbendazim (0.2%)	Cdz	Cdz	Cdz	Cdz
T_2	*Trichoderma* spp. (10^8 spores mL^{-1})	T.	T.	T.	T.
T_3	Stripping	Stripping	Stripping	……	……
T_4	*Trichoderma* + Carbendazim	T.	Cdz	T.	Cdz
T_5	*Trichoderma* + Stripping	T.	Stripping	T.	T.
T_6	Carbendazim+ Stripping	Cdz	Stripping	Cdz	Cdz
T_7	*Trichoderma* + Carbendazim + Stripping	T.	Stripping	Cdz	T.
T_8	Untreated check	……..	…….	…….	……

DAS = Days after sowing

3.3.7 Data recording

(a) Disease severity and percent disease control

Disease scores, lesion length and relative lesion length of test variety were recorded at 97 days after sowing. Relative lesion length was calculated by the formula as described in section 3.1.8. Disease scores were assessed by using 1-5 scoring scale as described in the previous section 3.1.8. Percentage of disease control was calculated by using the following formula (Asif 2013).

$$\text{Disease control (\%)} = \frac{\text{Disease score in untreated} - \text{Disease score in treatment}}{\text{Disease score in untreated}} \times 100$$

(b) Yield parameters

At harvest, yield parameters such as number of ear plant^{-1}, ear weight plant^{-1}, kernel weight plant^{-1} and 1000-kernel weight were recorded. Moisture content (%) of the test variety was measured by using grain moisture meter (GMK series). Shelling (%) was calculated by following equation (CIMMYT's International Maize Testing Program 1999).

$$\text{Shelling (\%)} = \frac{\text{Kernel dry weight}}{\text{Ear dry weight}} \times 100$$

Yield plant^{-1} was computed by using the following formula (Muis and Quimio 2006).

$$\text{Yield plant}^{-1}\text{ (kg)} = \frac{(100 - \text{Moisture content}) \times \text{Field weight plant}^{-1}\text{ (kg)} \times \text{Shelling(\%)}}{(100 - 15)\text{ at 15\% moisture content}}$$

(c) Economic assessment

In order to find out economically effective method to control the disease, performance of different control methods were adjudged on the basis of net profit and return per kyat investment. Yield data obtained were used for the economic analysis as described by Batsa (2004) and Asif (2013).

i. Additional yield (kg)

= Yield in treatment-Yield in untreated check

ii. Value of additional yield (kyats)

= Additional yield (kg) x selling price (kyats kg^{-1})

iii. Cost of treatment (kyats)

= It includes cost of fungicides, bio-control agents and cost of application including labour cost.

iv. Net profit (kyats)

= a - b

Where, a = value of additional yield (kyats)

b = cost of treatment (kyats)

v. Return per kyat investment

$$= \frac{a - b}{b}$$

3.4 Data analysis

For all experiments, the collected data were analyzed by Statistix (version 8.0) computer software program. Treatment means were compared by using LSD test at 5% level.

CHAPTER IV

RESULTS

4.1 Evaluation of Pathogenicity of *Rhizoctonia solani* Isolates

4.1.1 Isolation and identification of *Rhizoctonia solani* isolates

R. solani isolates namely, Tatkone-1, Tatkone-2, Lewe-1, Lewe-2, Lewe-3, Pyinmana and Kyaukme were cultured on PDA medium. Their characteristic of mycelium and sclerotia were identified according to Barnett and Hunter (1972). Mycelia of all isolates showed colorless at young stage and turned to light brown as they matured. The characteristics of hyphae were constricted at the point of branching and branched near distal septum. The mature hyphae were branched at right and acute angle to main branch (Plate 4.1).

On PDA media, the six isolates of *R. solani*, Tatkone-1, Tatkone-2, Lewe-1, Lewe-2, Pyinmana and Kyaukme produced aerial hyphae while the isolate, Lewe-3 was less aerial culture. The colony colors of all isolates were light to light brown (Plate 4.2). The five isolates of *R. solani* such as Tatkone-1, Tatkone-2, Lewe-1, Pyinmana and Kyaukme, were found to be produced sclerotia abundantly at 4 days after incubation. However, Lewe-2 isolate produced the sclerotia at 15 days after incubation while Lewe-3 isolate produced them at 30 days after incubation respectively. Mostly, sclerotia were spherical shape and dark brown to black colour. The sclerotia of all isolates were found on the surface of PDA media.

4.1.2 Pathogenicity test for *Rhizoctonia solani* isolates

All of *R. solani* isolates were pathogenic to the test varieties, Yezin-10, Yezin-11 and CPDK-888. All inoculated plants showed typical symptoms of BLShB on the second lower most leaf sheath and stem at 7 days after inoculation. Lesions were water-soaked at first and then, developed a straw-coloured centre with a brownish border. Finally, these enlarged and produced brown discoloured areas alternating with darker brown bands on the infected leaf sheaths. Severely infected leaves and sheaths were thin and papery and resemble from a distance, a shape of snake skin. Average disease score, lesion length and relative lesion length of each variety inoculated with seven *R. solani* isolates were shown in Table 4.1. The disease score, lesion length and relative lesion length were significantly different among the isolates and also among the varieties at 45 days after inoculation (Plate 4.3 and 4.4).

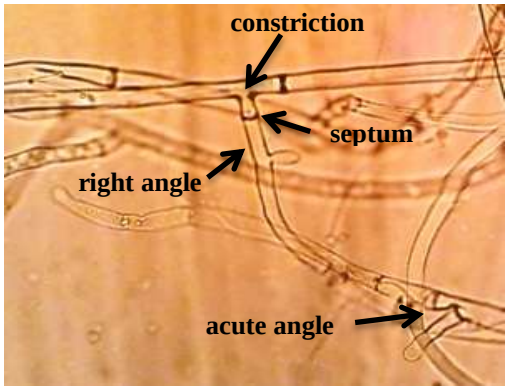
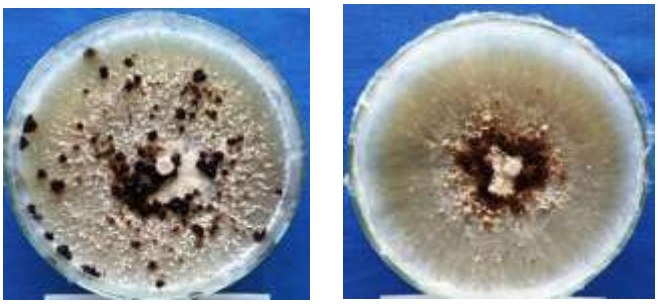
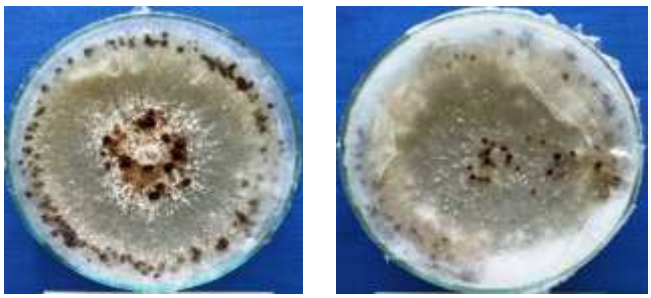


Plate 4.1 Typical mycelial feature of *Rhizoctonia solani* observed under microscope (400x)



