# ECO-FRIENDLY MANAGEMENT OF SHEATH BLIGHT OF RICE

**TANBIN HASAN SHUVO**

**DEPARTMENT OF PLANT PATHOLOGY**

**SHER-E-BANGLA AGRICULTURAL UNIVERSITY**

**SHER-E-BANGLA NAGAR, DHAKA-1207, BANGLADESH**

**JUNE, 2015**

# ECO-FRIENDLY MANAGEMENT OF SHEATH BLIGHT OF RICE

**BY**

**TANBIN HASAN SHUVO**

**REGISTRATION NO.: 09-03551**

A Thesis
*Submitted to the Faculty of Agriculture,*
*Sher-e-Bangla Agricultural University,*
*Dhaka, in partial fulfillment of the requirements for the degree of*

**MASTER OF SCIENCE (MS)**
**IN**

**PLANT PATHOLOGY**

**SEMESTER: JANUARY- JUNE, 2015**

**Approved by:**

| | |
|---|---|
| **Dr. Nazneen Sultana** | **Dr. Md. Rafiqul Islam** |
| **Professor** | **Professor** |
| **Supervisor** | **Co-Supervisor** |

**Assoc. Prof. Dr. Md. Belal Hossain**
Chairman
Examination Committee
Department of Plant Pathology
Sher-e-Bangla Agricultural University

**Dr. Nazneen Sultana**
**Professor**
**Department of Plant Pathology**
**Sher-e-Bangla Agricultural University**
**Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh**
**Mobile: +880-1733955171**
**E-mail: nazneensau@yahoo.com**

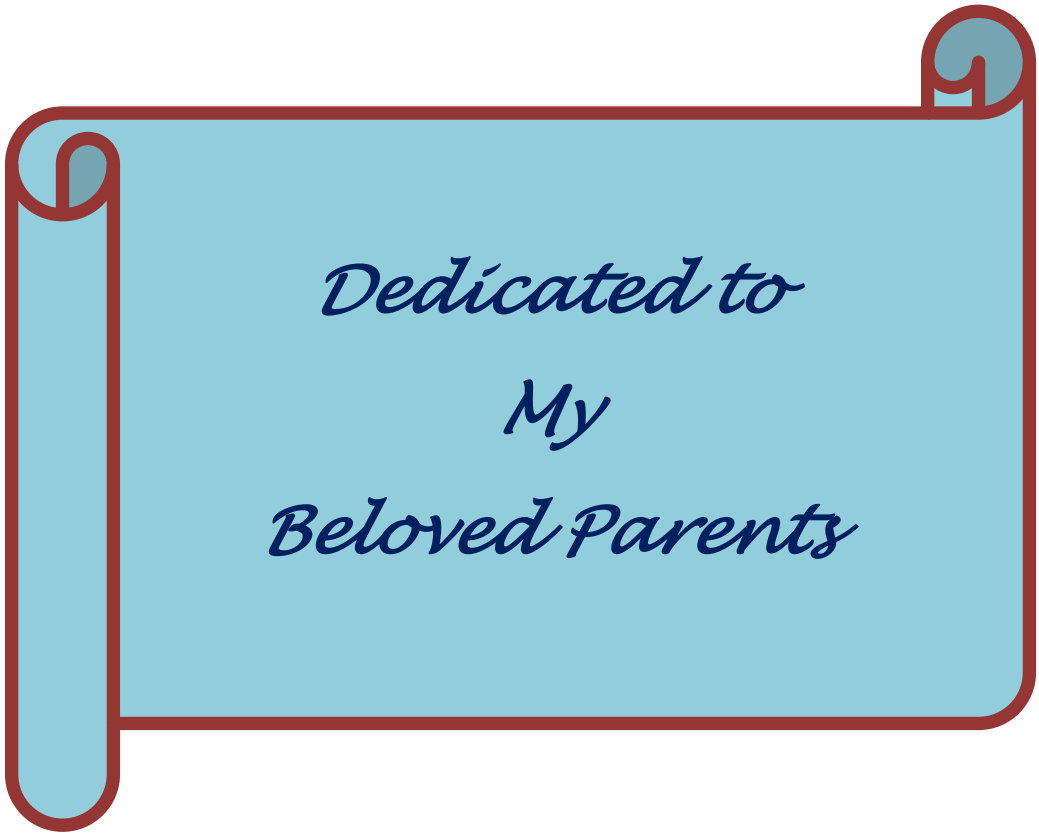
# CERTIFICATE

*This is to certify that thesis entitled, "ECO-FRIENDLY MANAGEMENT OF SHEATH BLIGHT OF RICE" submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (M.S.) IN PLANT PATHOLOGY, embodies the result of a piece of bona fide research work carried out by TANBIN HASAN SHUVO, Registration No.: 09-03551 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

*I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly acknowledged by him.*

*Dated: 26 May, 2016*
*Place: Dhaka, Bangladesh*

*(Dr. Nazneen Sultana)*
*Supervisor*
*Professor*
*Department of Plant Pathology*
*Sher-e-Bangla Agricultural University*


Dedicated to
My
Beloved Parents

## *ACKNOWLEDGEMENT*

*First of all, I would like to bow my heartfelt gratitude and praise to the Almighty ALLAH, the most beneficent and merciful who granted me to complete the dissertation work successfully.*

*I sincerely express my deepest sense of gratitude, respect, profound appreciation to my research supervisor Professor Dr. Nazneen Sultana, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for her kind and scholastic guidance, untiring effort, valuable suggestions, inspiration, co-operation and constructive criticisms through the entire period of the research work and the preparation of the manuscript of this thesis.*

*I would like to express my deepest respect and boundless gratitude to my co- supervisor Professor Dr. Md. Rafiqul Islam, Department of Plant Pathology and all the teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for their valuable suggestions, co-operation and constructive criticisms of the research work.*

*I would like to thank to my friends, Md. Mahbub Elahi, Nusrat Jahan Anonna, Tayeabur Rahman, Aslam Hossain Sheikh, Khalid Hasan for their helping hand during research work with thesis preparation. Thanks to my honorable brother Sayed Muhammad Mohsin, Md. Monir Hossain, Shefat-Al-Maruf and Abdullah Al Sayed of Sher-e-Bangla Agricultural University for their support and inspiration throughout my research period.*

*Finally, I would like to express my deepest sense of gratitude and feeling to my beloved father, mother, Elder brother Rezaul Karim, adorable sister China, dear Nephew Moniruzzaman and other relatives for their blessings, encouragements, sacrifices, affectionate feelings, dedicated efforts to reach this level.*

***June, 2015.*** ***The Author***

# ECO- FRIENDLY MANAGEMENT OF SHEATH BLIGHT OF RICE

## ABSTRACT

A study was undertaken to evaluate the potentiality of *Trichoderma harzianum* and *Bacillus subtilis* against sheath blight disease of rice in the laboratory of the Department of Plant Pathology, Sher-e- Bangla Agricultural University and pot experiment in the net house during October, 2014 to November, 2015. *Trichoderma harzianum* was isolated from the mushroom substrate and *B. subtilis* based formulation PRH was collected from local market. In *in vitro* test, *T. harzianum* and *B. subtilis* showed excellent performance and produced inhibition zone against *Rhizoctonia solani.* At 4 days after inoculation *T. harzianum* and *B. subtilis* produced 73% and 68% inhibition zone, respectively. In pot experiment nine treatments were used where seedling root treated and sprayed in the sheath at tillering stage with *T. harzianum* and *Bacillus subtilis* based formulation PRH, respectively. On the other hand *R. solani* treated with *Bacillus subtilis* based formulation PRH and sprayed at tillering stage on the sheath. *Rhizoctonia solani* applied in pot soil and inoculated rice plant at tillering stage. Spraying of *T. harzianum* at tillering stage gave the best performance regarding all parameters viz. decrease incidence and severity of sheath blight, lesion size, number of lesion, and increase grain yield compared to control. *Bacillus subtilis* also showed good performance. At 75 days after transplanting the lowest incidence (28.69%) and the lowest severity (7.11%) were recorded from soil inoculated with *R. solani* + tiller treated with PRH and seedling root treated with *T. harzianum* + tiller inoculated with *R. solani*, respectively. The highest grain yield of 396.64g/hills was obtained from the treatment where *T. harzianum* was applied at tillering stage and *R. solani* inoculated at sheath of the rice plant.

## LIST OF APPENDICES

# TABLES OF CONTENTS

## MATERIALS AND METHODS (Cont'd)

## LIST OF FIGURES

# LIST OF PLATES

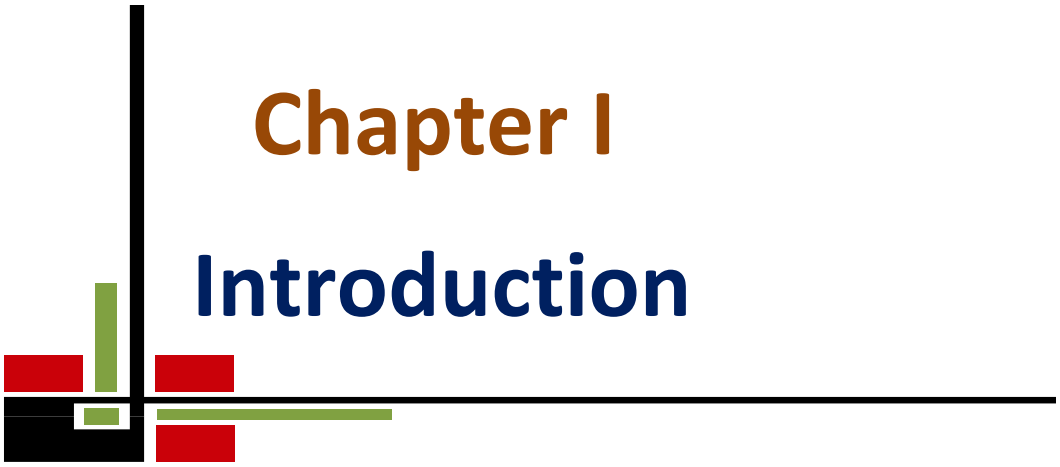
# LIST OF SYMBOLS AND ABBREVIATIONS

| ABBREVIATIONS | ACRONYMS |
|---|---|
| % | Percentage |
| *et al.* | And others |
| spp. | Species |
| J. | Journal |
| No. | Number |
| viz. | Namely |
| df. | Degrees of freedom |
| & | And |
| etc. | Etcetera |
| C | Degree Celsius |
| @ | At the rate of |
| cm | Centimeter |
| mm | millimeter |
| m | meter |
| cfu | Colony forming unit |
| ppm | Parts per million |
| LSD | Least Significant Difference |
| CV% | Percentages of Co-efficient of Variance |
| kg | kilogram |
| g | gram |
| ml | mililiter |
| wb | Wettable Powder |
| hr | Hour(s) |
| cv. | Cultivar (s) |
| i.e. | That is |
| T | Treatment |

## LIST OF SYMBOLS AND ABBREVIATIONS (Cont'd)

| ABBREVIATIONS | ACRONYMS |
|---|---|
| pv. | Pathovar |
| ft | Feet (s) |
| µl | Microliter |
| µm | Micrometer |
| *R. solani* | *Rhizoctonia solni* |
| *T. harzianum* | *Trichoderma harzianum* |
| *B. subtilis* | *Bacillus subtilis* |
| PRH | Plant Regulation Hormone |
| Rs | *Rhizoctonia solni* |
| Th | *Trichoderma harzianum* |
| Bs | *Bacillus subtilis* |
| SAU | Sher-e-Bangla Agricultural University |
| BAU | Bangladesh Agricultural University |
| BARI | Bangladesh Agricultural Research Institute |
| BBS | Bangladesh Bureau of Statistics |
| HSD | Honest Significant Difference |
| USA | United States of America |
| NA | Nutrient Agar (media) |
| PDA | Potato Dextrose Agar (media) |
| PSI | Per Square Inch |
| ANOVA | Analysis of variances |

# LIST OF TABLES



# Chapter I

# Introduction

# INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major cereal crops of the world. It is used as the staple food by the 60% of the world population. Rice is the most important cereal crop in Asia producing about 96% of the world rice production (IRRI, 2006). It is also the staple food of the people of Bangladesh. About 79.77% of cropped area of the country is used for rice production (BBS, 2013). Bangladesh is ranked as fourth in rice production with annual production of 47.72 million metric tons in the world (BBS, 2014). Rice provides 75% calories and 55% protein in the average daily diet of the people and shares 95% of cereal consumptions (Bhuiyan *et al.,* 2002).

There are many factors responsible for the low yield of rice in Bangladesh. Among them, vulnerability of the crop to pests and diseases is important. Rice diseases are caused by different groups of pathogenic microorganisms. Thirty six fungal, twenty one viral, six bacterial and six nematode diseases attack rice plants (Ou, 1985a). In Bangladesh, 31 rice diseases have been so far identified. Of which ten are considered as major (Shahjahan and Miah, 1987). The sheath blight disease of rice (*Rhizoctonia solani*) is prevalent in almost all rice growing countries of the world. It is one of the most destructive diseases of rice productivity, especially where rice production is intensive (Bowman *et al.,* 1992). Both local and high yielding varieties of rice are susceptible to sheath blight disease (Naidu, 1992). Sheath blight is also a limiting factor for rice cultivation in Bangladesh. High temperature and relative humidity during crop growing are favourable to sheath blight disease development. Short structure, high tillering and high nitrogen responsive varieties are comparatively more susceptible than those of the traditional ones of tall plants type with low tillering ability (Miah *et al*., 1985). Sheath blight causes substantial loss to rice both in quality and quantity of grains in the present ecosystem in Bangladesh. It attacks the rice plant at tillering stage by sclerotium, the primary source of inocula that contains over winters in soil and plant debris (Ou, 1985b). It

affects filling of the grains and emergence of panicles. It may causes about 25-32% yield losses in rice in Bangladesh (Shahjahan *et al*., 1986).

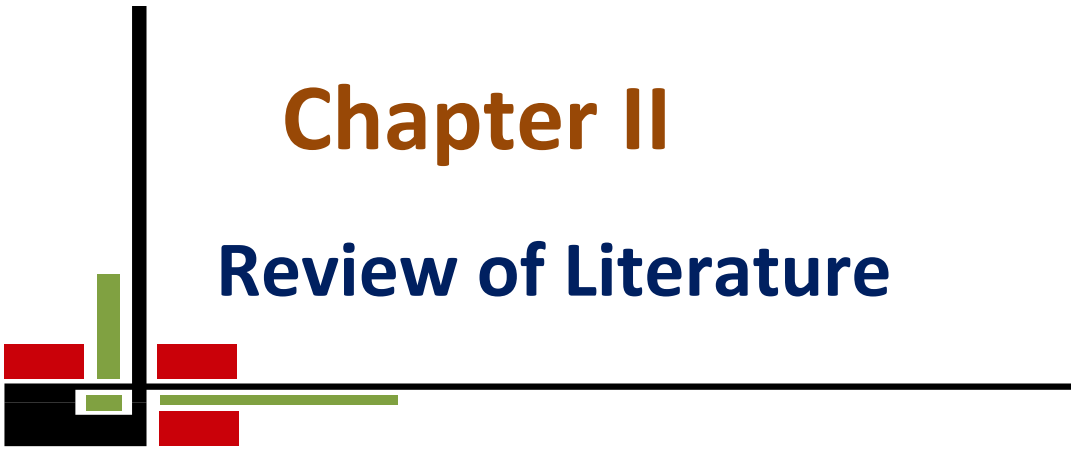
Among the cultivated rice varieties the level of resistance to sheath blight is low. Emphasis on development of strategies using cultural, chemical and /or biological means of control should be given. The conventional method for the fungal disease control relies mainly on synthetic fungicides. The chemical control using synthetic fungicides is less acceptable due to their increase incidence of development of resistance upon prolonged usage (El-Ghauouth, 1997; McGrath, 2001; Fernandez *et al*., 2006), lack of specificity towards the target pathogen, adverse effect on environment, beneficial microbes, humans and animals caused by their accumulation in environment (Leuroux, 2003). Thus, there is a need for alternative disease management strategies that provide effective control without their side effects on environment. Use of beneficial bacteria and fungi as biological control agents to suppress plant disease offers a potential eco-friendly alternative to the extensively used chemical fungicides (Kiss, 2003; Nagorska *et al*., 2007). The increasing awareness of fungicide related hazards has emphasized the need for adopting biological methods as an alternative disease control method, which is also eco-friendly (Khare *et al*., 2010). Biological control appears to be the best solution for long term sustainability and effective management of soil borne disease which can considerably minimize the disease (Howell, 2003). Successful management of *R. solani* on various crops by bio-agents was previously reported (Meena *et al*., 2003; Atef, 2008; Hajieghrari *et al*., 2008). On the other hand, cultural or biological means of control involved less cost. Again through the use of biological entities the population of particular pathogens can be kept below economic damage level. It will certainly be popular to the farmers as it has no health hazard and no chance of environmental population.

Under the above circumstances, alternative control measures are to be developed and biological control may be an efficient disease management strategy. Biological control is the best alternate to chemical fungicides, which aimed at maximum productivity with least negative environmental and

ecological consequences (Nagarajkumar *et al*., 2004). Several attempts were made in India and other countries to control sheath blight of rice using bio-control agents (Mew and Rosales, 1985; Krishnamurthy, 1997; Krishnamurthy and Gnanamanickam, 1998; Faltin *et al*., 2004; Singh and Sinha, 2006).