Tatkone-1 **Tatkone-2**

Lewe-1 **Lewe-2** **Lewe-3**



Pvinmana **Kyaukme**

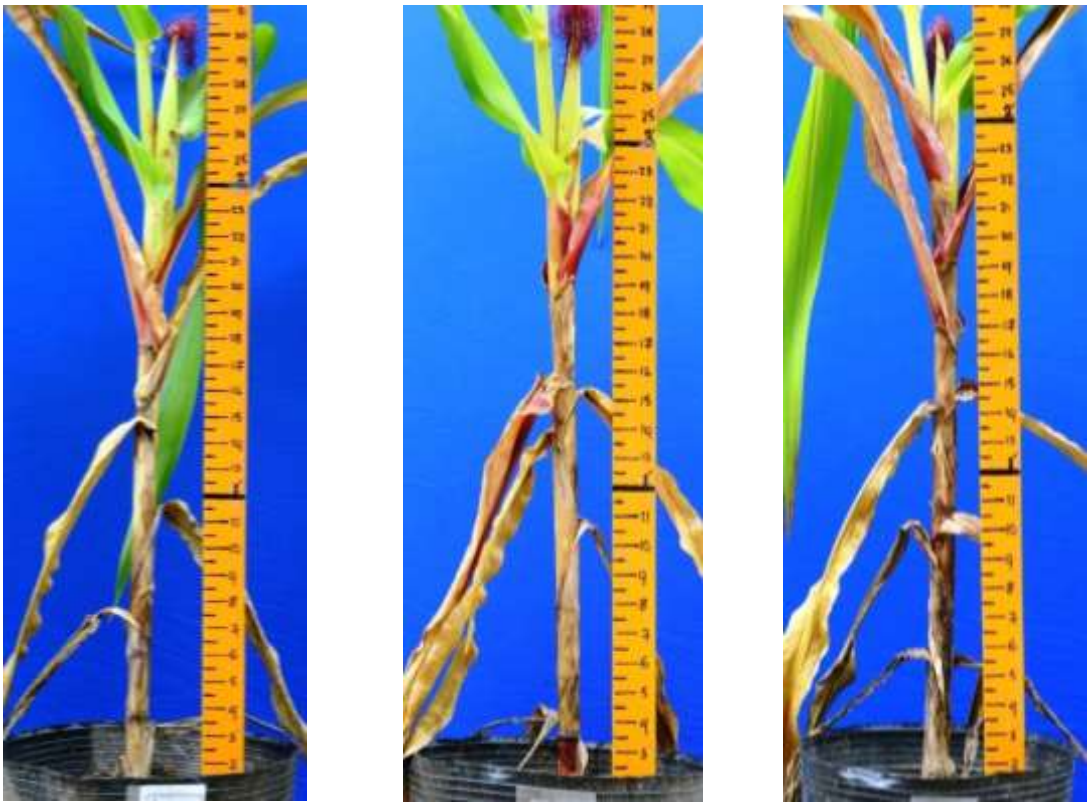
Plate 4.2 Colonies of different *Rhizoctonia solani* isolates on PDA media at 7 days after incubation

Table 4.1 Response of three hybrid maize varieties to seven isolates of *Rhizoctonia solani* at 45 days after inoculation

	Disease score*	Lesion length (cm)*	Relative lesion length (%)*
***R. solani* isolates**			
Tatkone-1	4.1bc**	54.3b	45.6b
Tatkone-2	4.3b	57.6b	49.0b
Lewe-1	4.0c	55.3b	46.5b
Lewe-2	2.9d	33.6c	28.1c
Lewe-3	1.2e	15.6d	13.0d
Pyinmana	4.9a	70.7a	60. 4a
Kyaukme	4.4b	56.9b	48.4b
LSD $_{0.05}$	**0.37**	**5.62**	**5.78**
Hybrid varieties			
Yezin-10	3.4b	45.0b	37.5b
Yezin-11	3.6b	48.3b	42.2a
CPDK-888	4.0a	54.1a	45.1a
LSD $_{0.05}$	**0.24**	**3.68**	**3.79**
Pr > F			
Isolate (A)	<0.001	<0.001	<0.001
Variety(B)	<0.001	<0.001	<0.001
A x B	0.45	0.28	0.54
CV (%)	**16.24**	**18.73**	**22.78**

* Means of seven replications

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



Yezin-10 **Yezin-11** **CPDK-888**

Plate 4.3 Disease severity of banded leaf and sheath blight on three hybrid maize varieties inoculated with *Rhizoctonia solani,* Pyinmana isolate at 45 days after inoculation

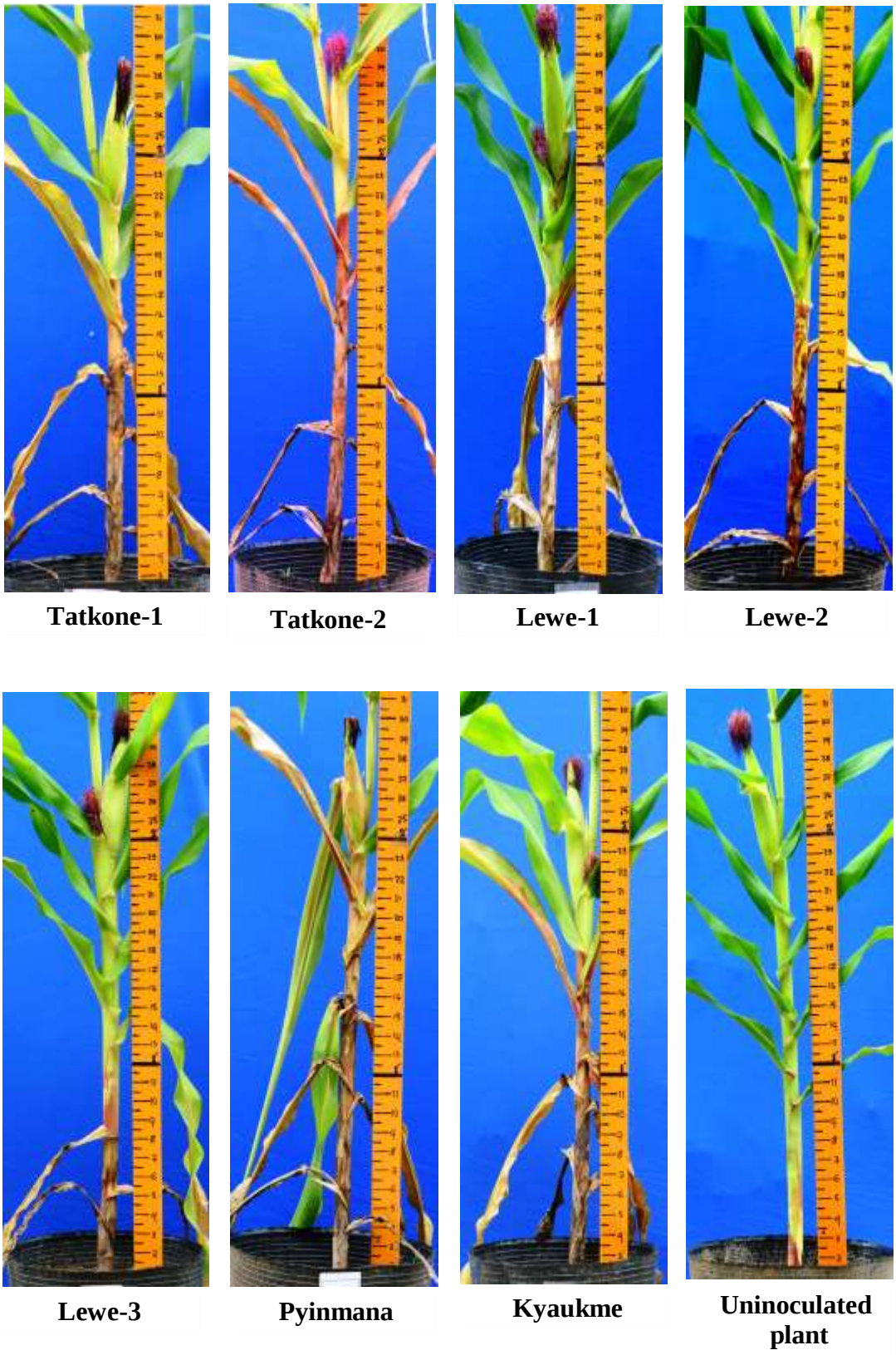


Plate 4.4 Disease severity of banded leaf and sheath blight on CPDK-888 variety inoculated with different *Rhizoctonia solani* isolates at 45 days after inoculation

The isolate collected from Pyinmana was observed as the most virulent one in all isolates due to the highest level of disease score (4.9), lesion length (70.7 cm) and relative lesion length (60.4%). In contrast, *R. solani*, Lewe-3 isolate was found to be the least virulent in terms of the lowest disease score (1.2), lesion length (15.6 cm) and relative lesion length (13%) on all tested varieties.