In biological control, living micro organisms such as bacteria or fungi are employed as antagonist, parasites or predators (Kwok *et al*., 1987). However, controlling of sheath blight of rice caused by *R. solani* through antagonistic *Trichoderma harzianum* and *Bacillus subtilis*, systematic research on isolation identification and multiplication are needed to explore the potential control agents against the target pathogens. *Trichoderma* is a fungus belongs to the order Moniliales of family Moniliaceae under the class Hypomycetes. *Bacillus* is gram positive bacteria belong to the order Bacillales of family Bacillaceae. They have an antagonistic character against many soil borne fungi such as *R. solani.* They are highly competitive for plant residues and thus exhaust the nutrient supply for pathogens (IRRI, 1987). *T. harzianum* can reduce the pathogen of sheath blight disease of rice (Rahman, 2007). *T. harzianum* and *T. virens* have successfully suppressed *R. solani* in several pathosystems (Boland, 1990). *T. viride, B. subtilis* and *B. cereuss*in consortium are effective for controlling *R. Solani* in field condition (Somani and Arora, 2010). *Bacillus subtilis* suppressed 23 type plant pathogens growth under laboratory condition (Stein, 2005; Nagorska *et al*., 2007).

Considering the above facts the present study was undertaken to find out a biocontrol agent, *T. harzianum* to control sheath blight of rice caused by *Rhizoctonia solani.*



# Chapter II

## Review of Literature

# REVIEW OF LITERATURE

## 2.1 Effect of antagonistic *T. harzianum* against *R. solani*

An experiment was conducted on efficacy of bio control agents, *T. viride* and *P. fluorescens* against sheath blight disease of rice (Pal *et al.*, 2015). The bio-control agents were applied as seed treatment and in combination of seed treatment and foliar spray and were also compared with standard chemicals like validamycin @ 2.5 ml/l and propiconazole @ 1ml/L. The result revealed that, seed treatment + 3 sprays with *T. viride* @ 1% was the most effective bio-control treatment recording 10.93% pooled percent disease index (PDI) against 34.41% in control plot and its performance was at par with the standard fungicide propiconazole @ 1%. The treatment also exhibited maximum increase in all the yield attributing factors recorded and gave a yield increase of 41.1% over control. The 1000 grain weight was also found highest (22.2 g) among all the treatments.

Razu (2014) studied the comparative efficacy of BAU-Biofungicide (a product of *T. harzianum,* at 2%), Garlic (*Allium sativum*) clove extract (5%), Allamanda (*Allamanda cathartica*) leaf extract (5%), Bion (25ppm), Amistar (0.1%) and Tilt 250EC (0.1%) on rice cv. BRRI dhan49 under field and laboratory conditions from July, 2013 to March, 2014. He found that the lowest sheath blight incidence was occured in BAU- Biofungicide treated plots.

The biocontrol abilities of water-soluble and volatile metabolites of three different isolates of *Trichoderma* (*T. asperellum, T. harzianum* and *Trichoderma* spp.) against soil borne plant pathogen *R. solani* was investigated both *in vitro* and *in vivo* by Asad *et al* (2014). They observed that mycelial growth inhibition of the pathogen was 74.4-67.8% with water-soluble metabolites as compared to 15.3-10.6% with volatile metabolites *in vitro*. *In vivo* antagonistic activity of *Trichoderma* isolates against *R. solani* was

evaluated on bean plants under laboratory and greenhouse conditions. They concluted that three isolates of *Trichoderma* could be used as effective biocontrol agents against *R. solani.*

Seema and Devaki (2012) used four fungal and one bacterial bioagents viz, *T. viride, T. harzianum, Aspergillus niger, Penicillium* sp. and *B. subtilis* were evaluated *in vitro* against *R. solani*. In the dual culture assay, the percentage inhibition of growth by *T. viride, T. harzianum, A. niger, B. subtilis* and *Penicillium* sp. on *R. solani* were 70, 67, 57, 50 and 44%, respectively. All the antagonists suppressed the formation of sclerotia. The volatile metabolite revealed that *T. viride* and *T. harzianum* showed 50% and 40% inhibition in mycelial growth respectively. They reported that *T. viride* and *T. harzianum* have excellent potential antagonists capable of controlling the pathogenicity of *R. solani*.

The effectiveness of the *Trichoderma* isolates as a biological control agent against *R. solani* was studied by Ali and Nadarajah (2012). *In vitro* tests of antagonistic activity and ability of *Trichoderma* isolates against *R. solani* via the dual culture technique showed that isolates 6, 7, 8, 20, 13, 22 and 17 had high antagonistic activities with reduced radial growth of *R. solani*. The suppression results of *Trichoderma* isolates 2, 7 and 9 on *R. solani* growth showed disease incidence of approximately 33.33% while disease severity results for the same isolates were 20, 15.67 and 20%, respectively. The combination treatment between *Trichoderma* isolates and *B. subtilis* showed that all *Trichordema* isolates are able to reduce pre and post emergence disease of seedlings. *Trichoderma* isolates 2, 7, 8, 9, 11 and 21 in combination with *B. subtilis* had excellent suppression of pre (8.67, 8.33, 13, 8.67, 8.67 and 8.67%) and post (9, 8.67, 9.33, 14, 9 and 14%) emergence disease in *R. solani* inoculated soil. This shows an overall reduction of disease incidence by 22-33% and severity of the disease by 15.33-22% when the *Trichoderma* isolates were used in combination with *B. subtilis* to suppress *R. solani* infestations.

Naeimi *et al.* (2011) used different *Trichoderma* strains in paddy field to control rice sheath blight. Effects of *Trichoderna* strains on disease incidence (percentage of infected tillers) and severity (RLH), yield, 1000 grains weight, plant height and number of tillers were studied and compared with chemical fungicide (propiconazole) and controls (inoculated and non-inoculated). The lowest disease severity and incidence 15 and 35 days after inoculation were recorded for *T. harzianum* AS12-2 and propiconazole respectively. They showed that the overall *T. harzianum* AS12-2, among three other indigenous *Trichoderma* strains was the most promising biocontrol agent for control of sheath blight under field condition and comparable to the common chemical fungicide.

Prasad and Kumar (2011) used three isolates of *Trichoderma* spp. against *R. solani*. They reported that the three potential *Trichoderma* spp. TN3 was found highly effective against *R. solani* under *in vitro* conditions. It was found most effective in reducing disease incidence and increasing grain yield.

Naeimi *et al.* (2010) conducted an experiment on biological control of *R. solani* AG1-1A, the causal agent of rice sheath blight with *Trichoderma* strains and showed that several strains belonging to *T. harzianum*, *T. virens* and *T. atroviride* showed excellent biocontrol activity. These potential antagonist strains were further evaluated for their effectiveness in controlling sheath blight under glasshouse conditions. Among the 55 selected strains, 7 significantly controlled the disease. *T. harzianum* AS12-2 was the most effective strain in controlling rice sheath blight, better even than propiconazole, the most commonly used fungicide in Iran.

An experiment was conducted by Bhat *et al.* (2009) ot evaluate of the bio-control agents against *R. solani* and sheath blight disease of rice under temperate ecology and observed the highest growth inhibition of *R. solani* was recorded by *T. harzianum* (65.87%) followed by *T. viride*, *G. virens* and *Trichothecium* spp., while *G. roseum* exhibited least effective against radial

growth of pathogen. Soil application of these bio-agents revealed that disease severity was highest when crop was treated with *Trichothecium* spp., followed by *G. roseum, G. virens, T. viride* and lowest (0.71%) by *T. harzianum*.

Rahman (2007) evaluated the effectiveness of *Trichoderma* spp. for controlling sheath blight of rice. *T. harzianum* showed linear overgrowth against *R. solani* at 10, 30 and 70 DAI. *Trichoderma harzianum* significantly reduced the pathogen of sheath blight disease of rice.

Hyperparasitic activity of *T. harzianum* against *R. solani* was demostrated by Reyes *et al.* (2007). They observed hyperparasitic and competitive activity of strains A-34 and A-53 of *T. harzianum,* which exhibited good potentialities for the control of these pathogens. Highly significant differences were found in statistical tests with a linear rate growing of 7.37 cm in A-34 strain and 7.10 cm in A-53 strain up to 96 h. Growth rate was 3.63 cm for *C. grisea* and 2.62 cm for *R. solani.*

The effectivity of *T. harzianum* against sheath blight (*R. solani*) of rice was tested by Khan and Sinha (2007). They found that *T. harzianum* application reduced the sheath blight severity and incidence, and increased rice grain yield.

Mathivanan *et al.* (2006) studied the effects of talc formulations of *Pseudomonas fluorescens* and *T. viride* on sheath blight disease and grain yield in rice. They found increased root and shoot lengths, dry weight and plant height following treatments of plants with *P. fluorescens* and *T. viride* either alone or in combination. Application of *P. fluorescens* and *T. viride* resulted in a significant reduction of sheath blight (*R. solani*) incidence comparable to the treatment with a systemic fungicide, Carbendazim.

Nakkeeran *et al.* (2006) conducted the various research activities on *Trichoderma* spp. in India. They identified the superior strains of *Trichoderma viride* suitable for the management of soil-borne diseases of cereals, pulses, oilseeds vegetable and other horticultural crops. They observed that the

chitinase gene of *T. viride* strain $MNT_7$ showed best performance against rice sheath blight .

Bhagawati (2005) found that *T. harzianum* and *T. viride* when added to soil in the presence of *R. solani* could suppress infection of sheath blight in rice. They observed that *T. harzianum* and *T. viride* showed the highest antagonistic activity against *R. solani* at pH 5.1-6.0 with less infection of sheath blight, highest plant growth and yield. In acidic pH, *Trichoderma* population was highest, while that of *R. solani* was lowest.

Khan and Sinha (2005a) used *Trichoderma* spp. and some commercial formulations of biocontrol agents against *R. solani* causing sheath blight of rice. Maximum reduction in disease severity (70.57%) and incidence (38.25%) were observed with foliar sprays of contaf (hexaconazole). Among bioagents foliar sprays with *T. harzianum* (a rice leaf isolate) was found most effective in reducing sheath blight (44.35-52.37%) and increasing grain yield (20.25-23.13%) and 1000 grain weight (6.36-7.35%).

Khan and Sinha (2005b) also tested different rate and time of application of *Trichoderma* spp. to control sheath blight. They observed that *T. harzianum* (rice leaf isolate) was more effective against sheath blight as compared to other isolates of *Trichoderma* spp. They also found that when application of *T. harzianum*, seven days before inoculation of *R. solani* resulted in maximum reduction (78.98%) of the disease and higher rates of *T. harzianum* (4 or 8 g/kg. of soil) were highly effective in reducing disease severity (32%) and incidence (81.26%).

Tewari and Singh (2005) applied *T. harzianum* in different methods against *R. solani* under glasshouse and field conditions in India. They observed that disease severity and incidence were significantly reduced by application of *T. harzianum* in all cases except for soil treatment under glasshouse conditions. For field application, foliar spray was found superior showing significantly

reduced disease severity (40.82%) compared to other treatments. Soil treatment was found least effective for control of sheath blight of rice under both glasshouse and field conditions.

Kazempour *et al.* (2003) used *T. harzianum*, *Gliocladium virens* and some fungicides (Benomyl, Carbendazim, Carboxin-Thiram, Edifenphos and Zineb) to control sheath blight of rice (*R. solani)* under field condition. They found that the *T. harzianum* reduce sheath blight disease by 19.8%.

Tang *et al.* (2001) conducted an experiment on the efficiency of *Trichoderma* spp. against the *R. solani* in China. They found that six strains greatly inhibited the growth of *R. solani* over 800 fungal strains or *Trichoderma* spp. in dual culture.

Das and Hazarika (2000) used *T. viride* and *T. harzianum* to managment sheath blight disease of rice. They observed *T. viride* and *T. harzianum* significantly decrease the infection of sheath blight diseases. Both antagonists exhibited higher efficacy in reducing sheath infection and increased grain yield when they were treated with either 2% (w/v) methyl cellulose or 2% (w/v) methyl cellulose and 0.1 M $MgSO_4$. They reported that the *T. harzianum* was more effective than *T. viride* in reducing sheath blight infection and increase in yield.

Wang *et al.* (2000) tested the efficacy of the *T. harzianum* isolates TC3 and NF9 against sheath blight of rice (*R. solani*). They observed both isolates were active against sheath blight (ShB) disease. The maximum control efficacy of isolates TC3 and NF9 to ShB were 77.56% and 83.09%, respectively.

Rodriguez *et al.* (1999) applied different cultural practices and *T. harzianum* to control sheath blight disease of rice. They observed that the disease incidence of sheath blight was significantly reduced (about 50%) with *T. harzianum* compare to cultural practices.

Sudhakar *et al.* (1998) reported that antagonistic fungi *T. viride, T. koningii* and *G. virens* were evaluated against *R. solani*, the sheath blight pathogen of rice. In dual culture technique, *T. viride* was superior in inhibiting the growth of the pathogen followed by *T. harzianum*, *G. virens* and *T. harzianum*. Lysis of the pathogen with biological control agents was observed. Many biological control agents were superior in controlling sheath blight disease when sprayed 24 hrs after inoculation of the plant with the pathogen than sprayed 24 hrs before inoculation. Maximum disease reduction was observed in *T. koningii* followed by *T. viride*, *T. viride* and *T. viride*, when sprayed 24 hrs after inoculation. In both the treatments, *T. koningii* showed superiority over other biological control agents, while the performance of *G. virens* was very poor irrespective of the treatments although the performance was better in inhibiting the pathogen in dual culture experiments.

### 2.2. Effect of antagonistic *B. subtilis* against *R. solani*

Shrestha *et al.* (2016) isolated 29 rice-associated bacteria (RABs) and screened based on their antagonistic activities against both *R. solani* and *B. glumae.* Among them 26 RABs showed antagonistic activity against *R. solani* where 7 out of 26 antagonistic RABs were closes to *B. methylotrophicus* and *B. subtilis.* These RABs were observed to inhibit the sclerotial germination of *R. solani* on potato dextrose agar and the lesion development on detached rice leaves by artificial inoculation of *R. solani.* These antagonistic RABs also significantly suppressed the disease development of sheath blight and bacterial panicle blight in a field condition.

Peng *et al.* (2014) tested *B. subtilis* NJ-18 against *R. solani* under laboratory, greenhouse and field tests were conducted to determine the effect of combining the biological control agent *B. subtilis* NJ-18 with the fungicide jinggangmycin for control of rice sheath blight. They observed growth of NJ-18 *in vitro* was not affected by jinggangmycin. In a greenhouse experiment, disease control was greater with a mixture of NJ-18 and jinggangmycin than with either alone; a mixture of NJ-18 at $10^8$ cfu/ml and jinggangmycin at 50 or 100 mg/ L reduced

lesion length by 35% and 20%, respectively, and the combinations showed a synergistic action. In three field trials, disease control was significantly greater with a mixture of NJ-18 at $10^8$ cfu/ml and jinggangmycin at 75 or 150 g a.i. $ha^{-1}$ than with either component alone.

Kadir *et al.* (2013) studied on bio-formulation of antagonistic bacterial consortium for controlling blast, sheath blight and bacterial blight diseases on rice. The bacterial isolates used in single or in mixture combination were apparent to reduce sheath blight, neck blast and bacterial leaf blight under *in vitro* test. They found that Talc-A5 (*Bacillus* /firmusE65, *Pseudomonas aeruginosa* C32b) formulation was effective against sheath blight and bacterial blight but showed lower effect on neck blast disease in the field.

Four strains of biological control agents (BCA) B-916 (*B. subtilis*), P7-14 (*P. fluorescens*), P9409 (*P. resinovorans*) and P10353 (*P. malculicola*) were tested *R. solani* by growing them on peptone potassium nitrate medium (PPM) ranged from 0.5 to 1000 ug/mL of four antibiotitics (ampicilin, hygromycin, kanamycin and rifampicin) (Maji and Shaibu, 2012). They observed that *R. solani* was effectively controlled by the antibiotics, BCAs and mutants depending on the compatibility of the BCA and antibiotics.

Kumar *et al*. (2012) evaluated the efficacy of integral, the commercial liquid formulation of *B. subtilis* strain MBI 600, against rice sheath blight and for plant growth promotion. In greenhouse studies, four log concentrations of integral (from $2.2\times10^6$ to $2.2\times10^9$ cfu/ml) were used as seed treatment (ST). After 25 days, seedlings were dipped (SD) into integral prior to transplanting. At 30 days after transplanting (DAT), leaf sheaths were inoculated with immature sclerotia of the pathogen. At 45 DAT, a foliar spray (FS) with integral was applied to some treatments. Sheath blight (ShB) severity was rated at 52 DAT, and seedling height and number of tillers per plant were rated at 60 DAT. The integral treatments of ST + SD + FS at $2.2\times10^9$ cfu/mL significantly suppressed ShB over other treatments. In field studies, integral provided

significant increase of seedling height in nursery, and number of tillers per plant, compared with the control. ShB severity was significantly suppressed with higher concentrations of integral compared to lower concentrations. Grain yield were the highest at an integral concentration of $2.2\times10^9$ cfu/ml. Overall, integral significantly reduced ShB severity, enhanced seedling growth, number of tillers per plant and grain yield as ST + SD + FS at the concentration of $2.2\times10^9$ cfu/ml under the conditions evaluated.

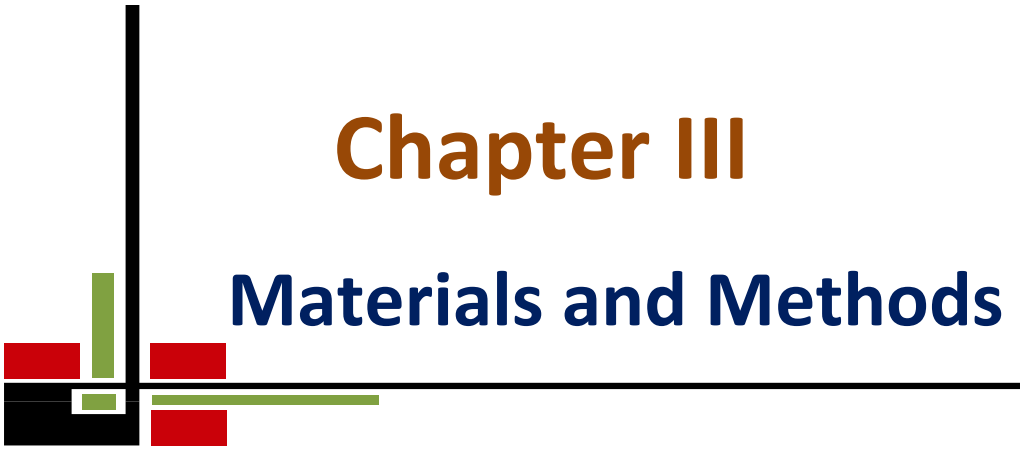
Fifty bacterial isolates were tested against sheath blight pathogen in PDA medium (Bashar *et al.*, 2010). Among the isolates of antagonistic bacteria 11 produced more than 15 mm inhibition zone and remarkable inhibition zone producing 10 isolates which selected to observe their antagonistic behavior by soaking the sclerotia of *R. solani* and rice seedlings in different hours into bacterial suspension of $3.84 \times 10^7$ cfu/ml.

An experiment were studied by Yang *et al.* (2009) on the activity and efficacy of *B. subtilis* strain NJ-18 against rice sheath blight. They observed that NJ-18 inhibited the *in vitro* radial extension of hyphae of the phytopathogenic fungi *R. solani* by producing antifungal metabolites that diffused through the agar and caused abnormal swelling of hyphae. In field experiments for controlling of sheath blight of rice, fermentation of NJ-18 at 5.0107 cfu/ml significantly reduced disease incidence and severity.

*In-vitro* efficacy of various rhizobacterial isolates were tested against *R. solani* (Kumar *et al.*, 2009). For biological control of the disease using plant growth promoting rhizobacteria (PGPR) is a potential alternative to the presently available chemical control methods. The present study focuses on screening of 70 rhizobacterial isolates of Bacillus for antagonistic activity against mycelial growth, sclerotial germination and sheath blight lesion development on leaf blades under *in vitro* conditions. Dual culture studies revealed that the mycelial growth of *R. solani* was inhibited up to 83% by these PGPR and 10 strains were found to exhibit antagonism of over 70%.

Potential bacterial and fungal antagonists suppress *R. solani* on agar medium. The selected bacterial (*P. fluorescens* and one isolate of *B. subtilis*) and fungal (*T. koningii*) antagonists were tested *in vivo* in a greenhouse experiment for suppression of sheath blight disease development (Kanjanamaneesathian, 1994). Seed treatment, rice root dipping, and plant spraying were used for both single and combined antagonist applications. Better suppression of sheath blight disease development was obtained from seed treatment and plant spraying. The two bacterial antagonist applications gave better sheath blight suppression than the fungal antagonist.

Gnanamanickam and Mew (1990) used more than 400 strains of bacteria isolated from IRRI rice fields were screened in the laboratory for antagonism towards *P. oryzae* and *R. solani*. They found 5 strains of *Bacillus* spp. which created an inhibition zones ranged from 20–31 mm against *R. solani.* These bacterial strains also suppressed sheath blight in detached rice leaves. They further evaluated in field tests for suppression of blast and sheath blight when applied as seed treatments or as seed treatments plus sprays. In three field experiments conducted in the IRRI farm, bacterial treatments afforded significant sheath blight control and performed better than validamycin, the fungicide routinely used for sheath blight control.



# Chapter III

## Materials and Methods

# Materials and Methods

### 3.1. Experimental site

The experiment was conducted in the MS laboratory of Plant Pathology Department, Faculty of Agriculture, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka, Bangladesh (Appendix I).

### 3.2. Experimental period

The experiments were conducted during the period on October, 2014 to November, 2015.

### 3.3. Collection of disease sample

Sheath blight infected rice plants were collected from experimental field of Agronomy, Sher-e-Bangla Agricultural University (SAU) on 10 November, 2014 (Plate 1).

#### 3.3.1. Preparation of potato dextrose agar (PDA)

PDA was prepared as described by Islam (2009). Exactly 200g peeled and sliced potato was boiled in 500ml water in a bowl for about half an hour. The extract of the potato was filtered through a cheese cloth. The other two ingredients viz. 20g dextrose and 10g agar were added in the extract and the volume was made up to1L mark. The prepared PDA was poured in 1000ml conical flask and sterilized. In autoclave 121˚C with 1.1kg/cm$^2$ pressure for 15 minutes (Appendix IV).

#### 3.3.2. Isolation and purification of *R. solani*

*Rhizoctonia solani* was isolated from sheath blight infected rice plants. Infected sheath was washed in running tap water until all soil particles and other waste substances were removed. After washing infected rice sheath was cut into small pieces (approximately 5 mm long) with a pair of sterilized scissors, kept in a sterilized polyethylene bag and brought to the laboratory for microscopic study and isolation work. The fungus was isolated from infected parts of the rice

plants following tissue planting method (Bashar *et al*., 2010). The cut pieces were surface sterilized with 0.1% $HgCl_2$ for 5 minutes and rinsed in sterilized water for three times. Excess water was removed from the cut pieces and sterilized pieces were plated on PDA medium and incubated at room temperature (26-35°C). After 3 days, hyphal tips of isolates were transferred to petri plates containing sterilized PDA at 15 ml/plate. The plates were incubated in the laboratory at room temperature. The isolates were purified following hyphal tip technique (Tuite, 1969) and stored in PDA slants in test tubes at 4°C for subsequent pathogenicity and antagonism tests.

### 3.4. Isolation and purification of *T. harzianum* from mushroom substrate

*T. harzianum* was isolated from mushroom substrate (Johnson and Curl, 1972). Mushroom substrate was placed on PDA media in sterilized petridish. After 5-7 days of incubation at room temperature (26-35°C) fungi was transferred to PDA slants, maintained at room temperature (26- 35°C), and subsequently it was used for antagonism testing against *R. solani.*

### 3.5. Used *Bacillus subtilis* formulation

Plant Regulation Hormone (PRH) formulation was used in the experiment. PRH is a liquid formulation containing live *B. subtilis* which used as a bio-control agent against *R. solani*. The product contained a minimum of $2.2\times10^6$ to $2.2\times10^9$ spores/ml and was packed in 200 ml bottles. It was collected from Natural Bio Agro Tech Co. (PVT) Ltd.

### 3.6. *In vitro* screening of *T. harzianum* and PRH formulation against *R. solani*

#### 3.6.1. *In vitro* screening of *T. harzianum* against *R. solani*

An *in vitro* test was conducted to find out the comparative antagonistic potential of *T. harzianum* against *R. solani* by dual culture technique (Dhingra and Sinclair, 1985). Discs of *R. solani* mycelium were cut (5 mm diameter) from the edge of an actively growing fungal colony with a cork borer. Test plates were prepared by pouring 20ml of PDA per plate. After solidification, one mycelial disc of *T. harzianum* and one disc of *R. solani* were placed simultaneously on the edge of the each PDA Petri plate at opposite direction. Three replicate plates were used. Control trial was done without *T. harzinum*n in the plate. The plates were incubated in the laboratory having ambient temperature of 25±5$^0$C. There after inhibition percentages of the *R. solani* were calculated based on the growth of the pathogen on fresh PDA plates following the formula as suggested by Sundakar *et al.* (1998).

Percentage inhibition of growth = $\frac{X-Y}{X} \times 100$

X= Mycelial growth of *Rhizoctonia solani* in absence of *Trichoderma harzianum*

Y= Mycelial growth of *Rhizoctonia solani* in presence of *Trichoderma harzianum*

#### 3.6.2. *In vitro* evaluation of PRH formulation against *R. solani*

Mycelial discs of *R. solani* mycelium were cut (5 mm diameter) from the edge of an actively growing fungal colony with a cork borer 5mm diameter. Test plates were prepared by pouring 20ml of PDA per plate. After solidification, one mycelial disc of *R. solani* was placed simultaneously on the edge of the each PDA Petri plate and at the opposite direction making a hole with a cork borer and filled it with PRH formulation. Three replicated plates were used. Control trial was done without PRH formulation in the plate. The plates were

incubated in the laboratory having ambient temperature of $25\pm5^0$ C. There after inhibition percentages of the *R. solani* were calculated based on the growth of the pathogen on PDA plates following the formula as suggested by Sundakar *et al.* (1998).

Percentage inhibition of growth = $\frac{X-Y}{X} \times 100$

X = Mycelial growth of *Rhizoctonia solani* in absence of PRH formulation**.**

Y = Mycelial growth of *Rhizoctonia solani* in presence of PRH formulation**.**

### 3.7. Treatments of the experiment

The experiment was conducted to determine the eco-friendly management of sheath blight of rice with antagonistic fungus and bacteria. Nine treatments were used. There are given below:

$T_1$ = Tiller inoculated with Rs *(control)

$T_2$ = Soil inoculated with Rs + Seedling root treated with PRH**

$T_3$ = Soil inoculated with Rs + Seedling root treated with Th***

$T_4$ = Soil inoculated with Rs + Tiller treated with PRH

$T_5$ = Soil inoculated with Rs + Tiller treated with Th

$T_6$ = Seedling root treated with PRH + Tiller inoculated with Rs

$T_7$ = Seedling root treated with Th + Tiller inoculated with Rs

$T_8$ = Tiller inoculated with Rs + Tiller treated with PRH

$T_9$ = Tiller inoculated with Rs + Tiller treated with Th

* Rs = *Rhizoctonia solani*

** PRH = Plant Revaluation Hormone (*Bacillus subtilis* based formulation)

***Th = *Trichoderma harzinum*

### 3.8. Design and layout

The experiment was laid out in completely randomized design with five replications. There were nine treatments combinations. The total numbers of unit pots were 45. Each treatment contains 5 pots with their individual plants.

### 3.9. Variety used

BRRI dhan56 variety was used in the experiment which is mostly cultivated in Aman season in Bangladesh. Seeds were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur.

### 3.10. Raising seedling

Seeds were soaked in a plastic pot with tap water for 24 hrs. Before sowing in seed bed, seeds were taken out from water followed by put in a gunny bag and kept at room temperature for 72 hours for sprouting. Seed bed was prepared on plastic tray (50×35 cm) containing autoclaved soil which was collected from the experimental field of Agronomy, Sher-e-Bangla Agricultural University. Before using the soil it was sterilized in the autoclaved (121˚C with 1.1kg/cm$^2$ pressure for 15 min.). As the soil was rich in organic matters, therefore no manuring was done. Sprouted seeds were sown in wet seed bed on 30 May, 2015 (Plate 2). Weeds were removed and irrigation was given in the seed bed as and when necessary. Twenty-day after sowing healthy, uniform seedling were selected for the pot experiment.


**Plate 1: Collected sheath blight disease samples with characteristics symptoms**

**A) Symptoms with panicle**
**B) Washed sample**


**Plate 2: Wet seed bed in the plastic tray**

**A. Sprouting seeds in the seed bed**
**B. Growing seedlings in seedbed**

### 3. 11. Soil sterilization

Soil was collected from the same field from where the tray soil was collected. The soil carried out to the experiment at field in the Plant Pathology Department, SAU and dried it. Decomposed cowdung from the dairy farm of SAU was added to it. The soil was mixed uniformly with cow dung (2:1). The dried soil was sterilized with formalin (40%) at the rate of 5 ml formalin diluted with 20 ml of water for 4 kg soil (Hossain, 2006). The formalin treated soil was covered with polythene sheet for 48 hrs and then exposed of 48 hrs for aeration before setting the experiment.

### 3.12. Application of manure and fertilizer

The entire quantity of cowdung was applied to the soil after being sterilized. Urea, triple super phosphate (TSP), murate of potash (MoP), zinc sulphate and gypsum were given at the rate of 375 g, 200 g, 200 g, 175 g and 100 g, respectively (100:60:40 kg/ha). TSP, Zinc sulphate, gypsum were given as basal during final pot preparation. Split application of urea and MP were done at 20, 40 and 60 days after transplanting.

### 3.13. Preparation of pots

The earthen pots were bought from the market which height was 14 inches and diameter 0.16$m^2$ per pot. Then pots were filled with sterilized soil @ 10kg/ pot.

### 3.14. Inocula production of *R. solani* and application in the pot soil

Inocula of *R. solani* were prepared on sterilized chickpea grains in 500 ml Erlenmeyer flask. Chickpea grains (l00 g) were soaked in the water were autoclaved at 121°C with 1.1kg/$cm^2$ pressure for 20 minutes. Sterilized grains were inocubated with 5 mm mycelial discs of *R. solani* cut from the edges of three days old culture *R. solani*. Each flask received 10 mycelial discs and incubated at 25±2°C for 20 days. For even colonization the flask were shaken by hand at 3 days interval. The colonized chickpea grains were air dried and stored at 4°C temperature and used as inocula. One day prior to inoculation

with *R. solani,* the soil was inoculated with the colonized *R. solani* chickpea @ 5g per pot (Faruk *et al.*, 1999).

### 3.15. Preparation of *T. harzianum* suspension and seedling treatment

For preparing inoculum suspension of *T. harzianum*, the bioagent was multiplied on PDA medium. Conidia were collected of 7 days old culture by scrubbing and mixed with sterilize water and sieved through 80 mesh sieve. Suspensions of *T. harzianum* containing $1.5x10^8$ spores/ml were used in the experiment for seedling root inoculation. Twenty days old rice seedlings were uprooted, root system was washed with sterile water and dipped in suspension of *T. harzianum* for 30 minutes and transplanted in pots (Tewari and Singh, 2005).

### 3.16. Seedlings root treatment with PRH formulation

Ten milliliter core PRH formulation was diluted with 100 ml water. Root system of seedling was kept in the PRH formulation about 24 hours and treated seedlings transplanted in the pots soil. Each pot received two seedlings (Plate 3). Weeding and watering was when necessary.

### 3.17. Inoculation with *R. solani* at tillering stage

Thirty eight days after transplanting seedlings were inoculated with *R. solani* (Kumar *et al*., 2012). For inoculation, rice plants inoculated with *R. solani* immature mycelial sclerotia were placed beneath the leaf sheath (Plate 4.A). The inoculated sheath was covered immediately with aluminum foil (Plate 4.B). When typical lesions appeared (after 3 days) the aluminum foil was removed. Seven days after inoculation, the lesion length on the sheath of the inoculated plants was measured (Park *et al.,* 2008).

### 3.18. Application of *T. harzianum* at tillering stage

Spores suspension of *T. harzianum* was prepared at $10^8$ spores/ml and applied as a foliar spray over seedlings after 7 days of inoculation with *R. solani* (Tewari and Singh, 2005). *Trichodema harzianum* was sprayed on 45 day old rice plants in each pot with a hand-held sprayer. Each rice plant received approximately 5 ml of *T. harzianum* suspension.

### 3.19. Application of PRH formulation at tillering stage

At 7 days after inoculation, PRH formulation was applied as a foliar spray over the rice plants at $2.2\times10^9$ spores/ml. For foliar sprays, 10 ml of PRH formulation mixed with 100 ml of sterilize water and sprayed at 45 days after transplanting (DAT) with a hand-held sprayer. Each rice plant received approximately 5 ml of *B. subtilis* formulation.