Among the three hybrid maize varieties, Yezin-10, Yezin-11 and CPDK-888, higher disease score (4.0), lesion length (54.1cm) and relative lesion length (45.1%) were found on CPDK-888, followed by Yezin-10 and Yezin-11 against all of *R. solani* isolates.

There was no interaction between the isolates and the varieties. When the response of CPDK-888 to each isolate of *R. solani* was determined, the highest disease score, lesion length and relative lesion length were shown by Pyinmana isolate followed by Kyaukme, Tatkone-2, Tatkone-1, Lewe-1, Lewe-2 and Lewe-3. In Yezin-10 and Yezin-11 varieties against each isolate, similar trends of disease severity were observed (Figure 4.1).

4.2 Effect of *Trichoderma* spp. on *Rhizoctonia solani* (*in vitro* and *in vivo*)

4.2.1 Isolation and identification of *Trichoderma* spp.

Four indigenous *Trichoderma* spp. were successfully cultured on Martin's media from rhizospheric soil samples and designated as *Trichoderma*-1, *Trichoderma*-2, *Trichoderma*-3 and *Trichoderma*-4 respectively. Various fungal colonies of *Trichoderma* spp. were started to appear at 3 days after incubation of soil suspensions. From which, each colony of *Trichoderma* spp. was separately subcultured and purified on PDA media.

Colonies of the indigenous *Trichoderma* spp. appeared cottony while *T. harzianum*, was flatter, more compact and less cottony (Plate 4.5). The conidial productions of the indigenous *Trichoderma* spp. were restricted to the center of the colonies and that of all colonies appeared to be light to dark green. However, the conidial production in *T. harzianum* was diffused, dispersed and not formed concentric rings. When the colonies of four *Trichoderma* spp. were observed, diffusible yellow pigments were found to be produced by only *Trichoderma*-4.

The microscopic features of all *Trichoderma* spp. were observed under the microscope with 400x magnification (Plate 4.6). The four indigenous *Trichoderma*

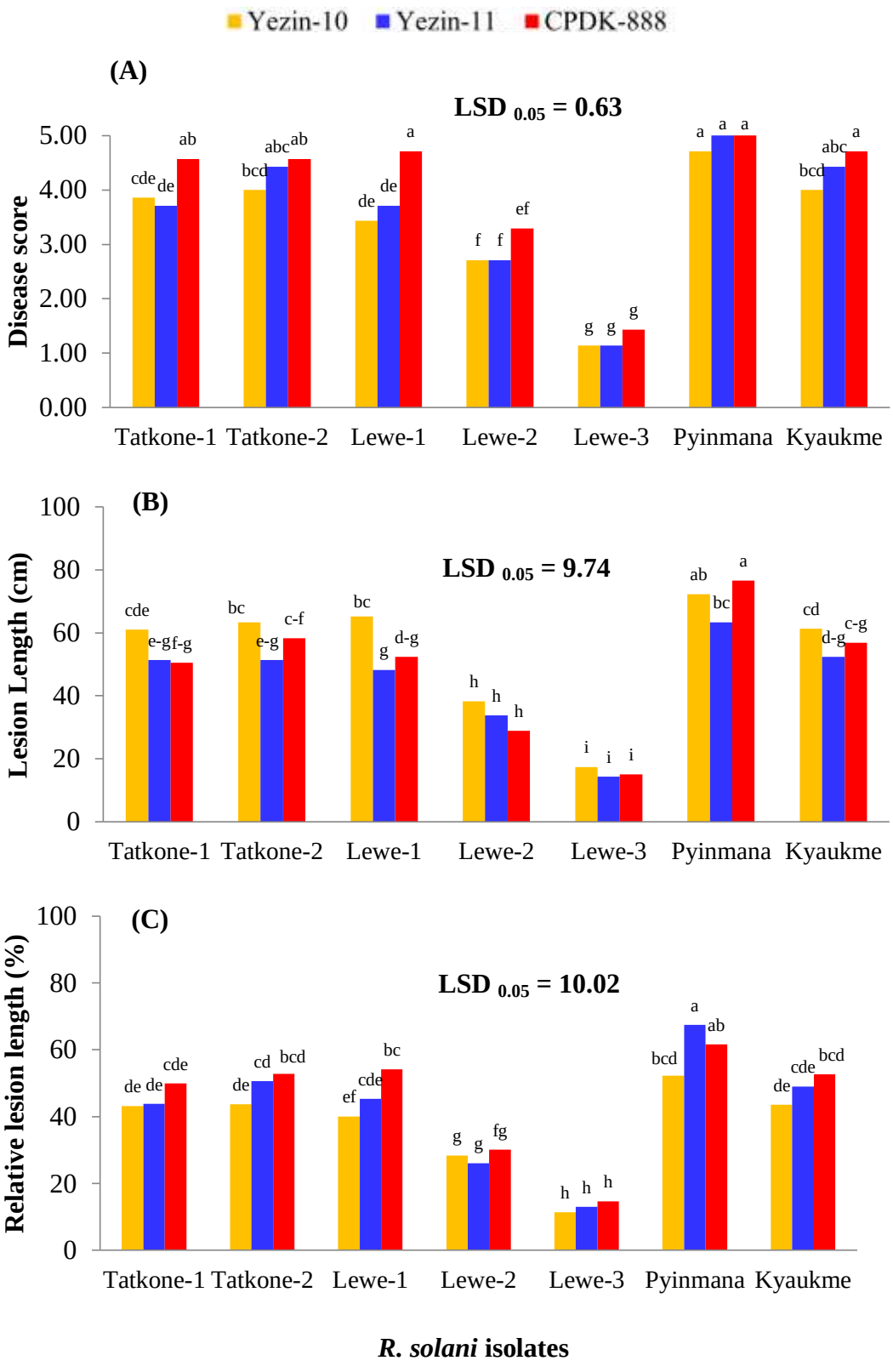
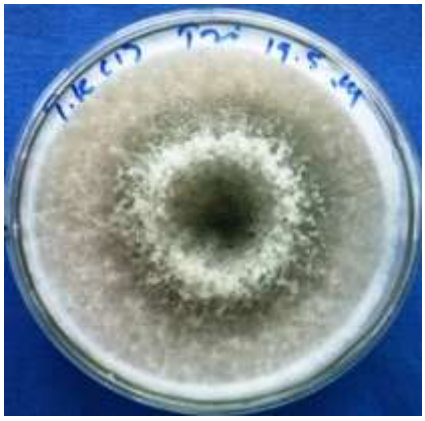
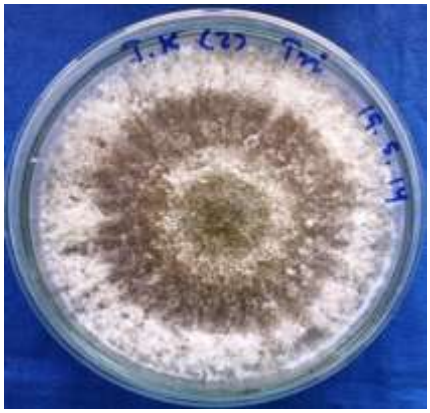


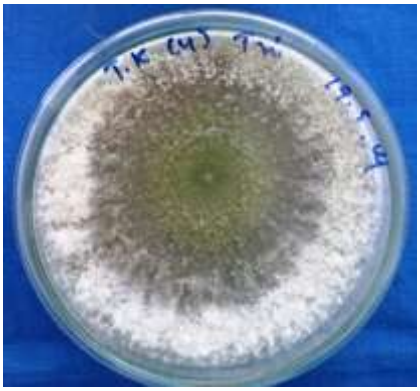
Figure 4.1 (A) Disease score, (B) Lesion length and (C) Relative lesion length of banded leaf and sheath blight disease on three test varieties inoculated with seven isolates of *Rhizoctonia solani*