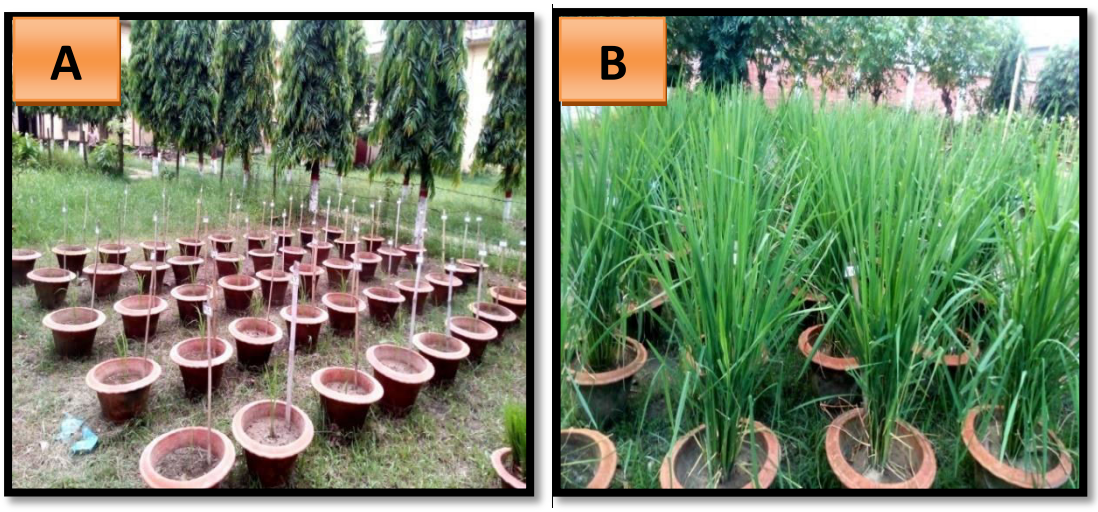



**Plate 3: Transplanted seedling in pots**

**A. Seedling at 7 DAT**

**B. Seedling at 50 DAT**


**Plate 4: Inoculation of *R. solani* beneath the leaf sheath**

**A. Sclerotia beneath the leaf sheath**

**B. Inoculated zone covered with aluminium foil**

### 3.20. Collection of data on disease severity and disease incidence

The pots under the experiment for rice plant was arrange following complete randomized design with nine treatments. Data on sheath blight incidence (%) and severity were recorded and expressed in percentage according to Mansoor *et al.* (2007):

$$\% \text{ Disease incidence} = \frac{\text{Number of sheath blight infected tiller /hill}}{\text{Total number of tiller/hill}} \times 100$$

Percent disease severity in sheath was calculated in the portion of plant tissues infected in relation to the amount of tissue examined. Disease severity data were collected on the following parameters (Agrios, 2005):

$$\% \text{ Disease severity} = \frac{\text{Area of tissues infected sheath}}{\text{Area of tissues inspected sheath}} \times 100$$

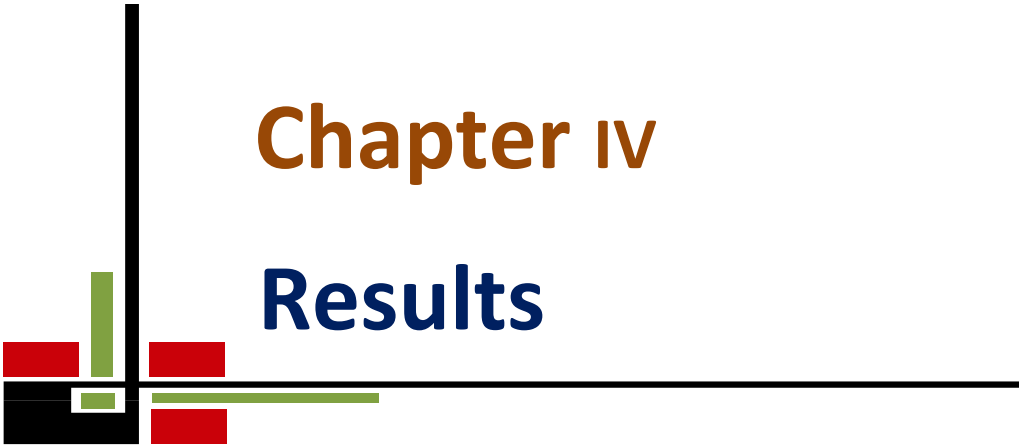
Data on number of total tillers, infected tillers, infected sheath and lesions per hill and length and width of lesion were recorded.

### 3.21. Harvest of the crop

At ripening stage the crop was harvested. The plants sun dried, grains were separated manually and sun dried. Data on fresh and dry grain weigh and dry straw weight were recorded.

### 3.22. Statistical Analysis

The collected data were analyzed using computer software statistix 10.Tukey HSD (Honest Significant Difference) test was performed to determine the level of significant differences and to separate the means within the parameters (Ahmed, 2014).



# Chapter IV

# Results

# RESULTS

## 4.1. Symptoms of sheath blight disease of rice

Symptoms of sheath blight appeared on inoculated rice leaf sheath as oval or ellipsoidal greenish gray lesions, usually 1-3 cm long, initially just above the soil. Cobra snake skin like lesion develops on infected sheath (Plate 5).

## 4. 2. Isolated and purified *R. solani*

The colony of *R. solani* on PDA (potato dextrose agar) medium was white initially (Plate 7.A) and then turned into brown, and the mycelium grew superficially on agar medium (Plate 7.B). *Rhizoctonia solani* grew very fast (45 mm in radius within 3 days) at room temperature (26-35°C). Sclerotia were produced on agar surface usually 3 days after the mycelium covered the whole plate. Sclerotia on PDA medium were grey when they were immature, and turned to deep brown 2 days later. Shape of sclerotia was varied on PDA medium but usually was spherical except for the flat side attached to the agar surface. Both single and aggregated sclerotia were observed on the agar medium. No conidia and clamp connections were found but produced creamy perpendicular hypha with constriction at base which observed under compound microscope (Plate 8).

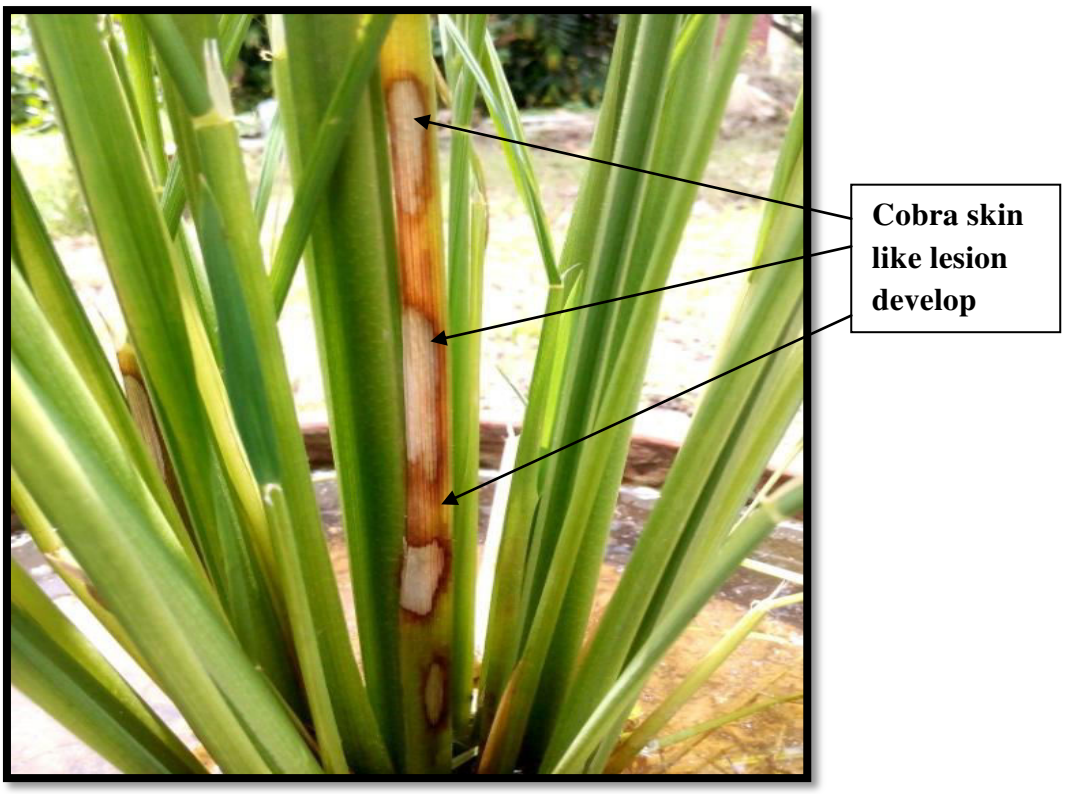



**Plate 5: A typical symptoms of sheath blight disease of rice caused by *R. solani***

**Plate 6. Preservation of *R. solani* on PDA slant**

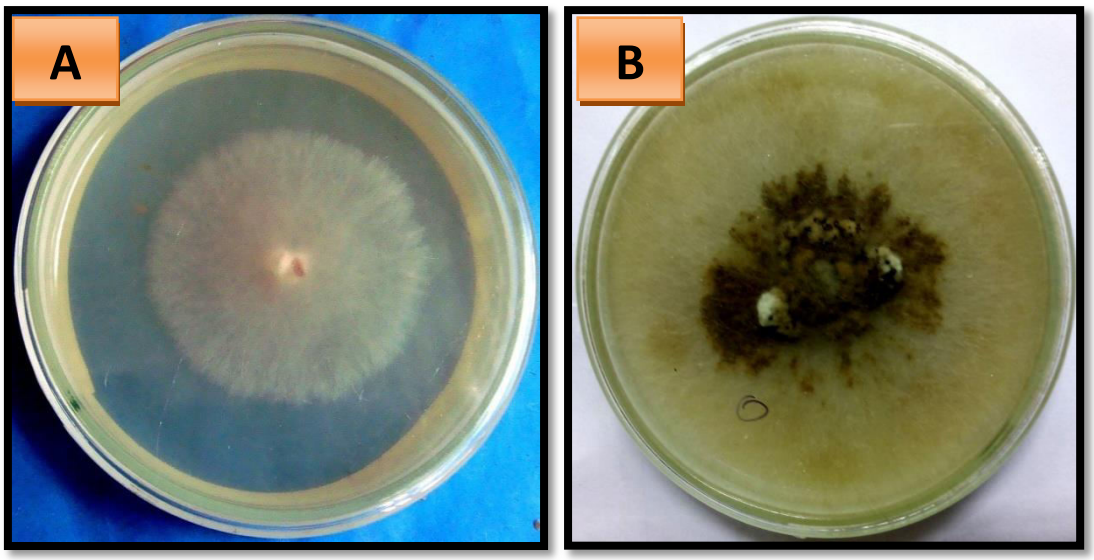



**Plate 7: A pure culture of *R. solani* on PDA medium**

**A. Young mycelial colony**
**B. Brownish sclerotia forming on mature colony**

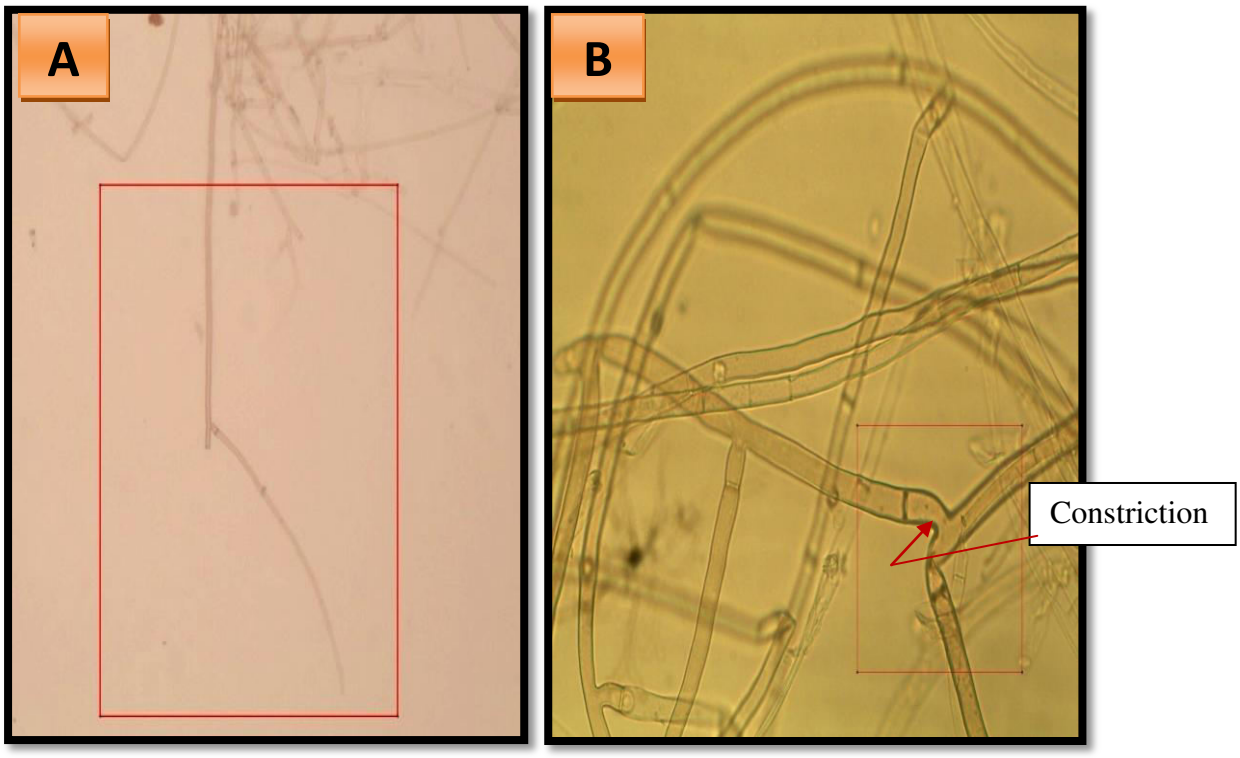



**Plate 8: Microscopic view of mycelial body of *R. solani***

**A. Microscopic view in (10x) magnification**
**B. Microscopic view in (40x) magnification**

### 4.3. Isolated and purified *T. harzianum*

Isolated *T. harzianum* produced radial mycelial growth on PDA. At the early stage whitish to greenish mycelia appeared (Plate 9.A). Next a deep green colour developed in central part and gradually extended to the periphery. Finally, it appeared a whitish green colour (Plate 9.B). Mostly globose to subglobose conidia developed on phialides produced in the opposite direction in each point which observed under compound microscope (Plate 10).

### 4.4. *In vitro* screening of *T. harzianum* against *R. solani*

Antagonists were tested between *T. harzianum* and *R. solani. Trichoderma harzianum* reduced the mycelial growth and suppressed sclerotia production of *R. solani* in dual culture. *Trichoderma harzianum* showed more than 50% inhibition of the radial growth of *R. solani* as compared to control (Plate 11.C). After 72 hrs of incubation percent inhibition zone was 44% and after 96 hrs it was 73%. After 7 days of inoculation *T. harzianum* grew over *R. solani* (Plate 11).

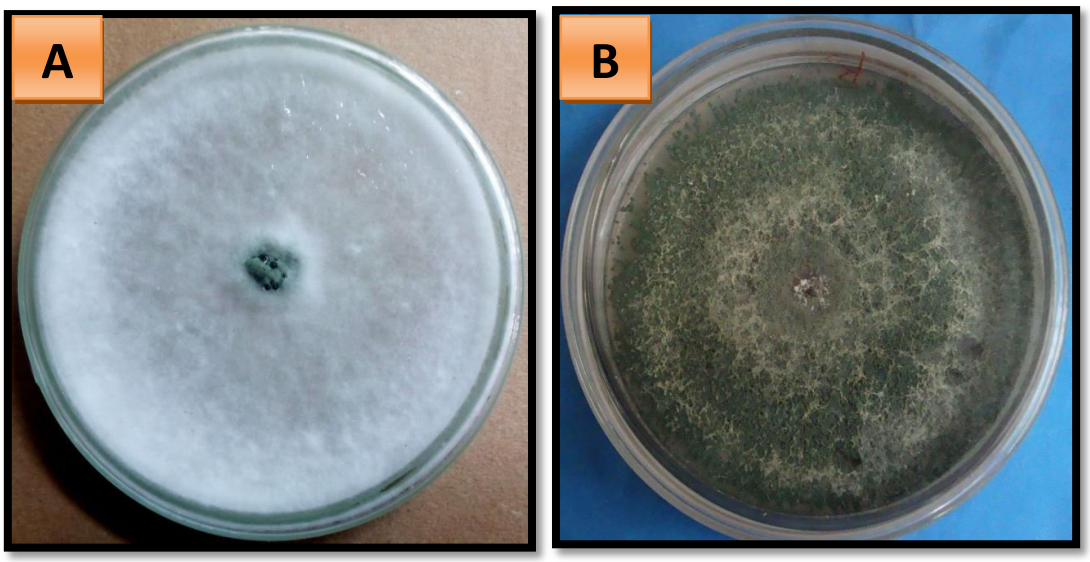



**Plate 9: Pure culture of *T. harzianum* on PDA medium**

**A. Young colony** **B. Mature colony**

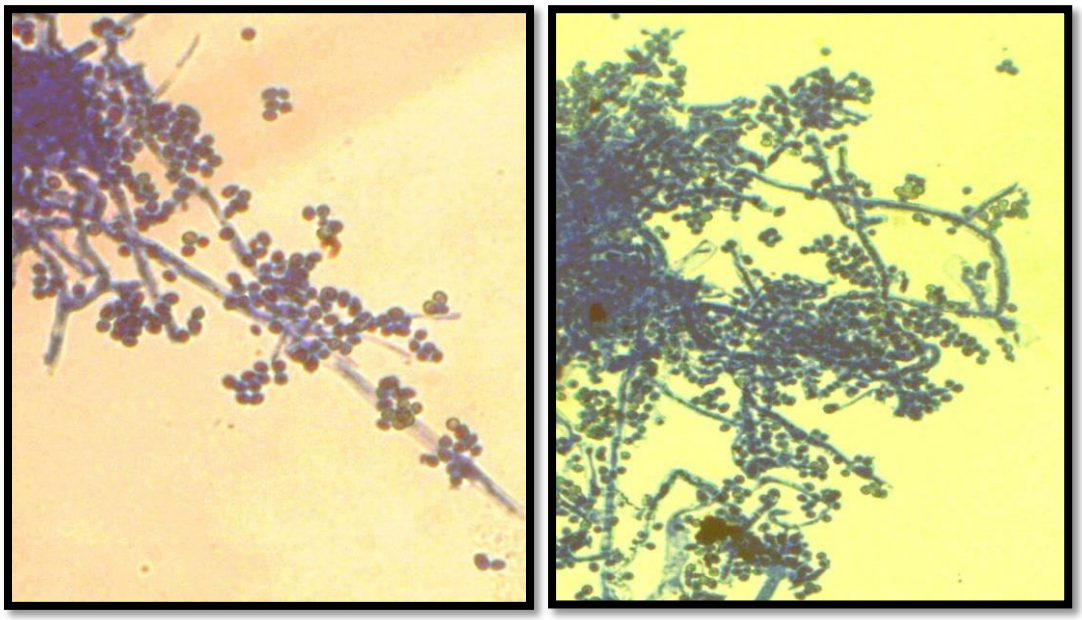


**Plate 10: Microscopic view of *T. harzianum* (10x)**

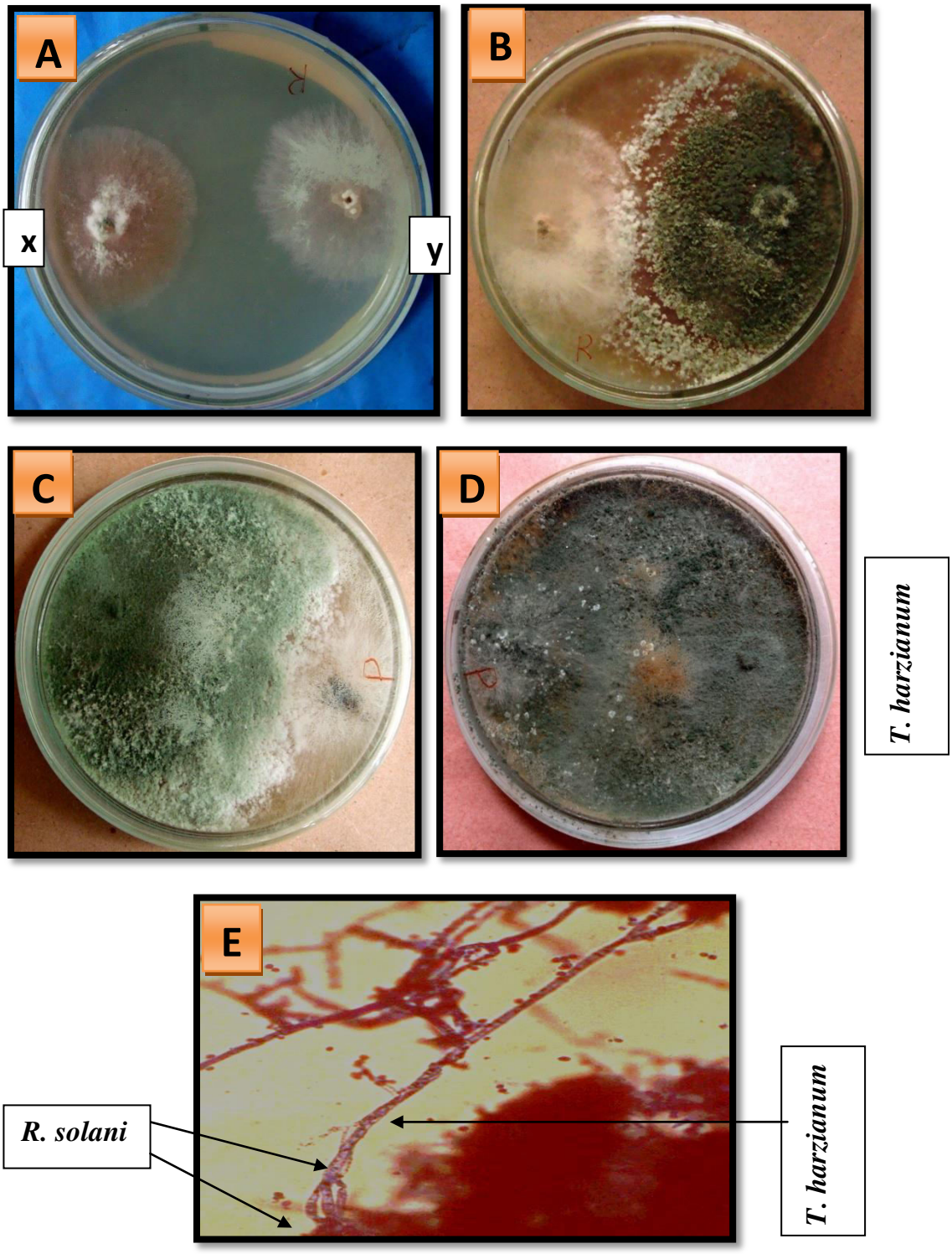



**Plate 11: Dual culture of .*T harzianum* (x) and *R. solani* (y)**

A. Growing of *T. harzianum* and *R. solani*
B. *T. harzianum* showing inhibition zone against *R. solani*
C. *T. harzianum* grew over *R. solani*
D. *T. harzianum* fully grew over *R. solani*
E. *T. harzianum* hyphae coiled around the mycelium of *R. solani* (Microscopic view in 10x)

### 4.5. *In vitro* screening of *B. subtilis* against *R. solani*

*Bacillus subtilis* reduced the mycelia growth and suppressed sclerotia production of *R. solani* in dual culture. Clear inhibition zone was observed between *B. subtilis* and *R. solani. Bacillus subtilis* showed more than 50% inhibition of the radial growth of the test pathogen *R. solani* as compared with the control. After 72 hours of incubation percent inhibition zone was 37% and after 96 hrs percent inhibition zone was recorded 68%.

### 4.6. Response of different treatments on development of sheath blight disease of rice

Response of rice variety BRRI dhan56 inoculated with *R. solani* on the development of sheath blight disease were found to be statistically different. Number of infected tillers and sheaths were found significantly on different days after transplanting (DAT).

#### 4.6.1. Effect of different treatments on incidence of sheath blight disease of rice in pot

The effect of different treatments on incidence of sheath blight disease of rice in pot was recorded at 45, 60 and 75 days after transplanting (DAT). The disease incidence showed significant variations among the treatments. At 45 DAT diseases incidence ranged 10.34-44.33%. Among the treatments, the highest incidence (44.33%) in $T_1$ (control) and the lowest incidence (10.34%) was found in $T_9$ which was statistically similar to $T_8$ and $T_7$. After 60 DAT, the highest incidence (66.12%) was found in $T_1$ and the lowest incidence (21.05%) in $T_6$ which was statistically similar to $T_9$, $T_4$, $T_5$ and $T_7$. At 75 DAT, the highest incidence (100%) was also found in $T_1$ and the lowest incidence (28.69%) in $T_4$ which was statistically similar to $T_5$, $T_6$, $T_7$, $T_9$ and $T_3$. It was noted that the disease incidence was gradually increased with the increase of age of plant (Fig.1).

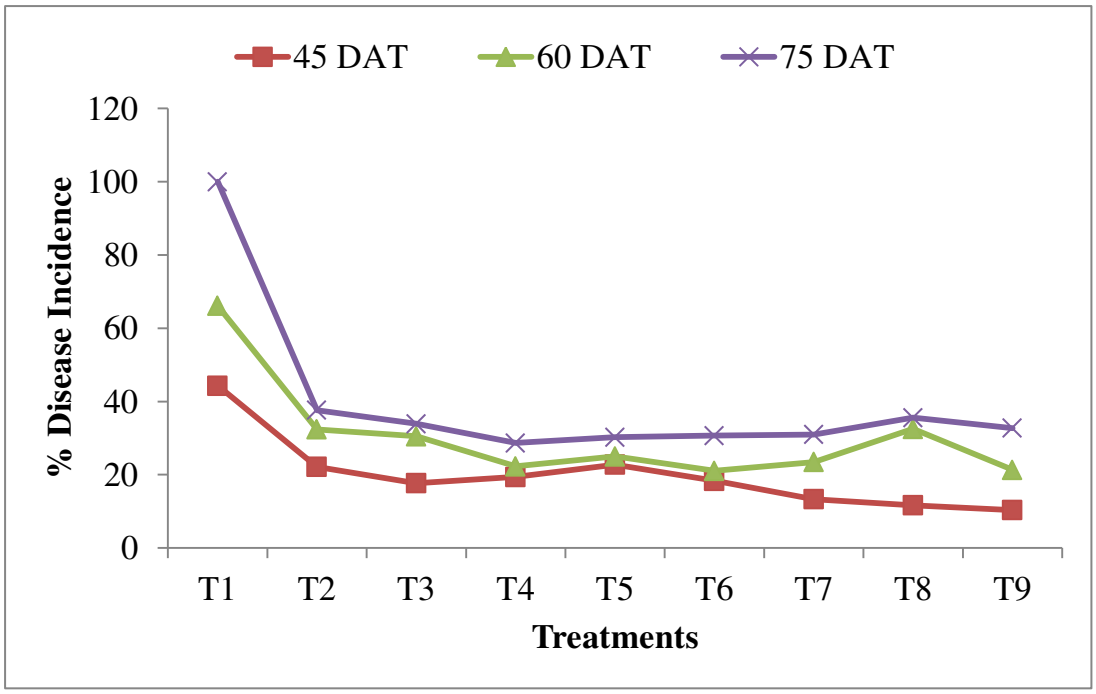



**Fig.1. Effect of different treatments on percent disease incidence of sheath blight disease of rice in pot at different days after transplanting**

**$T_1$** = Tiller treated with Rs* (control), **$T_2$** = Soil inoculated with Rs + Seedling root treated with PRH**, **$T_3$** = Soil inoculated with Rs + Seedling root treated with Th***, **$T_4$** = Soil inoculated with Rs + Tiller treated with PRH, **$T_5$** = Soil inoculated with Rs + Tiller treated with Th, **$T_6$** = Seedling root treated with PRH + Tiller treated with Rs, **$T_7$** = Seedling root treated with Th + Tiller treated with Rs, **$T_8$** = Tiller treated with Rs + Tiller treated with PRH, **$T_9$** = Tiller treated with Rs + Tiller treated with Th

* **Rs** = *Rhizoctonia solani*

** **Th** = *Trichoderma harzianum*

*** **PRH** = Plant Regulation Hormone (*Bacillus subtilis* based formulation)

### 4.6.2. Effect of different treatments on percentage severity of sheath blight disease of rice

The effect of different treatments on severity of sheath blight disease of rice was recorded at 45, 60 and 75 DAT. The disease severity showed significant variations among the treatments. At 45 DAT diseases severity ranged 3.03-10.02%. Among the treatments, the highest severity (10.02%) was found in $T_5$ which was statistically similar to $T_4$ and $T_6$. The lowest severity (3.03%) was recorded in $T_9$ which was statistically similar to $T_8$ and $T_7$. At 60 DAT, the highest severity (30.81%) was found in $T_1$ (control) and the lowest (4.40%) in $T_9$ which was statistically similar to $T_7$, $T_8$ and $T_3$. At 75 DAT, the highest severity (60.09%) was also found in $T_1$ (Plate 12) and the lowest (7.11%) in $T_7$ which was statistically similar to $T_9$ and $T_8$. It was noted that the disease severity was gradually increased (Fig.2).

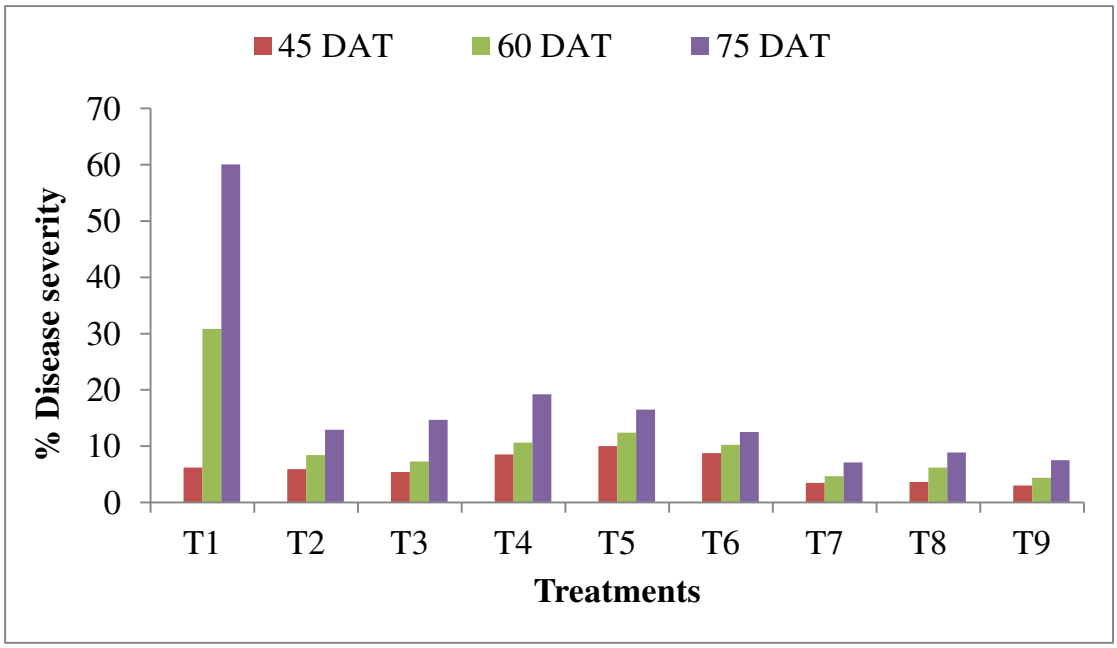



**Fig.2. Effect of different treatments on percentage severity of sheath blight disease of rice at different days after transplanting**

**$T_1$** = Tiller treated with Rs* (control), **$T_2$** = Soil inoculated with Rs + Seedling root treated with PRH**, **$T_3$** = Soil inoculated with Rs + Seedling root treated with Th***, **$T_4$** = Soil inoculated with Rs + Tiller treated with PRH, **$T_5$** = Soil inoculated with Rs + Tiller treated with Th, **$T_6$** = Seedling root treated with PRH + Tiller treated with Rs, **$T_7$** = Seedling root treated with Th + Tiller treated with Rs, **$T_8$** = Tiller treated with Rs + Tiller treated with PRH, **$T_9$** = Tiller treated with Rs + Tiller treated with Th

* **Rs** = *Rhizoctonia solani*

** **Th** = *Trichoderma harzianum*

*** **PRH** = Plant Regulation Hormone (*Bacillus subtilis* based formulation)

**45 DAT** **60DAT**

**75 DAT**

**Plate 12. Sheath blight disease severity in sheath at 45, 60 and 75 DAT in control plants**

### 4.6.3. Effect of different treatments on percentage of infected sheath of rice

The effect of different treatments on percent of infected sheath of rice recorded at 45, 60 and 75 DAT. The percent infected sheath showed significant variations among the treatments. At 45 DAT percent infected sheath ranged 3.50-19.04%. Among the treatments, the highest infected sheath (19.04%) was found in $T_1$ (control) and the lowest (3.50%) in $T_8$ which was statistically similar to $T_9$, $T_7$ and $T_3$. At 60 DAT, the highest infected sheath (40.12%) was found in $T_1$ and the lowest (8.47%) in $T_7$ which was statistically similar to all other treatments. At 75 DAT, the highest infected sheath was also found in control (71.10%) and the lowest (10.35%) in $T_3$. It was noted that the disease infected sheath gradually increased with the age of plant (Table 1).

**Table 1. Effect of different treatments on percentage of infected sheath of rice at different days after transplanting**

| Treatments | Percentage of Infected Sheath | | | | | |
|---|---|---|---|---|---|---|
| | 45 DAT | | 60 DAT | | 75 DAT | |
| $T_1$ | 19.04 | a | 40.12 | a | 71.10 | a |
| $T_2$ | 7.60 | bc | 9.50 | b | 11.63 | bc |
| $T_3$ | 6.06 | cd | 8.77 | b | 10.35 | c |
| $T_4$ | 7.37 | bc | 12.38 | b | 14.25 | b |
| $T_5$ | 9.13 | b | 11.71 | b | 13.54 | bc |
| $T_6$ | 8.11 | bc | 10.24 | b | 12.48 | bc |
| $T_7$ | 3.81 | d | 8.47 | b | 10.86 | bc |
| $T_8$ | 3.50 | d | 10.33 | b | 13.72 | bc |
| $T_9$ | 3.64 | d | 8.83 | b | 12.59 | bc |
| Critical Value for Comparison | 2.62 | | 4.14 | | 3.75 | |
| CV (%) | 16.54 | | 4.84 | | 9.50 | |
| Level of Significance | ** | | ** | | ** | |

Each data represents the mean value of five replications.

Values followed by the same letter within a column are not significantly different ($p \leq 0.05$) according to Tukey's HSD range test.

**$T_1$** = Tiller treated with Rs* (control), **$T_2$** = Soil inoculated with Rs + Seedling root treated with PRH**, **$T_3$** = Soil inoculated with Rs + Seedling root treated with Th***, **$T_4$** = Soil inoculated with Rs + Tiller treated with PRH, **$T_5$** = Soil inoculated with Rs + Tiller treated with Th, **$T_6$** = Seedling root treated with PRH + Tiller treated with Rs, **$T_7$** = Seedling root treated with Th + Tiller treated with Rs, **$T_8$** = Tiller treated with Rs + Tiller treated with PRH, **$T_9$** = Tiller treated with Rs + Tiller treated with Th

* **Rs** = *Rhizoctonia solani*

** **Th** = *Trichoderma harzianum*

*** **PRH** = Plant Regulation Hormone (*Bacillus subtilis* based formulation)

### 4.6.4. Effect of different treatments on number of lesion in infected sheath

The effect of different treatments on number of lesion in infected sheath of rice in 100 tillers recorded at 45, 60 and 75 DAT showed significant variations among the treatments. At 45 DAT lesion number ranged from 12.32 to 170.71. Among the treatments, the highest number of lesion (170.71) was found in $T_1$ (control) and the lowest (12.32) in $T_2$ followed by $T_5$, $T_6$, $T_7$ and $T_9$. At 60 DAT, the highest number of lesion (291.40) was found in $T_1$ and the lowest (27.40) in $T_2$ followed by $T_6$, $T_5$ and $T_3$. At 75 DAT, the highest number of lesion (366.95) was also found in $T_1$ and the lowest (40.48) in $T_2$ which was statistically similar to $T_3$. It was noted that the number of lesion gradually increased with the age of plant (Fig.3).