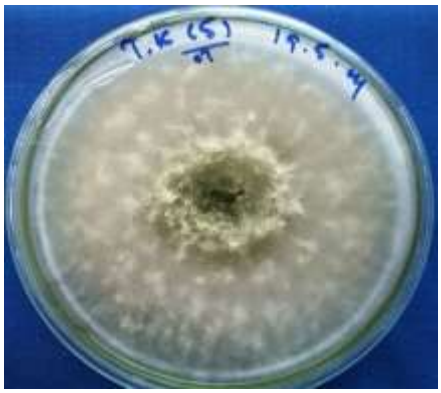
(A) *Trichoderma*-1



(B) *Trichoderma*-2



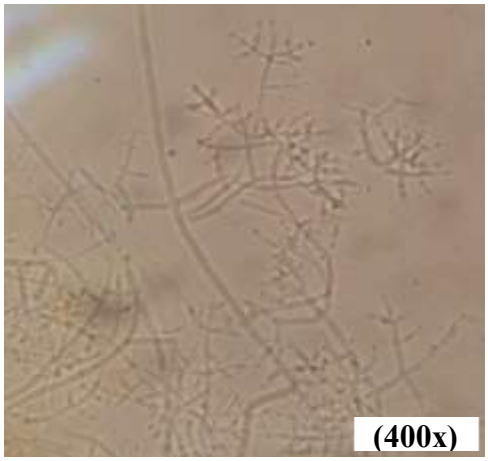
(C) *Trichoderma*-3



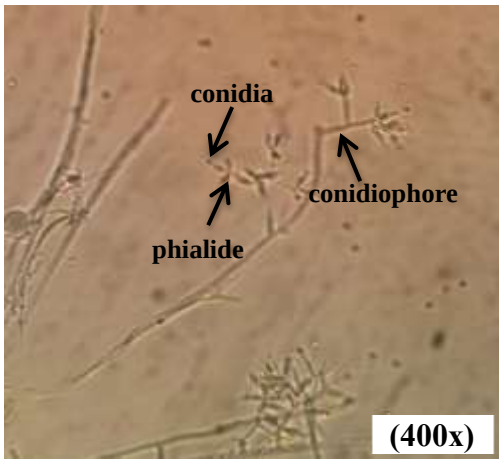
(D) *Trichoderma*-4

(E) *T. harzianum*

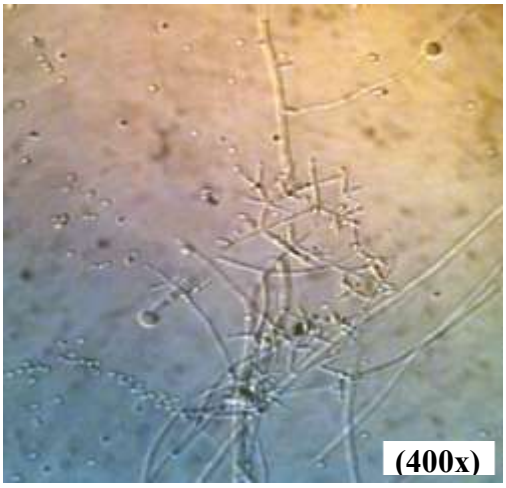
Plate 4.5 Colonies of *Trichoderma* spp. isolated from soil (A-D) and *T. harzianum* from commercial inoculant (E) on PDA media at 3 days after incubation



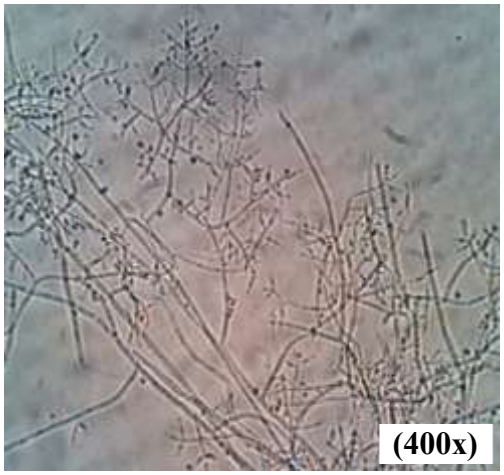
(A) *Trichoderma*-1



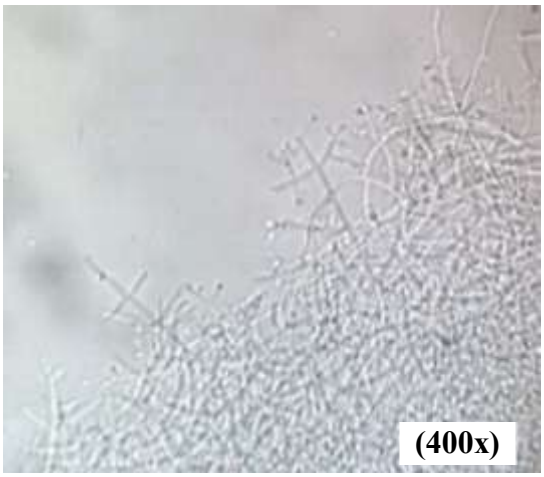
(B) *Trichoderma*-2



(C) *Trichoderma*-3



(D) *Trichoderma*-4



(E) *T. harzianum*

Plate 4.6 Conidia, phialides, conidiophores and mycelia of *Trichoderma* spp. isolated from soil (A-D) and *T. harzianum* from commercial inoculant (E) observed under microscope

spp. have subglobose to globose shapes of conidia and conidiophores with paired or lateral primary branches. The primary branches of the four indigenous *Trichoderma* spp. were formed in nearly right angle (90°) to the main axis. Their phialides are flask-shaped and normally held in whorls of two to three phialides. The same morphological characters of conidia and conidiophore were also found in *T. harzianum* (Plate 4.6).

4.2.2 Effect of *Trichoderma* spp. on mycelial growth of *Rhizoctonia solani* (*in vitro*)

Four indigenous *Trichoderma* spp. from soil and *T. harzianum* from commercial inoculant were used for this study. The inhibitory effect of *Trichoderma* spp. on *R. solani* (Pyinmana isolate) was evaluated on PDA media by dual culture technique as described by Dennis and Webster (1971b). Radial mycelium growth of *R. solani* and percent inhibition of mycelial growth by *Trichoderma* spp. were significantly different among the treatments. All *Trichoderma* spp. exhibited antagonistic effect on *R. solani* by reducing mycelial growth of *R. solani* and by inhibiting its mycelial growth over untreated check (Plate 4.7).

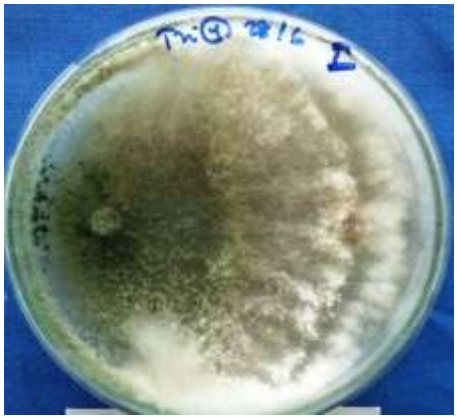
Mycelial growth of *R. solani* in dual culture plate ranged from 0.2 cm to 3.2 cm while that in untreated plate (*R. solani* only) resulted 7.5 cm. This caused 57% to 97% inhibition percent of mycelial growth of *R. solani* by *Trichoderma* spp. Four days after incubation, growth of *R. solani* was inhibited by *Trichoderma*-1, *Trichoderma*-2, *Trichoderma*-3, *Trichoderma*-4 and *T. harzianum* and attained a growth of 0.4 cm, 2.5 cm, 0.2 cm, 0.7 cm and 3.2 cm respectively. Inhibition percent by *Trichoderma*-1, *Trichoderma*-2, *Trichoderma*-3, *Trichoderma*-4 and *T. harzianum* against *R. solani* was 95%, 67%, 97%, 91% and 57% respectively (Table 4.2). Among them, three spp., *Trichoderma*-1, *Trichoderma*-3 and *Trichoderma*-4 showed lower mycelial growth and higher percent inhibition on mycelial growth of test pathogen than that of the others *Trichoderma* spp.

4.2.3 Effect of *Trichoderma* spp. on banded leaf and sheath blight (*in vivo*)

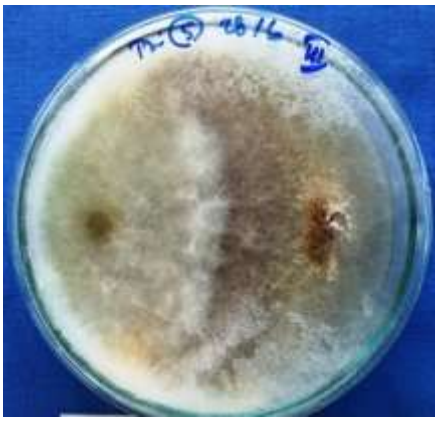
Four indigenous *Trichoderma* spp. and *T. harzianum* were evaluated for their effectiveness on disease severity of CPDK-888 against the most virulent *R. solani* isolate (Plate 4.8). Inoculation of *Trichoderma* spp. and *R. solani* was done by soil infestation method at 7 days before sowing of the most susceptible hybrid maize vatiety, CPDK-888.

Trichoderma*-1** ***R. solani

Trichoderma*-2** ***R. solani

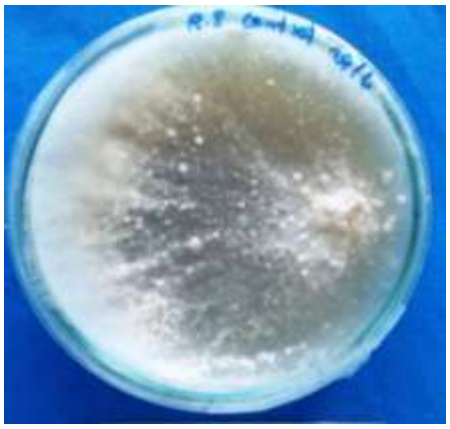


Trichoderma*-3** ***R. solani



Trichoderma*-4** ***R. solani

T. harzianum ***R. solani***



***R. solani* (pathogen only)**

Plate 4.7 Inhibitory effect of *Trichoderma* spp. on mycelial growth of *Rhizoctonia solani* at 4 days after incubation

Table 4.2 Inhibitory effect of *Trichoderma* spp. on mycelial growth of *Rhizoctonia solani in vitro* (dual culture test)

Treatments	Radial growth of *R. solani* (cm)*	Percent inhibition (%)*
Trichoderma-1	0.4d**	95a**
Trichoderma-2	2.5c	67b
Trichoderma-3	0.2d	97a
Trichoderma-4	0.7d	91a
T. harzianum	3.2b	57c
Untreated (check)	7.5a	-
LSD $_{0.05}$	**0.53**	**7.70**
F pr.	**<0.001**	**<0.001**
CV (%)	**16.79**	**7.20**

* Means of five replications

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

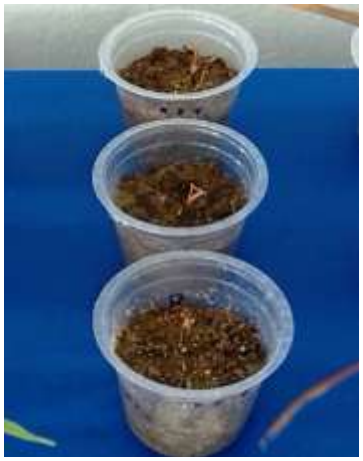
Treated with *Trichoderma*-1

Treated with *Trichoderma*-2

Treated with *Trichoderma*-3

Treated with *Trichoderma*-4

Treated with *T. harzianum*



Untreated (check)

Plate 4.8 Plant growth of maize variety, CPDK-888 treated with *Trichoderma* spp., sown on *R. solani* infested soil at 30 DAS

Although lesion length of test variety treated with each of *Trichoderma* spp. was not significantly different from that of untreated check, the relative lesion length of test variety was significantly different among the treatments (Table 4.3). In comparison with untreated check, the test variety treated with each *Trichoderma* was found to be reducing in lesion length ranged from 2.2 cm to 4.9 cm and relative lesion length ranged from 15.8% to 55.9% which caused percent disease control ranged from 16% to 76%. Out of four indigenous *Trichoderma* spp., the test variety treated with *Trichoderma*-1, *Trichoderma*-3 and *Trichoderma*-4 gave lower lesion length (2.6 cm, 2.7 cm and 2.2 cm) and significantly lower relative lesion length (21.0%, 21.9% and 15.8%) that caused higher percent disease control (69%, 67% and 76% respectively) than others two, *Trichoderma*-2 and *T. harzianum.* Out of the five test *Trichoderma* spp., the lowest disease severity and the highest percent disease control were found on the test variety treated with *Trichoderma*-4.

4.3 Evaluation of Effectiveness of Different Disease Control Measures on Banded Leaf and Sheath Blight of Maize

4.3.1 Disease score and relative lesion length

Disease severity of test variey treated with each disease control measure was significantly different among the treatments regarding in the disease score and relative lesion length (Table 4.4).

Out of all disease control measures, T_1 (Carbendazim), T_4 (combination of *Trichoderma* and Carbendazim), T_6 (combination of stripping and Carbendazim) and T_7 (combination of *Trichoderma*, Carbendazim and stripping) were resulted in lower mean value of disease score of 1.4, 1.5, 1.0 and 1.5 and relative lesion length of 12.3%, 12.8%, 7.2% and 12.1% respectively than that of the other treatments.

The test variety treated with T_2 (*Trichoderma*) and T_3 (Stripping) showed higher disease score of 2.9 and 2.8 and relative lesion length of 27.8% and 26.6 % respectively than the other treatments. The untreated check gave the highest disease score of 4.7 and relative lesion length of 62.3%. A highly significant positive correlation ($r = 0.92$) was observed between disease score and relative lesion (Figure 4.2).

All treatments were found to be effective in controlling the disease, showing percent disease control ranged from 37.5% to 78.6%. Among the treatments,

Table 4.3 Effect of *Trichoderma* spp. on banded leaf and sheath blight disease of maize at 30 days after sowing *in vivo* (pot experiment)

Treatments	Lesion length (cm)*	Relative lesion length(%)*	Percent disease control (%)*
Trichoderma-1	2.6b**	21.0b**	69
Trichoderma-2	4.9a	50.8a	24
Trichoderma-3	2.7b	21.9b	67
Trichoderma-4	2.2b	15.8b	76
T. harzianum	4.8a	55.9a	16
Untreated (check)	3.7ab	66.7a	
LSD $_{0.05}$	**2.10**	**24.89**	
F pr.	**0.05**	**<0.001**	
CV (%)	**46.18**	**49.33**	

* Means of five replications

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

Table 4.4 Effectiveness of different disease control measures on banded leaf and sheath blight of maize variety (CPDK-888) against *Rhizoctonia solani*