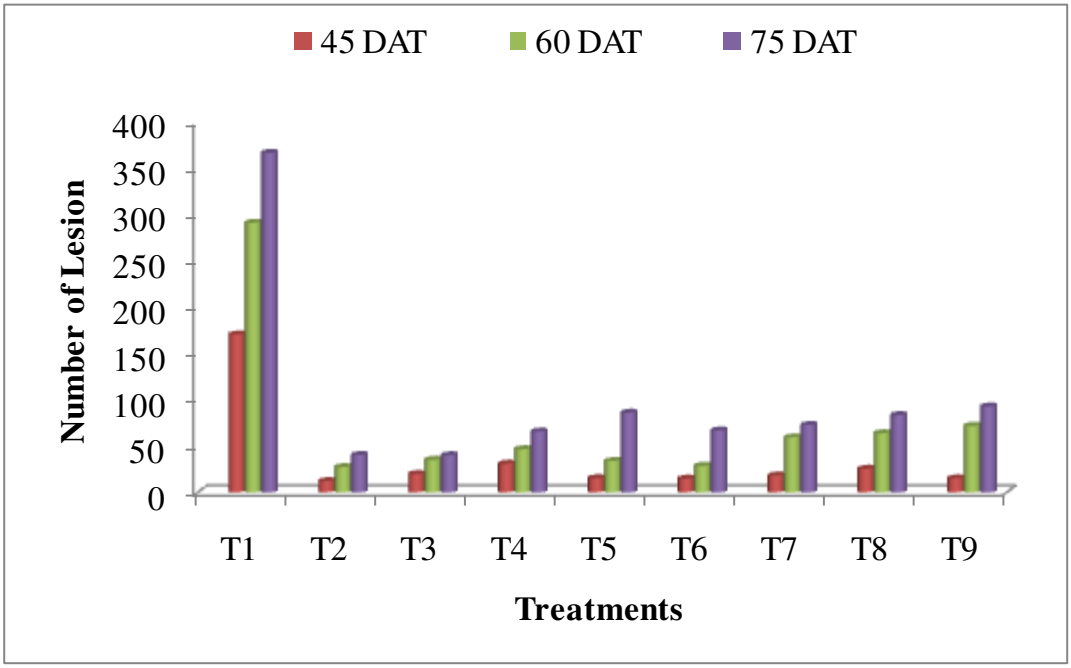



**Fig.3. Effect of different treatments on number of lesion in infected sheath at different days after transplanting**

**$T_1$** = Tiller treated with Rs* (control), **$T_2$** = Soil inoculated with Rs + Seedling root treated with PRH**, **$T_3$** = Soil inoculated with Rs + Seedling root treated with Th***, **$T_4$** = Soil inoculated with Rs + Tiller treated with PRH, **$T_5$** = Soil inoculated with Rs + Tiller treated with Th, **$T_6$** = Seedling root treated with PRH + Tiller treated with Rs, **$T_7$** = Seedling root treated with Th + Tiller treated with Rs, **$T_8$** = Tiller treated with Rs + Tiller treated with PRH, **$T_9$** = Tiller treated with Rs + Tiller treated with Th

* **Rs** = *Rhizoctonia solani*

** **Th** = *Trichoderma harzianum*

*** **PRH** = Plant Regulation Hormone (*Bacillus subtilis* based formulation)

### 4.6.5. Effect of different treatments on height of lesion in infected sheath

The height of lesion in infected sheath was showed significant variations among the treatments. At 45 DAT lesion was 3.40-11.6 mm. Among the treatments, the highest height of lesion (11.6 mm) was found in $T_1$ (control) which was statistically similar to $T_5$ and the lowest (3.40 mm) in $T_2$ which was statistically similar to $T_3$. At 60 DAT, the highest height of lesion (29.38 mm) in $T_1$ and the lowest (6.00 mm) in $T_2$ followed by $T_3$. At 75 DAT, the highest height of lesion (35.31 mm) was also found in $T_1$ and the lowest (11.34 mm) in $T_3$ which was statistically similar to $T_2$. It was noted that the disease height of lesion was gradually increased with the age of plant (Table 2).

**Table 2. Effect of different treatments on height of lesion in infected sheath at different days after transplanting**

| Treatments | Height of Lesion (mm) | | |
|---|---|---|---|
| | 45 DAT | 60 DAT | 75 DAT |
| $T_1$ | 11.68 a | 29.38 a | 35.31 a |
| $T_2$ | 3.40 e | 6.00 d | 12.30 e |
| $T_3$ | 5.10 de | 8.54 d | 11.34 e |
| $T_4$ | 7.34 bcd | 19.72 b | 25.59 b |
| $T_5$ | 9.62 ab | 15.71 c | 18.47 cd |
| $T_6$ | 7.56 bcd | 15.98 c | 21.92 bc |
| $T_7$ | 8.21 bc | 20.82 b | 23.34 b |
| $T_8$ | 6.02 cd | 15.17 c | 17.10 d |
| $T_9$ | 6.86 cd | 15.82 c | 16.53 d |
| Critical Value for Comparison | 2.55 | 3.60 | 4.10 |
| CV (%) | 16.75 | 10.55 | 9.73 |
| Level of Significance | ** | ** | ** |

Each data represents the mean value of five replications.

Values followed by the same letter within a column are not significantly different ($p \leq 0.05$) according to Tukey's HSD range test.

**$T_1$** = Tiller treated with Rs* (control), **$T_2$** = Soil inoculated with Rs + Seedling root treated with PRH**, **$T_3$** = Soil inoculated with Rs + Seedling root treated with Th***, **$T_4$** = Soil inoculated with Rs + Tiller treated with PRH, **$T_5$** = Soil inoculated with Rs + Tiller treated with Th, **$T_6$** = Seedling root treated with PRH + Tiller treated with Rs, **$T_7$** = Seedling root treated with Th + Tiller treated with Rs, **$T_8$** = Tiller treated with Rs + Tiller treated with PRH, **$T_9$** = Tiller treated with Rs + Tiller treated with Th

* **Rs** = *Rhizoctonia solani*

** **Th** = *Trichoderma harzianum*

*** **PRH** = Plant Regulation Hormone (*Bacillus subtilis* based formulation)

### 4.6.6. Effect of different treatments on width of lesion in infected sheath

The width of lesion in infected sheath showed significant variations among the treatments. At 45 DAT width of lesion ranged from 1.78-4.66 mm. Among the treatments, the highest width of lesion (4.66 mm) was found in $T_1$ (control) which was statistically similar to $T_5$ and the lowest (1.78 mm) in $T_3$ which was statistically similar to $T_2$. At 60 DAT, the highest width of lesion (4.98 mm) was found in $T_1$ that was statistically similar to $T_5$, $T_7$ and $T_8$ and the lowest (2.76 mm) in $T_3$ followed by $T_2$, $T_4$, $T_6$ and $T_9$. At 75 DAT, the highest width of lesion (5.56 mm) was found in $T_1$ and the lowest (3.26 mm) in $T_2$ which was statistically similar to $T_3$, $T_5$, $T_8$ and $T_9$. It was noted that the disease width of lesion was gradually increased with the age of plant (Table 3).

**Table 3. Effect of different treatments on width in infected sheath at different days after transplanting**

| Treatments | Width of Lesion (mm) | | |
|---|---|---|---|
| | 45 DAT | 60 DAT | 75 DAT |
| $T_1$ | 4.66 a | 4.98 a | 5.56 a |
| $T_2$ | 2.16 cd | 2.89 cd | 3.26 d |
| $T_3$ | 1.78 d | 2.76 d | 3.36 cd |
| $T_4$ | 3.06 bc | 3.79 bcd | 4.40 b |
| $T_5$ | 3.56 ab | 4.12 ab | 4.19 bcd |
| $T_6$ | 2.90 bc | 3.67 bcd | 4.21 bc |
| $T_7$ | 3.24 bc | 4.02 abc | 4.32 b |
| $T_8$ | 3.08 bc | 3.95 abc | 4.09 bcd |
| $T_9$ | 2.94 bc | 3.31 bcd | 3.89 bcd |
| Critical Value for Comparison | 1.10 | 1.135 | 0.94 |
| CV (%) | 17.35 | 14.64 | 10.84 |
| Level of Significance | ** | ** | ** |

Each data represents the mean value of five replications.

Values followed by the same letter within a column are not significantly different ($p \leq 0.05$) according to Tukey's HSD range test.

**$T_1$** = Tiller treated with Rs* (control), **$T_2$** = Soil inoculated with Rs + Seedling root treated with PRH**, **$T_3$** = Soil inoculated with Rs + Seedling root treated with Th***, **$T_4$** = Soil inoculated with Rs + Tiller treated with PRH, **$T_5$** = Soil inoculated with Rs + Tiller treated with Th, **$T_6$** = Seedling root treated with PRH + Tiller treated with Rs, **$T_7$** = Seedling root treated with Th + Tiller treated with Rs, **$T_8$** = Tiller treated with Rs + Tiller treated with PRH, **$T_9$** = Tiller treated with Rs + Tiller treated with Th

* **Rs** = *Rhizoctonia solani*

** **Th** = *Trichoderma harzianum*

*** **PRH** = Plant Regulation Hormone (*Bacillus subtilis* based formulation)

### 4.6.7. Yield performance of rice cv. BRRI dhan56 as influenced by different treatments against sheath blight disease in per pot

In case of fresh grain weight, the highest yield (399.64g/hill) was recorded from $T_9$ and lowest (308.42g/hill) in $T_1$ (control). In case of dry grain weight, the highest yield (356.64g/hill) was recorded from $T_9$ that was statistically similar to $T_5$ and $T_2$ and lowest (267.24g/hill) in $T_1$. In case of dry straw weight, the highest yield (430.86g/hill) was recorded from $T_9$ that was statistically similar to $T_2$ and $T_7$ and lowest (327.88g/hill) in $T_1$ (Table 4).

**Table 4. Yield performance of rice cv. BRRI dhan56 as influenced by different treatments of rice against sheath blight disease**

| Treatments | Yield (g/hill) | | |
|---|---|---|---|
| | Fresh Grain Weight | Dry Grain Weight | Dry Straw Weight |
| $T_1$ | 308.42 f | 267.24 d | 327.88 e |
| $T_2$ | 379.13 bc | 346.18 ab | 426.49 ab |
| $T_3$ | 346.36 d | 306.65 c | 414.88 c |
| $T_4$ | 327.75 e | 296.10 c | 373.90 d |
| $T_5$ | 392.83 ab | 351.46 a | 418.26 bc |
| $T_6$ | 325.94 e | 301.53 c | 378.26 d |
| $T_7$ | 365.48 c | 336.51 b | 420.22 abc |
| $T_8$ | 347.67 d | 305.63 c | 373.75 d |
| $T_9$ | 399.64 a | 356.64 a | 430.86 a |
| Critical Value for Comparison | 16.99 | 13.8 | 10.87 |
| CV (%) | 2.31 | 2.10 | 1.32 |
| Level of Significance | ** | ** | ** |

Each data represents the mean value of five replications.

Values followed by the same letter within a column are not significantly different ($p \leq 0.05$) according to Tukey's HSD range test.

**$T_1$** = Tiller treated with Rs* (control), **$T_2$** = Soil inoculated with Rs + Seedling root treated with PRH**, **$T_3$** = Soil inoculated with Rs + Seedling root treated with Th***, **$T_4$** = Soil inoculated with Rs + Tiller treated with PRH, **$T_5$** = Soil inoculated with Rs + Tiller treated with Th, **$T_6$** = Seedling root treated with PRH + Tiller treated with Rs, **$T_7$** = Seedling root treated with Th + Tiller treated with Rs, **$T_8$** = Tiller treated with Rs + Tiller treated with PRH, **$T_9$** = Tiller treated with Rs + Tiller treated with Th

* **Rs** = *Rhizoctonia solani*

** **Th** = *Trichoderma harzianum*

*** **PRH** = Plant Regulation Hormone (*Bacillus subtilis* based formulation)

# Chapter v

# Discussion

# DISCUSSION

Experiment was carried out to find out the possibility of using *T. harzianum* and *B. subtilis* as a bio-control agent to control sheath blight of rice caused by *R. solani*. The results of the present study revealed that bio-control agents had great impact on percent growth inhibition of *R. solani* and their interaction of rice and also growth promoting characters of rice plants.

*R. solani*, the causal agent of sheath blight disease of rice, was isolated from infected rice sheath collected from Agronomy field of Sher-e-Bangla Agricultural University (SAU) and followed by tissue planting method. Singh *et al.*, 2003; Shahram *et al.*, 2010; Bashar *et al.*, 2010 and Yugander *et al.*, 2015 isolated the *R. solani* from infected rice plant. Morphological tests were done to identify *R. solani.*

Antagonistic *T. harzianum* isolated from mushroom substrate and identified following the keys of Hermosa *et al*., 1999; Choi *et al*., 2003; Bhat *et al.*, 2009; Hatvani *et al*., 2012 and Shah *et al*., 2012.

*B. subtilis* based formulation was collected from Natural Bio Agro Tech Co (Prv) Ltd. Kumar *et al*. (2012) used commercial liquid formulation of *B. subtilis* strain against rice sheath blight disease (*R. solani*) and for plant growth promotion.

In *in vitro* screening *T. harzianum* showed best performance by reducing the growth of *R. solani* and produced highest inhibition (73%). *Trichoderma harzianum* and *T. viride* completely overgrew on *R. solani* and produced inhibition zone of 67 % and 70 %, respectively in dual culture (Seema and Devaki, 2012). Ali and Nadarajah (2012) showed that *T. harzianum* have antagonistic activity against *R. solani.* Ali *et al.* (2014) reported that *T. harzianum* inhibited the mycelial growth of *R. solani* by 74.4-67.8%. Bhat *et al.* (2009) recorded the highest growth inhibition (65.87%) of *R. solani* by *T. harzianum* followed by *T.*

*viride*. Rahman (2007) stated that *T. harzianum* showing linear overgrowth against *R. solani* and significantly reduced the pathogen of sheath blight disease of rice. According to Reyes *et al*. (2007) *T. harzianum* showed hyperparasitic activity against *R. solani. Trichoderma harzianum* and *T. viride* showed the highest antagonistic activity against *R. solani* (Bhagawati, 2005). Alarcon *et al.* (2005) observed that *T. harzianum* showed antagonistic effect and hyperparasitism against *R. solani.* Gogoi and Ali (2005) found that *T. harzianum* inhibited the growth of the rice sheath blight pathogen (*R. solani*). Tang *et al.* (2001) used six stains of *Trichoderma* spp. greatly inhibited the growth of *T. solani* in dual culture.

*B. subtilis* formulation PRH also showed good result and produced inhibition zone and suppressed mycelial growth of *R. solani* in dual culture. This is in accordance with Shrestha *et al.* (2016) and they found that *B. subtilis* inhibited the sclerotial germination of *R. solani* on potato dextrose agar. Bashar *et al.* (2010) tested 50 isolated of bacteria against *R. solani* and observed 10 isolates produced remarkable inhibition zone (15 mm) against *R. solani. Bacillus subtilis* strain NJ-18 inhibited the radial extension of hyphae of the phytopathogenic fungi *R. solani* by producing antifungal metabolites that diffused through the agar and caused abnormal swelling of hyphae (Yang *et al.*, 2009). In dual culture the mycelial growth of *R. solani* was found 83% overgrown by *Bacillus* spp. (Kumar *et al.*, 2009). Radheshyam *et al*., 1990 and Moita *et al*., 2005 reported that *Bacillus* spp. produced antifungal metabolites and protect plants from fungal infection. Gnanamanickam and Mew (1990) found 5 strains of *Bacillus* spp. which created an inhibition zones ranged from 20–31 mm against *R. solani.*

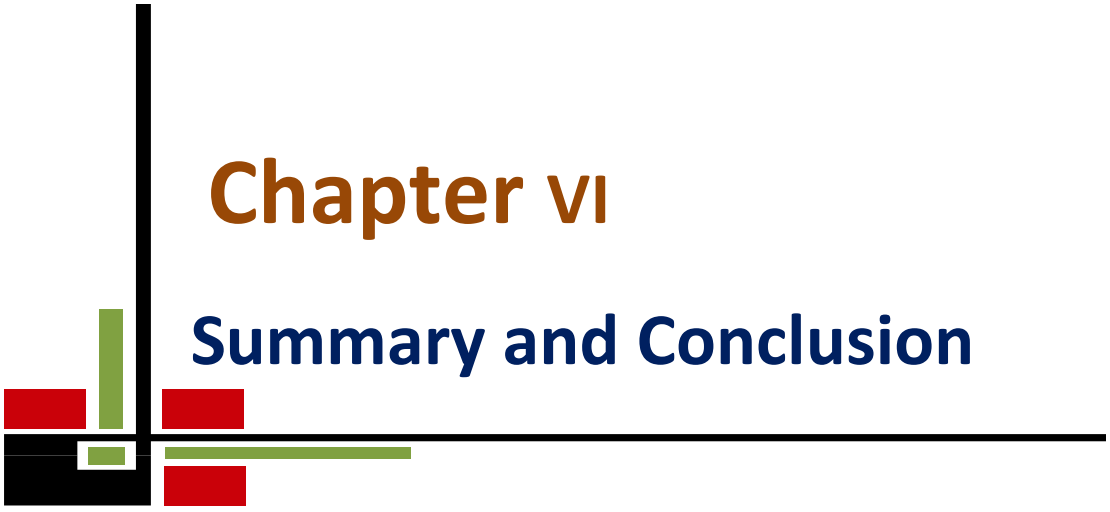
Lowest sheath blight disease incidence and severity at different days after transplanting were recorded in *T. harzianum* and *B. subtilis* treated plants compared to control. These results supported by Ali and Nadarajah (2012) who recorded disease incidence 22-33% and severity 14.33-22% in *T. harzianum* and *B. subtilis* treated plants against *R. solani*. Khan and Sinha (2006) applied *T.*

*harzianum* that reduced the sheath blight incidence (81.26%) and severity (78.98%), and increased rice grain yield. *Trichoderma harzianum* significantly reduced disease severity (40.82%) compared to other treatments (Tewari and Rajbir, 2005). According to Kumar *et al.* (2012) commercial liquid formulation of *B. subtilis* had potential effect against *R. solani* and reduced sheath blight incidence and severity.