	Treatments	Relative lesion length at 97 DAS (%)*	Disease score at 97 DAS*	Yield plant^{-1} (g)*	1000-kernel weight (g)*	Cost of treatment plant^{-1} (kyats)	Net profit plant^{-1} (kyats)	Cost and benefit ratio (C:B)
T_1	Carbendazim (Cdz)	12.3c**	1.4cd**	33.3a**	261.9a**	1.2	4.8	1 : 4.0
T_2	*Trichoderma* (*T.*)	27.8b	2.9b	31.1a	283.7a	1.0	4.5	1 : 4.5
T_3	Stripping	26.6b	2.8b	34.5a	260.8a	1.4	4.9	1 : 3.6
T_4	*T.* + Cdz	12.8c	1.5cd	31.2a	251.4a	1.1	4.4	1 : 4.0
T_5	*T.* + Stripping	14.2c	1.8c	30.9a	258.0a	1.5	4.0	1 : 2.7
T_6	Cdz + Stripping	7.3c	1.0d	36.8a	268.1a	1.6	5.3	1 : 3.3
T_7	*T.* + Cdz + Stripping	12.1c	1.5cd	32.7a	274.5a	1.5	4.4	1 : 2.9
T_8	Untreated (check)	62.3a	4.7a	8.1b	208.6b	0.0	-	-
	LSD 0.05	**10.12**	**0.62**	**8.35**	**36.02**			
	Pr>F	**<0.001**	**<0.001**	**<0.001**	**0.02**			
	CV%	**26.35**	**15.94**	**15.99**	**7.96**			

***** Means of three replications

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

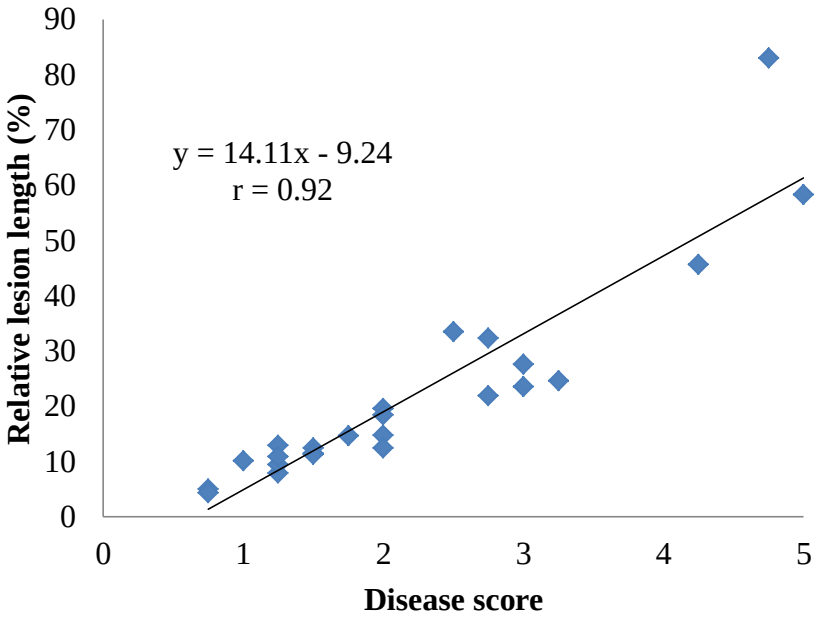


Figure 4.2 Relationship between disease score and relative lesion length of CPDK-888 against banded leaf and sheath blight disease as affected by application of different disease management practices

T_6 (combination of Carbendazim and stripping) showed the highest percent disease control of 78.6%, followed by T_4 (combination of *Trichoderma* and Carbendazim) resulted in 67.9%, T_7 (combination of *Trichoderma*, stripping and Carbendazim) resulted in 69.7% and T_5 (combination of *Trichoderma* and stripping) gave 60.7%. Out of single control measures, higher percent disease control of 70% was found on the plants treated with T_1 (Carbendazim) while the plants treated with T_2 (*Trichoderma*) and T_3 (Stripping) showed lower percent disease control resulting in 37.5% and 39.3%. In all treatments, integrated controls gave more percent disease control than single controls, *Trichoderma* alone and stripping alone (Figure 4.3).

4.3.2 Kernel yield

Effect of different disease control measures and its impact on kernel yield and 1000-kernel weight of CPDK-888 was described in Table 4.4 and Plate 4.9. The resulted yield plant^{-1} and 1000-kernel weight were significantly different between the plants treated by all disease control measures and untreated plants. The test variety treated with all disease control measures gave a comparatively higher yield plant^{-1} ranging from 30.9-36.8 g and 1000-kernel weight ranged from 251.4-283.7 g compaired with that of untreated plant (Table 4.4). Among all treatments, the lowest yield plant^{-1} of 8.1 g and 1000-kernel weight of 208.6 g were found in untreated plants. A significant negative correlation (r = - 0.63) was found between disease score and kernel yield plant^{-1} (Figure 4.4).

4.3.3 Economic assessment and economic return

The test plants treated with any control measure did not give losses over untreated check and gave more net profit (kyats) and return per kyat investment. Among the different disease control measures, the highest cost and benefit ratio (C:B) was obtained from yield of the test plants treated with T_2 (*Trichoderma*) giving return of 4.5 per kyat investment followed by T_1 (Carbendazim) and T_4 (combination of *Trichoderma* and Carbendazim) resulting return of 4.0 per kyat investment. Although T_6 (combination of Carbendazim and stripping) gave more net profit than the others, it returned of 3.3 per kyat investment as well as T_3 (stripping) which returned of 3.6 per kyat investment. The lower cost benefit ratio was found in T_5 (combination of *Trichoderma* and stripping) and T_7 (combination of *Trichoderma*, stripping and Carbendazim) resulting C:B of 2.7 and 2.9 per kyat investment respectively.

T. = *Trichoderma*-4, Cdz = Carbendazim

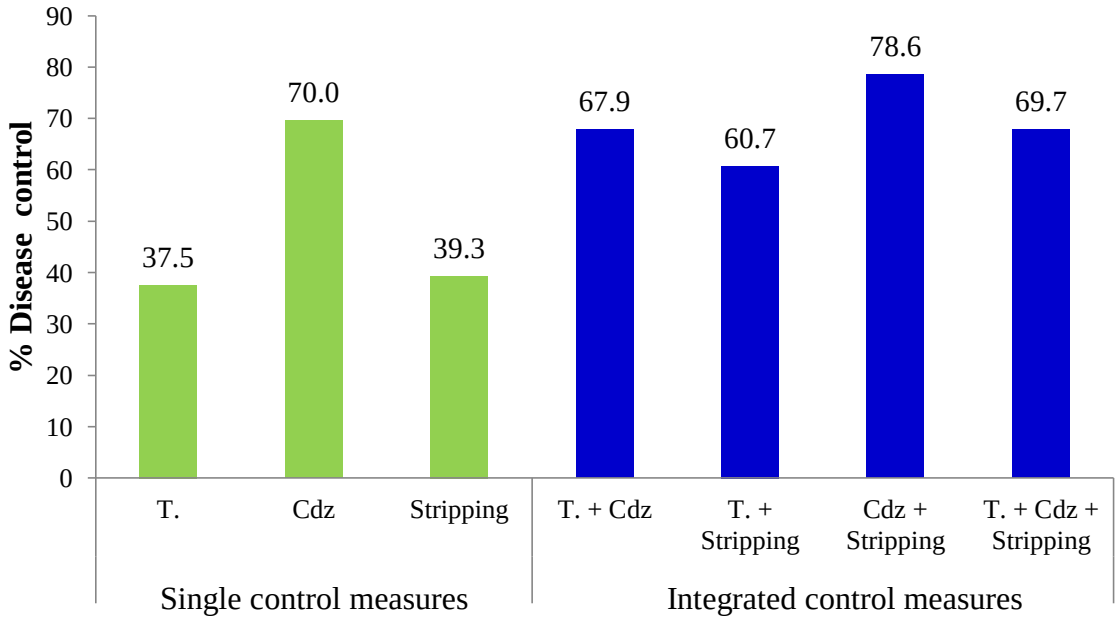


Figure 4.3 Percent disease control of single control measures and their combination effect on banded leaf and sheath blight inoculated on CPDK-888 at 97 days after sowing

Treated with Carbendazim

Treated with *Trichoderma*

Treated with stripping

Treated with *Trichoderma* and Carbendazim

Treated with *Trichoderma* and stripping

Treated with Carbendazim and stripping

Treated with Carbendazim, *Trichoderma* and stripping

Untreated check

Plate 4.9 Ear and kernel performance of CPDK-888 against banded leaf and sheath blight disease as affected by different control methods