*T. harzianum* and *B. subtilis* based formulation reduced lesion size and number compared to control. This is supported by Peng *et al.* (2014) found that *B. subtilis* reduced sheath blight lesion length by 35% and 20%.

*T. harzianum* treated hills gave the highest yield per hill followed by *B. subtilis* when *T. harzianum* applied in the sheath during tillering stage. Pal *et al.* (2015) reported that seed treatment and foliar spray with *T. harzianum* decreased percent disease index (PDI) by 34.41% and increased the yield up to 41.1% as compared to control. According to Prasad and Kumar (2011) *T. harzianum* was most effective against *R. solani* which reduced disease incidence and increased grain yield. *Trichoderma harzianum* showed effective in reducing sheath blight (44.35-52.37%) and increasing grain yield (20.25-23.13%) (Khan and Sinha, 2005). Kumar *et al.* (2012) recorded highest grain yield was in seedling root treated plants with commercial liquid formulation of *B. subtilis* strain MBI 600 ($2.2\times10^9$ cfu/ml).

For the above study it can be clearly pointed out the spraying of *T. harzianum* at tillering stage and seedling root treatment with PRH formulation showed better performance by reducing disease incidence and severity of sheath blight and increased the grain yield. Thus *B. subtilis* and *T. harzianum* could be used against *R. solani* and other pathogens as eco-friendly management.



# Chapter VI

## Summary and Conclusion

# SUMMARY AND CONCLUSION

A laboratory and pot experiments were carried out to find efficacy of two bio agents against sheath blight of rice caused by *R. solani. Trichoderma harzianum* was isolated from mushroom substrates and *B. subtilis* was collected from local market as a liquid formulation. Infected sheath of rice plant having typical symptoms were collected from naturally infected plants and isolated *R. solani* in the laboratory.

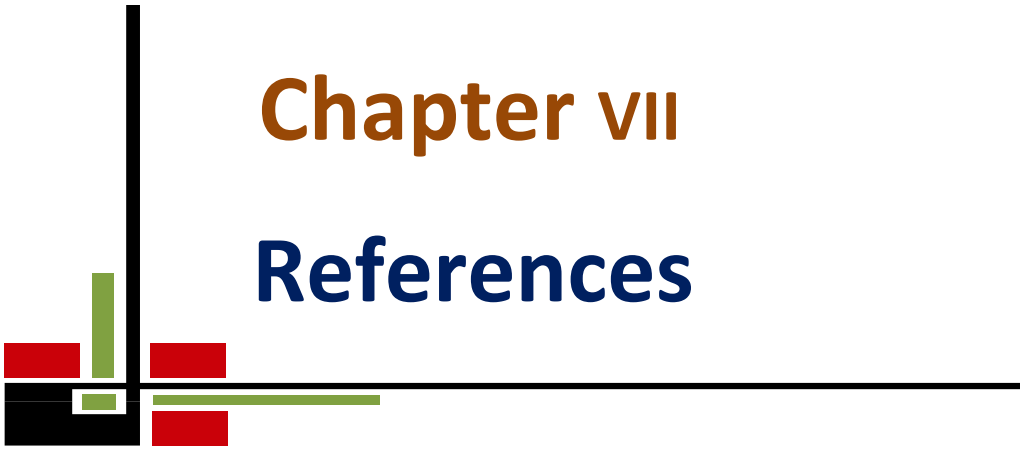
Antagonistic activity of *T. harzianum* and *B. subtilis* were studied following dual culture plate technique against *R. solani* on PDA. Antagonistic effect of *T. harzianum* and *B. subtilis* against *R. solani* showed the remarkable inhibition the mycelial growth of the pathogen on PDA. *Trichoderma harzianum* was found to be highest antagonistic effect to inhibit the mycelial growth of the pathogen on PDA. At 4 DAI (Days After Inoculation) *T. harzianum* and *B. subtilis* produced inhibition zone against *R. solani* by 73% and 68%, respectively.

A pot experiment was conducted in the net house of Department of Plant Pathology, Sher-e-Bangla Agricultural University, to find out effect of bio-agents or sheath blight incidence and yield of rice.

Nine treatments were used in this experiment. BRRI dhan56 was grown in pot in Sher-e-Bangla Agricultural University, Dhaka during the period from May 2015 to October 2015. Seedlings root dipped and foliar sprayed with *T. harzianum* and *B. subtilis* suspension in different stage. Among the treatments used, all treatments (except control) have showed potentiality antagonist against sheath blight disease of rice and significantly reduced the percent disease incidence and severity. The highest disease incidence was recorded from control plants at 45, 60 and 75 days after transplanting (DAT) which was 44.33%, 66.12% and 100%, respectively. The lowest disease incidence was recorded in $T_4$ (Soil inoculated with *R. solani* + Tiller treated with PRH) which was 19.40%, 22.30% and 28.69% at 45, 60 and 75

days after transplanting, respectively. Disease severity was recorded also highest in control plants. The highest disease severity was recorded from control plants at 45, 60 and 75 days after transplanting which was 6.20%, 30.81% and 60.09%, respectively. The lowest disease severity was recorded in $T_7$ (seedling root treated with *T. harzianum* + tiller inoculated with *R. solani*) which was 3.50%, 4.66% and 7.11% at 45, 60 and 75 days after transplanting, respectively. It was noted each control plant showed highest percent of disease incidence and severity compared to other treatments. Untreated control plant also showed lowest yield compared to *T. harzianum* and *B. subtilis* treated plants. The findings of the present study revealed that *T. harzianum* and *B. subtilis* were found effective in controlling sheath blight of rice. Therefore, tremendous prospects lying behind the use of *T. harzianum* and *B. subtilis* as bio-fungicide to control this disease with additional benefit of avoiding extra risk of applying fungicides that cause environmental pollutions.

There is a scope to use biological agent like *T. harzianum* and *B. subtilis* for management of sheath blight disease of rice. Further study is needed to test their efficacy under natural field condition and a comprehensive research should be undertaken to develop an effective method of controlling sheath blight disease of rice using antagonists.



# Chapter VII

# References

# REFFERENCES


Agrios, G. N. (2005). Plant pathology. 5th Ed. Elsevier Academic Burlington, Mass.USA.

Ahmed, M. M. (2014). Investigation on fruit tree diseases of selected homestead garden of haor areas under Habiganj district. M.S. thesis, Sher-e- Bangla Agricultural University, Dhaka, Bangladesh.

Ali, H. Z. and Nadarajah, K. (2012). Evaluating the efficacy of *Trichoderma* and *Bacillus* isolates as biological control agents against *Rhizoctonia solani. Research Journal Applied Sciences.* **7**(9): 255-259.

Asad, S. A., Ali, N., Hameed, A., Khan, S. A., Ahmad, R., Bilal, M. and Tabassum, A. (2014). Biocontrol efficacy of different isolates of *Trichoderma* against soil borne pathogen *Rhizoctonia solani***.** *Polish Journal of Microbiology.* **63**(1): 95-103.

Atef, N. M. (2008). *Bacillus subtilits* and *Trichoderma harzianum* as wheat inoculants for biocontrol of *Rhizoctonia solani*. *Australian Journal of Basic and Applied Sciences*. **2**: 1411 –1417.

Bashar, M. A., Hossain, M. A., Rahman, M. M., Uddin, M. N. and Begum, M. N. (2010). Biological control of sheath blight disease of rice by using antagonistic bacteria. *Bangladesh Journal of Scientific and Industrial Research.* **45**(3): 225-232.

BBS (2013). Annual Agricultural Statistics, Agriculture Wing, Bangladesh Bureau of Statistics, Ministry of Planning, Govt. of Bangladesh. p.139.

BBS (2014). Statistical year book of Bangladesh. Ministry of Planning, Government of People's Republic of Bangladesh. Bangladesh Bureau of statistics follow this.

Bhagawati, R. (2005). Effects of pH on antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* on sheath blight development and their survival. *Oryza* **42**(4): 290.

Bhat, K. A., Anwar, A. and Wani, A. H. (2009). Evaluation of biocontrol agents against *Rhizoctonia solani* and sheath blight disease of rice under temperate ecology. *Plant Diseases Research*. **24**(1): 15-18.

Bhuiyan, N. I., Paul, D. N. R. and Jabber, M. A. (2002). Feeding the extra millions by 2025-Challenges for rice research and extension in Bangladesh. A key note paper presented in the national workshop on rice research and extension-2002 held at BRRI, Gazipur, Bangladesh.

Boland, G. J. (1990). Biological control of plant diseases with fungal antagonists: challenges and opportunities. *Canadian Journal of Plant Pathology*. **12**(3): 295-299.

Bowman, B. J., Dschida, W. J. and Bowman, E. J. (1992). Vacuolar ATPase of Neurospora crassa: electron microscopy, gene characterization and gene inactivation/mutation. *The Journal of Experimental Biology*. **172**(1): 57-66.

Choi, I. Y., Hong, S. B. and Yadav, M. C. (2003). Molecular and morphological characterization of green mold, *Trichoderma* spp. isolated from oyster mushrooms. *Mycobiology*. **31**(2): 74-80.

Das, B. C. and Hazarika, D. K. (2000). Biological management of sheath blight of rice. *Indian Phytopathology*. **53**(4): 433-435.

Dhingra, O. D. and Sinclair, J. B. (1985). Basic plant pathology methods. CRC Press. Inc. Boca Raton Florida, USA. pp. 13-44.

El-Ghaouth, A. (1997). Biologically based alternatives to synthetic fungicides for the control of postharvest diseases. *Journal of Industrial Microbiology and Biotechnology*. **19**(3): 160-162.

Faltin, F., Lottmann, J., Grosch, R. and Berg, G. (2004). Strategy to select and assess antagonistic bacteria for biological control of *Rhizoctonia solani*. *Canadian Journal of Microbiology*. **50**(10): 811-820.

Faruk, M. I., Bari, M. A., Rahman, M. A., Nahar, M. S. and Khanam, N. N. (1999). Suppression of root knot (Meloidogyne spp.) of tomato using antagonistic isolates of *Trichoderma* species. *Bangladesh Journal Plant Pathology*. **15**: 39-42.

Fernandez, O. D., Perez, G. A., Lope, R. F., Romero, D., De Vicente, A. and Tores, J. A. (2006). Occurrence and distribution of resistance to QOI fungicides in populations of *Podosphaera fusca* in south central Spain. *European Journal of Plant Pathology*. **115**(2): 215-222.

Gnanamanickam, S. S. and Mew, T. W. (1990). Biological control of rice diseases (blast and sheath blight) with bacterial antagonists: an alternate strategy for disease management. *Pest Management in Rice*. **102**(2): 87-110.

Hajieghrari, B., Torabi-Giglou, M., Mohammadi, M.R. and Mahdi, D. (2008). Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. *African Journal of Biotechnology*. **7**: 967-972.

Hatvani, L., Sabolic, P., Kocsube, S., Kredics, L., Czifra, D., Vagvolgyi, C. and Kosalec, I. (2012). The first report on mushroom green mould disease. *Archives of Industrial Hygiene and Toxicology*. **63**(4): 481-487.

Hermosa, M. R., Grondona, I. and Monte, E. (1999). Isolation of *Trichoderma harzianum* from commercial mushroom compost in Spain. *Plant Disease*. **83**(6): 591-591.

Hossain, M. A. (2006). Isolation and analysis of plasmid profile from *Ralstonia solanacearum* and *Pseudomonas* spp. and its reaction to antibiotics. MS Thesis, Department of Plant Pathology. Bangladesh Agricultural University, Mymensingh, Bangladesh. pp. 24-47.

Howell, C.R. (2003). Mechanism employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. *Plant Disease.* **87**: 4-10.

IRRI (2006). World Rice Statistics. Intlernational Rice Research Institute, Philipines.

IRRI, (1987). IRRI Annual Report for 1986. International Rice Research Institute, Los Banos, Laguma, Philipines.

Islam, T. (2009). Population dynamics of *Phomopsis vexans, Sclerotium rolfsii, Fusarium oxysporum* f. sp *lycopersici* and *Trichoderma* in the soil of egg plant field. An M.S. Thesis submitted to the Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh. pp.48-57.

Johnson, L. F. and Curl, E. A. (1972). Methods for research on the ecology of soil-borne plant pathogens. Methods for research on the ecology of soil-borne plant pathogens. pp. 247.

Kadir, T. S., Hikmawati, N., Mubarik, N. R., Susilowati, D. N., Zaffan, Z. R. and Suryadi, Y. (2013). Bioformulation of antagonistic bacterial consortium for controlling blast, sheath blight and bacterial blight diseases on rice. *Asian Journal Plant Pathology.* **7**(3): 92-108.

Kanjanamaneesathian, M. (1994). Biological control of sheath blight of rice with bacterial and fungal antagonists (Doctoral dissertation, Lincoln University).

Kazempour, M. N., Pedramfar, H. and Elahinia, S. A. (2003). Effect of certain fungicides and isolates of antagonistic fungi on *R. solani*, the Causal agent of rice sheath blight. *JWSS-Isfahan University of Technology*. **6**(4): 151-158.

Khan, A. A. and Sinha, A. (2007). Biocontrol potential of *Trichoderma* species against sheath blight of rice. *Indian Phytopathology*. **60**(2): 208-213.

Khan, A. A. and Sinha, A. P. (2005a). Integration of fungal antagonist and organic amendments for the control of rice sheath blight. *Indian Phytopathology*. **59**(3): 363-365.

Khan, A. A. and Sinha, A. P. (2005b). Influence of different factors on the effectivity of fungal bioagents to manage rice sheath blight, in nursery. *Indian Phytopathology*. **58**(3): 289-293.

Khan, A.A. and Sinha, A.P. (2006). Influence of fertilizers on *Trichoderma harzianum* against sheath blight of rice. India. *Annals of Plant Protectiom Sciences*. **14**(2): 481-482.

Khare, A., Singh, B.K. and Upadhyay, R.S. (2010). Biological control of *Pythium aphanidermatum* causing damping off of mustard by mutants of *T. viridae* 1433. *Journal of Agricultural Technology*. **6**:231-243.

Kim, H. S., Park, J. S W., Choi, K. H., Choi, G. L., Ban, S. J., Lee, C. H. and Kim, C. S. (2003). Isolation and characterization of *Bacillus* strains for biological control. *Journal of Microbiology*. **41**(3): 196.

Kiss, L. (2003). A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. *Pest Management Science*. **59**(4): 475-483.

Krishnamurthy, K. (1997). Biological control of rice blast and sheath blight by *Pseudomoans* spp. Survival and migration of biocontrol agents and the induction of systemic resistance in biological disease suppression (Doctoral dissertation, Ph. D. Thesis, university of Madras, India).

Krishnamurthy, K. and Gnanamanickam, S.S. (1998). Biological control of sheath blight of rice: Induction of systemic resistance in rice by plant-associated *Pseudomonas* spp. *Curro Science.* **72**: 331-334.

Kumar, K. V. K., Reddy, M. S., Kloepper, J. W., Lawrence, K. S., Groth, D. E., Miller, M. E. and Du, B. (2009). *In vitro* efficacy of various rhizobacterial isolates against *R. solani*, the causal agent of rice sheath blight disease. Scientific Publishers, India.

Kumar, K. V. K., Yellareddygari, S. K., Reddy, M. S., Kloepper, J. W., Lawrence, K. S., Zhou, X. G. and Miller, M. E. (2012). Efficacy of *Bacillus subtilis* MBI 600 against sheath blight caused by *Rhizoctonia solani* and on growth and yield of rice. *Rice Science*. **19**(1): 55-63.

Kwok, O.C., Fahy, P.C., Ioitink, H.A.J. and Kuter, G.A. (1987). Interactions between bacteria and *T. harzianum* in suppression of *Rhizoctonia* damping off inbark compost media. *Phytopathology*. **77**(8): 1206- 121 2.

Leroux, P. (2003). Modes of action of agrochemicals against plant pathogenic organisms. *Comptes rendus biologies*. **326**(1): 9-21.

M. Seema and N.S. Devaki (2012). *In vitro* evaluation of biological control agents against *Rhizoctonia solani*. *Journal of Agricultural Science and Technology*. **8**(1): 233-240.

Maji, E. A. and Shaibu, A. A. (2012). Effects of antibiotics on biological control agents and their efficacy to control rice sheath blight (*R. solani* AG-I. 1). *Journal of Agricultural Science and Technology*. **8**(3): 993-997.

Mansoor, Yamasaki, S., Nishioka, M., Shiraishi, I. Iiyama, K. and Matsuyama, N. (2007). Antibacterial activities of *Pseudomonads* against plant pathogenic organisms and efficacy of *Pseudomonas aeruginosa* (ATCC7700) against bacterial wilt of tomato. *Annals of the Phytopathological Society of Japan.* **63**(6): 417-424.

Mathivanan, N., Prabavathy, V.R. and Vijayanandraj, V.R. (2006). Application of talc formulations of *Pseudomonas fluorescens* and *Trichoderma viride*, India. *Journal of Phytopathology*. **154**(11/12): 697- 701.

McGrath, M. T. (2001). Fungicide resistance in cucurbit powdery mildew: experiences and challenges. *Plant disease*. **85**(3): 236-245.

Meena, R. L., Rathore, R. S. and Mathur, K. (2003). Efficacy of biocontrol agents against *R. solani* f. sp. Sasakii causing banded leaf and sheath blight of maize. *Journal of Mycology and Pathology.* **33**: 310 –312.

Mew T.W. and A.M. Rosales (1985). Inflence of *Trichoderma* on survival of *Thanatephorus cucumeris* in association with rice in the tropics. In: Ecology and Management of Soil-borne Pathogens. American Phytopathological Society, St. Paul, MN, USA. pp. 117–120.

Mew, T. W. and Rosales, A. M. (1992). Control of *Rhizoctonia* sheath blight and other diseases by rice seed bacterization. In: Tjamos E S, Papavizas G C, Cook Research Journal of Biological Control of Plant Diseases. New York: Plenum Press: pp. 113–123.

Naeimi, S., Khosravi, V., Hoda, H., Shokri Marzangoo, R., Oskou, T., Omrani, M. and Bagheri, F. (2011). Biological control of rice sheath blight with promising *Trichoderma* strains in paddy fields. *AGRIS.* **21**(2): 34.

Naeimi, S., Okhovvat, S. M., Javan-nikkhah, M., Vagvolgyi, C., Khosravi, V. and Kredics, L. (2010). Biological control of *Rhizoctonia solani* AG1-1A, the causal agent of rice sheath blight with *Trichoderma* strains. *Phytopathologia Mediterranea*. **49**(3): 287−300.

Nagarajkumar, M., Bhaskaran, R. and Velazhahan, R. (2004). Involvement of secondary metabolites and extracellular lytic enzymes produced by *P. fluorescens* in inhibition of *R. solani*, the rice sheath blight pathogen. *Microbiological Research*. **159**(1): 73-81.

Nagorska, K., Bikowski, M. and Obuchowki, M. (2007). Multicellular behaviour and production of a wide vaiety of toxic substances support usage of *Bacillus subtilis* as a powerful biocontrol agent. *Acta Biochimica Polonica.* **54**(3): 495-508.

Naidu, V.D. (1992). Influence of sheath blight of rice on grain and straw yield in some popular local varieties. *Journal of Research Assam Agricultural University.* **10**: 78-80.

Nakkeeran, S., Fernando, D. W. G. and Siddiqui, Z. A. (2005). Plant growthpromoting rhizobacteria formulations and its scope in commercialization for the management of pests and diseases. *In:* Siddiqui Z. A. PGPR: Biocontrol and Biofertilization. Aligarh, India: Springer Publications. pp. 257–296.

Nakkeeran, S., Marimuthu, T., Chandrasekar, G., Sankaralingam, G. and Rnukadevi, P. (2006). Achievements in biological control of diseases with antagonistic organisms, India. pp.350-362.

Ou S.H., (1985a). Rice diseases 2nd edition. Commonwealth Mycological Institute, Kew, UK.p. 380.

Ou, S.H. (1985b). Rice diseases. Second Edition. Commonwealth Mycological Institute. The Cambrian News (Aberystwyth) Ltd. Kew. Surrey, England. pp. 272-286.

Pal, R., Biswas, M. K., Mandal, D. and Naik, B. S. (2015). Management of sheath blight disease of rice through bio control agents in west central table land zone of Odisha. *International Journal*. **3**(11). 747-753.

Park, D. S., Sayler, R. J., Hong, Y. G., Nam, M. H. and Yang, Y. (2008). A method for inoculation and evaluation of rice sheath blight disease. *Plant Disease*. **92**(1): 25-29.

Peng, D., Li, S., Wang, J., Chen, C. and Zhou, M. (2014). Integrated biological and chemical control of rice sheath blight by *Bacillus subtilis* NJ-18 and jinggangmycin. *Pest Management Sci*ence. **70**(2): 258-263.

Prasad, B. N. and Kumar, M. R. (2011). Comparative efficacy of different isolates of *Trichoderma* spp. against *Rhizoctonia solani*, incitant of sheath blight of rice. *Indian Journal Fundamental and Applied Life Science*. **1**(3): 107-111.

Rahman, Z. (2007). Biological control of sheath blight of rice using antagonistic *Trichoderma*. M.S. Thesis. Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh. pp. 1-58.

Razu, M.A. (2014). Eco-friendly management of rice diseases. M.S. Thesis, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Reyes, R., Rodriguez, T.G., Pupo, A.D., Alarcon, L. and Limonta, Y. (2007). *In vitro* effecacy of *Trichoderma harzianum* for biocontrol of *Rhizoctonia solani* and *Pyricularia grisea* isolated from rice crop *(Oryza saliva* L.). *Fitosanidad*. **11**: 29-33.

Rodriguez, H.A., Nass, H. Cardona, R. and Aleman, L. (1999). Altematives to control sheath blight caused by *Rhizoctonia solani* in rice. *Fitopatologia Venezolana*. **12**(1): 18-2l.

Romero, D., Vicente, A., Rakotoaly, R.H., Dufour, S.E., Veening, J.W., Arrebola, E., Cazorla, F.M., Kuipers, O., Paquot, M. and Garcia, A.P. (2006). The iturin and fengycin families of Lipopeptides are key factors in antagonism of *Bacillus subtilis* towards *Podosphaera fusca. Molecular Plant Microbe Interactions*. **118**(2): 323-327.

Seema, M. and Devaki, N. S. (2012). *In vitro* evaluation of biological control agents against *Rhizoctonia solani*. *Journal of Agricultural Technology*. **8**(1): 233-240.

Shah, S., Nasreen, S. and Sheikh, P. A. (2012). Cultural and morphological characterization of *Trichoderma* spp. Associated with green mold disease of *Pleurotus* spp. in Kashmir. *Research Journal of Microbiology*. **7**(2): 139.

Shahjahan, A. K. M., Duve, T. and Bonman, J. M. (1987). Climate and rice diseases. Weather and Rice. International Rice Research Institute, Los Banos, Philippines. pp. 125-137.

Shahjahan, A. K. M., Duve, T. and Bonman, J. M. (1987). Climate and rice diseases. Weather and Rice. International Rice Research Institute, Los Banos, Philippines. pp. 125-137.

Shahjahan, M. and Miah, M. A. H. (1986). The problems of *Pyrilla perpusilla* and the impact of its natural enemies. *Bangladesh Journal of Zoology*. **14**(1): 9-13.

Shrestha, B. K., Karki, H. S., Groth, D. E., Jungkhun, N. and Ham, J. H. (2016). Biological control activities of rice associated *Bacillus* sp. Strains against sheath blight and bacterial panicle blight of rice. *Plos one*. **11**(1): 235-252.

Singh, V., Singh, U. S. and Singh, K. P. (2003). Relative virulence of rice isolates of *R. solani* on rice and wheat. *Indian Phytopathology*. **56**(2): 186-187.

Somani, A. K. and Arora, R. K. (2010). Field efficacy of *Trichoderma viride*, *Bacillus subtilis* and *Bacillus cereus* in consortium for control of *Rhizoctonia solani* causing sheath blight disease of rice. *Indian Phytopathology*. **63**(1): 23-25.

Stein, T. (2005). *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Molecular Microbiology*. **56**(4): 845-857.

Sudhakar, R., Rao, K.C. and Reddy, C.S. (1998). Studies on antagonism of *Trichoderma* and *Gliocladium* species against *Rhizoctonia solani* of rice. *Indian Journal Plant Protection*. **26**(1): 25-29.

Sundar, A.R., Das, N.D. and Krishnaveni, D. 1995. *In vitro* Antagonism of *Trichoderma* spp. against two fungal pathogens of Castor. *Indian Journal Plant Protection*. **23**(2): 152-155.

Tang, J., Tian, B., LingXia, M., Ping, W. and Hong C. (2001). Biocontrol of *Rhizoclonia solani* with *Trichoderma* spp. CRRN. *Chinese Rice Research Newsl.* **9**: 8.

Tewari, L. and Singh, R. (2005). Biological control of sheath blight of rice by *Trichoderma harzianum* using different delivery systems. *Indian Phytopathology*. **58**(1): 35-40.

Tuite, J. 1969. Plant Pathological Methods: Fungi and Bacteria Burgess Pub.C0., Minneapolis, Minn., USA. p. 293.

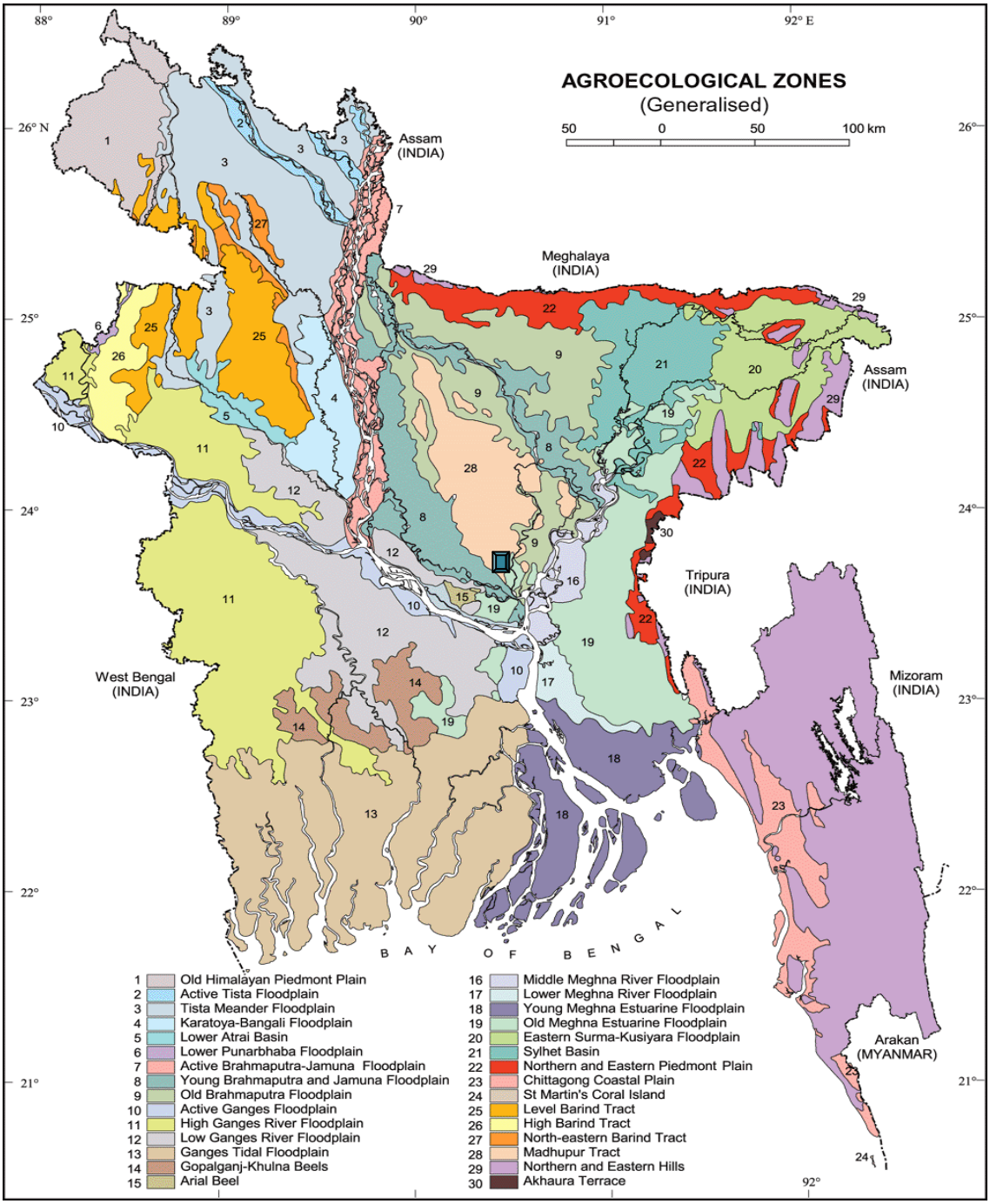
Wang, Y., Shen, Y. and Xu, T. (2000). Study on *Trichoderma harzianum* strains to control rice sheath blight, China. *Acta Phytophylacica Sinica*. **27**(2): 97-101.

Yang, D., Wang, B., Wang, J., Chen, Y. and Zhou, M. (2009). Activity and efficacy of *Bacillus subtilis* strain NJ-18 against rice sheath blight and Sclerotinia stem rot of rape. *Biological Control*. **51**(1): 61-65.

Yugander, A., Ladhalakshmi, D., Prakasham, V., Mangrauthia, S. K., Prasad, M. S., Krishnaveni, D. and Laha, G. S. (2015). Pathogenic and genetic variation among the isolates of *R. solani* (AG 1-IA), the rice sheath blight pathogen. *Journal of Phytopathology*. **163**(6): 465-474.

# APPENDICES

## Appendix I. Map showing the experimental site under the study




The experimental site under study

**Appendix II. Monthly average relative humidity, maximum and minimum temperature, rainfall and sunshine hour during the experimental period (May 2015 to October 2015)**

| Month | Average RH (%) | Average Temperature($^0$C) | | Total Rainfall | Average Sunshine Hours |
|---|---|---|---|---|---|
| | | Min. | Max. | | |
| May | 81 | 32.1 | 34.5 | 339.4 | 4.7 |
| June | 84 | 31.4 | 33.4 | 415.6 | 4.8 |
| July | 89 | 30.5 | 32.8 | 512.5 | 4.7 |
| August | 91 | 29.1 | 32.2 | 415.8 | 4.6 |
| September | 90 | 27.2 | 30.4 | 350.3 | 4.6 |
| October | 93 | 26.8 | 29.5 | 201.9 | 4.5 |

**Source:** Bangladesh Meteorological Department (Climate division), Agargaon Dhaka-1207.

## Appendix III. The mechanical and chemical characteristics of collected soil for the experiment

### a. Mechanical composition:

| Particle size | constitution |
|---|---|
| Sand | 40% |
| Silt | 40% |
| Clay | 20% |
| Texture | Loamy |

### b. Chemical composition:

| Soil characters | Value |
|---|---|
| Organic matter | 1.44 % |
| Potassium | 0.15 meq/100 g soil |
| Calcium | 3.60 meq/100 g soil |
| Magnesium | 1.00 meq/100 g soil |
| Total nitrogen | 0.072 |
| Phosphorus | 22.08 µg/g soil |
| Sulphur | 25.98 µg/g soil |
| Boron | 0.48 µg/g soil |
| Copper | 3.54 µg/g soil |
| Iron | 262.6 µg/g soil |
| Manganese | 164 µg/g soil |
| Zinc | 3.32 µg/g soil |

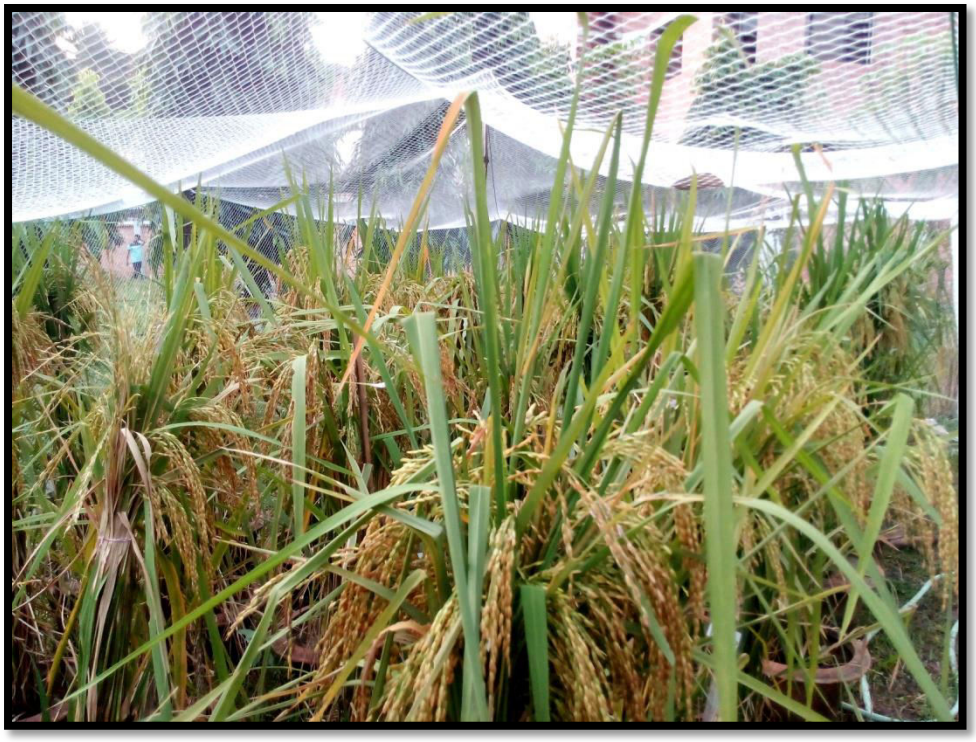
**Source:** Soil Resources Development Institute (SRDI), Khamarbari, Dhaka

## Appendix IV. Composition of PDA media

The compositions of the media used in this thesis work are given below: Unless otherwise mentioned all media were autoclaved t 121 0c for 15 minutes at 15 lb pressure.

| **Material** | **Volume** |
|---|---|
| Distilled water | 1000 ml |
| Potato | 200 g |
| Dextrose | 20 g |
| Agar | 20g |

**Appendix V. A view of the experimental site in pot experiment**



**Appendix VI. Ripening rice in net house**

**Appendix VII. A view of severely infected rice plant by *R. solani***

**Appendix VIII. During data collection from experiment field**