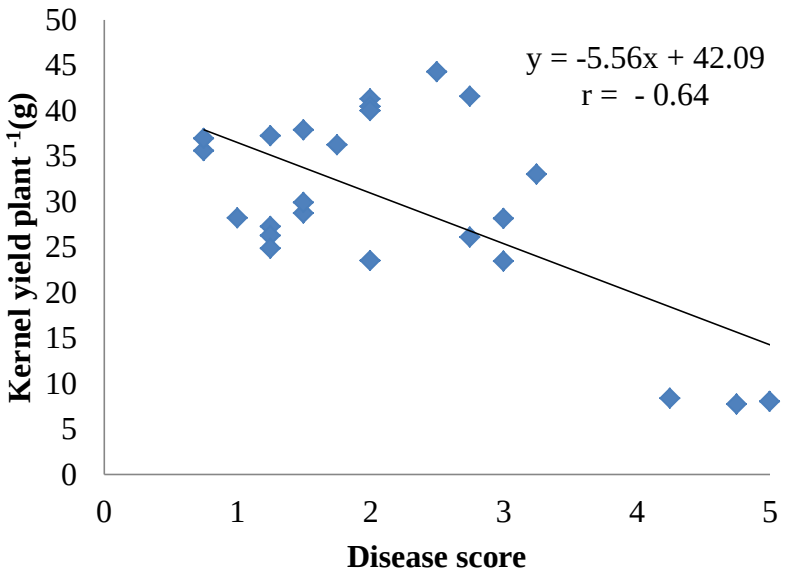


Figure 4.4 Relationship between disease score and kernel yield plant^{-1} of CPDK-888 against banded leaf and sheath blight disease as affected by application of different disease management practices

CHAPTER V

DISCUSSION AND CONCULSION

Microscopic studies revealed that the isolates in this study possessed a known feature of mycelial characters of *R. solani* which are accordance with the characters reported by Baker (1970) and Sneh *et al*. (1991). The most virulence in Pyinmana isolates, moderately virulence in Takone-1, Tatkone-2, Lewe-1 and Kyaukme isolates and less virulence in Lewe-2 and Lewe-3 isolates were generally found based on their disease severity on the three test varieties. The results indicated that the disease severity produced by the pathogenic isolates of *R. solani* was significantly different from each other. Bard *et al.* (1996) and Pascual *et al*. (2000) reported that fifty-two isolates belonged to anastomosis group AG1-IA which caused banded leaf and sheath blight in maize (*Zea mays* L.), but they showed considerable variation in virulence (30% to 80% in relative lesion length). Singh *et al*. (2002) have also studied differences in virulence within the population of *R. solani* infecting rice and wheat. This explanation might reflect to the pathogenic variability among *R. solani* isolates in the present study.

Out of the three hybrid maize varieties, Yezin-10, Yezin-11 and CPDK-888, the highest disease severity was recorded on CPDK-888 variety against all test isolates of *R. solani*. The present findings are accordance with that of Maung Maung Thein (2003) who described that CPDK-888 showed high disease severity as superior as Yezin 4 variety (susceptible check) among test entries against BLShB. Therefore, it suggested that CPDK-888 could be utilized as susceptible check for further research.

Vanderplank (1984) suggested that the presence of a significant interaction between variety and isolate is evidence for a differential (vertical) host-pathogen relationship. Lack of a significant interaction was associated with non-differential (horizontal) host-pathogen relationship which implied that differences in cultivar susceptibility are consistent relative to one another, regardless of pathogen isolates. In the present study, there was no significant interaction between the *R. solani* isolates and the varieties in terms of disease severity. Therefore, the test isolates and varieties in this study might have non-differential (horizontal) host-pathogen relationship showing consistent pathogenicity of each isolate and susceptiblility of each variety.

Discovery of new biological control agents and demonstration of their value in reducing disease infection have opened new promising avenues for practical applications in agriculture and for promoting environmental safety (Boland 1990). *Trichoderma* spp. are common inhabitants of rhizosphere and well recognized as biocontrol agents for soil borne plant pathogens (Samuels *et al*. 2002b; Wuezowsky *et al*. 2003; Harman *et al*. 2004).

When the morphological characteristics of four indigenous *Trichoderma* spp. were identified under microscope according to Rifai (1969), they were found to be less variation. Anees *et al*. (2010) described that *Trichoderma* spp. have relatively few variation in morphological characters that may cause overlapping and misidentification of *Trichoderma* isolates to species level. Moreover, morphological characters are influenced by culture conditions (Diguta *et al*. 2011). Therefore, in this study, species level of *Trichoderma* could not be identified because information from morphological study alone might be insufficient to precisely identify as *Trichoderma* sp.

In *in vitro* condition, all test *Trichoderma* spp. were effective in controlling *R. solani* ranging from 57-97% in inhibition by dual culture technique. Among them, *Trichoderma*-1, *Trichoderma*-3 and *Trichoderma*-4 showed higher inhibition of 91-97% on the mycelium of *R. solani.* These results are in agreement with Chet (1987) and Harman (2006) who reported that the genus *Trichoderma* is especially known for its antagonistic activity against *R. solani*. In contrast to this, Chet (1990) and Zhang Guang *et al*. (2005) reported that out of a total of 18 *Trichoderma* isolates obtained from maize rhizosphere, only 5 isolates were effective and their suppression rates on mycelial growth of *R. solani* were 39-85%.

In *in vivo* condition, all *Trichoderma* spp. were effective in reducing disease severity of banded leaf and sheath blight on test variety, CPDK-888. However, the percent disease control on *R. solani* was varied within isolates of *Trichoderma* spp. ranging from 16-76%. However, the four indigenous *Trichoderma* spp. from soil were found to be more effective in controlling the disease and also showing more plant height than the plants treated with commercial *T. harzianum.* Therefore, it suggested that isolation of *Trichoderma* spp. from indigenous soil is important rather than introducing one which might not exhibit its ability to the fullest extent.

Sharma *et al.* (2012) also described that different *Trichoderma* spp. may differ in their potential to control the disease and Lorito *et al.* 2006 who reported that

Trichoderma can function at the same time both as microbial antagonists and plant symbionts. Species of *Trichoderma* also have growth promoting capabilities that may or may not be integral to biological control (Yedidia *et al*. 1999; Benitez *et al.* 2004). Meena *et al*. (2003a) also stated that soil application by *T. harzianum* against banded leaf and sheath blight gave disease control of 42.9% in maize crop. In *in vitro* and *vivo* experiments, the three indigenous *Trichoderma* spp., possessed excellent antagonistic ability. Among them, *Trichoderma* 4 was selected to use as effective bio-control agent for integrated management study.

Evaluation on effectiveness of IDM against banded leaf and sheath blight disease was the major outline of this experiment. In single control measures, spraying of Carbendazim alone reduced the disease severity up to 70% and gave high kernel yield on the test variety as well as integrated treatment. This result agreed with that of Akhtar *et al.* (2010) who point out Carbendazim was found the most effective in controlling the disease as foliar application. Efficacy of Carbendazim reported by Kumar and Jha (1999) and Meena *et al.* (2003a) also support this finding. Application of fungicide is economically viable when susceptible varieties are grown and climatic conditions favour the disease severity (Asif 2013).

Another treatment, spraying of *Trichoderma* alone which was able to reduce significantly the disease up to 37% with lower disease score of 2.9 compared with untreated plants. However, it was less effective in controlling the disease compared with that of the other disease control measures. Saxena (2002) also pointed out that foliar sprays of *Trichoderma harzianum* alone could not significantly reduce the severity of BLShB but could exhibit some reduction in disease level (score 3). Moreover, AL-Kurtany *et al.* 2009 described that in severe plant diseases, *Trichoderma* sp. has a little or no effect against pathogens and in this situation, chemical fungicides must be applied. However, Mehta *et al*. (1993), Myint Myint San *et al*. (2008) and Asif (2013) reported that maximum percent disease control (20-65%) was attained by foliar spray of *T. harzianum* against BLShB of maize. This indicated that *Trichoderma* spp. which was applied by spraying of spore suspension was found to be effective in controlling soil borne disease that can also infect above ground portion of the plant.

Another single control method, stripping of the lower diseased leaves and sheaths before flowering showed the disease control of 39.3% over untreated control. This finding is supported by Kato and Inove (1995) who observed that in Japan,

resistance to this disease has been obtained after the fall of the lower sheath. Myint Myint San *et al.* (2008) also reported that stripping of the lower 2-3 disease leaves and sheaths at 14 days before tasselling was more pronounced in minimum disease severity and maximum grain yield of susceptible variety against BLShB. However, Asif (2013) described that leaf stripping was not found effective in reducing the disease. In the present study, stripping treatment showed the disease score of 2.8 as high as that by *Trichoderma* alone. It was done up to two times before flowering which might give more time to check the further spread of the disease to the upper leaves and the ear. However, under more favourable condition for disease development, stripping alone and *Trichoderma* alone might give some extent of disease severity regarding damage level. Saxena (2002) reported that cultural practice, removal of lower leaves alone could not significantly reduce the disease.

Combination of different control measures including Carbendazim, *Trichoderma* and stripping of diseased leaves and sheaths provided higher percent disease control ranged from 60.7% to 78.6% compared with single control practice except Carbendazim alone. Among the integrated control measures, the highest percent disease control (78.6%) was found in the plants treated by combination of Carbendazim and stripping. Zang (1994) suggested some cultural and chemical methods for integrated disease control of *R. solani.* However, in this treatment, Carbendazim was sprayed up to three times and so it might possess more total chemical usage leading to detrimental effect on environment.

Combination of Carbendazim and *Trichoderma* and also combination of *Trichoderma,* Carbendazim and stripping showed higher percent disease control of 67.9% and 69.7% respectively as superior as Carbendazim alone. Although the total chemical usage was reduced in both treatments, effectiveness of these integrated control measures was as superior as that of four sprays of Carbendazim alone. Therefore, both of these treatments were reduced in chemical usage regarding to less detrimental effect of the chemical on environment and so, it might be sustainable for IDM system. These findings are in agreement with Hjeljord and Tronsmo (1998) who mentioned that reduced amount of fungicide can stress and weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonist.

Locke *et al*. (1985) that combined use of biocontrol agents and chemical pesticides has attracted much attention in order to obtain synergestic or additive effects in the control of soilborne diseases. Similar trands were observed by many

workers. Srinivas and Ramakrishnan (2002) have reported that combination of biocontrol agents and commonly used fungicides showed positive association by reducing infection compared to fungicide and the fungal antagonists individually. Asif (2013) found that Carbendazim (0.1%) followed by *Trichoderma* spray significantly reduced the disease up to 40%. Dumitras (1984) also suggested that isolates of *T. viride* were found highly antagonistic to *R. solani* and the effectiveness can be increased (20%) by combining biocontrol with fungicides treatment.

Combination of *Trichoderma* and stripping method also gave higher percent disease control (60.7%) than each individual control measure reducing the disease severity of the test variety. Although stripping method alone and *Trichoderma* alone were less effective in controlling the disease severity, combination with each other was found to be more effective in controlling the disease. Khattabi *et al*. (2001), Khan and Shahzad (2007) and AL-Kurtany *et al*. (2009) suggested that antagonistic activity of biocontrol agents might be effective if it is integrated with other control practice and may result in acceptable levels of disease control. These findings are supported by Harman *et al.* (2004) who reported that biological and cultural control measures are two alternatives feasible options to synthetic pesticides in an integrated disease management programme.

Out of the eight treatments, significantly higher yield and 1000-kernel weight of the test variety were obtained in the plants treated by all disease control methods than that of untreated plants. However, kernel yield and 1000-kernel weight of test variety were not significantly different among the treatments. This might be due to the relatively lower disease severity (ranged from 1 to 2.9 in disease score) produced by the plants treated with different control measures. In this case, infection was not reached up to ear shoot on treated plants leading to produce healthy ear or kernel. In untreated plants, the disease severity was very high (4.7 in disease score) and so, the whole ears of these plants become distorted, shrink in shape and reduced in kernel number and light in weight. These findings are in accordance with that of Asif (2013) who reported that when the disease score become above 3.0, leaves showed premature drying, drooped down and grains were not formed and light in weight leading to reduction in yield.

When correlation was established, disease score was significantly negative correlated with kernel yield. It indicated that kernel yield of test variety were influenced by disease score and so, reduction in kernel yield was not avoidable on

high disease severity. A similar finding of negative correlation between disease severity and grain yield of maize was reported by Thakur *et al.* (1973), Lal *et al.* (1980), Liang *et al.* (1997) and Asif (2013). At disease score levels ranging from 3 to 5, a direct correlation with other yield parameters was exhibited in a yield loss of 5 to 97.4 % (Lal *et al.* 1980; Liang *et al.* 1997).

According to economic analysis, each control measure gave each of more net profit (kyats). In this study, a degree of variation in cost and benefit ratio was observed between all disease control measures, which ranged from 2.7 to 4.5 kyats. Net profit was dependent on the treatments taken by person and on the yield performance of the variety and cost of the treatment (Asif 2013).

In some treatments, especially in the disease control measures containing stripping method, net profit (kyats) was found to be higher than that of others. However, lower cost and benefit ratio was returned by the treatments including stripping method because of paying more cost than that for others. Asif (2013) also presented that stripping method was found to be more cost and lost by economic analysis. Among the treatments, the highest cost benefit ratio (C:B) was obtained in four sprays of *Trichoderma* alone which gave return of 4.5 per kyat investment followed by four sprays of Carbendazim alone and total four sprays of *Trichoderma* and Carbendazim alternately which gave each return of 4.0 per kyat investment. Similar trends were observed in the reports by Sinha (1992) who observed that Carbendazim was the most efficacious, gave the highest net profit and C:B ratio of 4.9 per rupee investment. Asif (2013) reported that return of 3.4 per rupee investment was found in spraying of Carbendazim alone and return of 2.6 per rupee investment was found in *T. harzianum* alone for management of banded leaf and sheath blight disease.

Based on these findings, all disease controls were found to be effective in reducing disease severity and increasing the yield of test variety and also profitable over untreated check. However, integrated disease control measures were found to be more effective in controlling the disease than single control measures. This reduced the total chemical usage leading to sustainable agriculture. Previous reports indicated that IDM includes use of chemicals along with other control measures to increase yield and reducing adverse effects on environment (Asif 2013). The present findings can help in improvement of antagonistic capability of indigenous *Trichoderma* spp. which can be used with reduced application of selected fungicides for the control of the disease. It can be stated that combination of bio-control agent with chemical or

cultural methods has significant effect on control of BLShB. It suggested that use of each combination in the present study might be ecologically safe and culturally more acceptable among the farmers.

Therefore, it can be concluded that alternate sprayings *Trichoderma* and Carbendazim could be applied as the most suitable method in terms of economic and environmental aspects to control banded leaf and sheath blight of maize. However, further investigation should be carried out for application and evaluation of integrated management practices against BLShB of maize on field level.

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APPENDICES

Appendix 1 Composition of water agar (WA) media

Agar	20g
Water	1 liter

Appendix 2 Composition of potato dextrose agar (PDA) media

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

Appendix 3 Composition of Martin's media

KH_2PO_4	1 g
$MgSO_4.7\ H_2O$	0.5 g
Peptone	5 g
Dextrose	10.0 g
Rosebengal	0.033 g
Agar	15 g
Streptomycin	100 mg
Water	1 L

Appendix 4 Composition of rice hull rice grain substrate (RHRG)

Rice hull	80 g
rice grain	130 g
tap water	150 ml