

BIOLOGICAL CONTROL OF SCLEROTIUM ROT OF SUGARBEET

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ABSTRACT

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Efficacy of IPM Lab Biopesticide for controlling Sclerotium rot of sugarbeet was studied. *Sclerotium rolfsii* was identified as causal organism of Sclerotium rot of sugarbeet. Incidence of Sclerotium rot of sugarbeet was observed at two locations. The highest disease incidence (24%) was observed at BAU farm, Mymensingh in compared to BSRI farm (5%). In an *in-vitro* assay it was observed that the growth of *S. rolfsii* was inhibited by *Trichoderma harzianum* based IPM lab biopesticide indicating antagonistic effect against *S. rolfsii*. From the pot experiment, it was observed that plants treated with biopesticide (*Trichoderma* based) reduced Sclerotium rot infection. A field experiment was conducted to investigate the efficacy of *Trichoderma* based biopesticide to control Sclerotium rot of sugarbeet.

The biopesticide was mixed up with soil during final land preparation at four doses viz. 0, 40, 60 and 80 kg/ha. After germination of sugarbeet plants, periodically infections were recorded and results showed that incidence of Sclerotium rot of sugarbeet was very low in biopesticide treated plots, where the best performance was recorded with biopesticide treatment at 80 kg/ha. The highest dose (80 kg/ha) of biopesticide used in this experiment showed significantly better growth, the highest yield (65.0 t/ha) and lower Sclerotium rot incidence (10.67%) than the non-treated plots. Thus, *Trichoderma* based IPM Lab biopesticide is effective in reducing Sclerotium rot of sugarbeet.

Key words: Sugarbeet, Sclerotium rot, biopesticide, biological control

INTRODUCTION

Sugarbeet (*Beta vulgaris*) is a biennial root crop and the only species of agricultural importance in *Beta* genus. Sugar beet provides at least 30% of the world sugar for human consumptions ranking second to sugarcane in terms of sugar production in the world (FAO 2011). Most importantly, sugarbeet is the most salt tolerant terrestrial crop species (Colmer and Flowers 2008). Therefore, cultivation of tropical sugarbeet might be a very good alternative crop for drought prone barind area and salinity prone coastal area of Bangladesh. The total demand of sugar in Bangladesh is 1.8 million metric tons where the average production is only 0.8 million metric tons (BSRI 2010). So it is very important to move towards an alternative crop to produce more sugar per year. Cultivation of tropical sugarbeet might be a successful initiative towards that purpose in the country. But the soil borne fungal pathogen *Sclerotium rolfsii* attacks sugarbeet tubers a few weeks prior to harvest, causing up to 50% -80% losses in crop yield and quality (Khettabi *et al.* 2004). It is difficult to manage root rot disease caused by *Sclerotium* because of the wide host range and pervasive presence of sclerotia in tropical to subtropical areas. If chemical fungicides are

applied during harvesting period, then fungicide residues may pose significant risks to food safety. Again, the indiscriminate use of chemicals for controlling disease of crop plants resulted environmental pollution, health hazards etc. Moreover, huge amount of foreign currency is needed to purchase plant protecting chemicals. As an alternate means of avoiding these problems, application of biological agents are being used for combating the diseases with the aim of increasing crop production. Biological control represents a natural and ecological approach in controlling diseases that reduces chemical inputs and their effects (Mukhopadhyay 1994). *Trichoderma* based biopesticides have gained considerable recognition as biological agent. Several strains of *Trichoderma* have been found to be effective as bio-control agent of various soil borne plant pathogenic fungi such as *Fusarium*, *Sclerotium*, *Rhizoctonia* etc. (Chet and Inber 1994). *Trichoderma harzianum* isolate shows low ability in coiling round hyphae of *Sclerotium rolfsii*, but it is very effective in penetrating or growing inside them. *T. harzianum* adversely affect them even without penetration (Ferrata and D'Ambra 1985). IPM Lab biopesticide has been demonstrated to control a wide range of soil borne plant pathogens including *Sclerotium rolfsii* (Nabi 2010; Islam 2008). Therefore, the present research was undertaken

to determine the incidence of Sclerotium rot of sugarbeet at BAU farm and BSRI, Ishurdi and to control Sclerotium rot of sugar beet by using *Trichoderma harzianum* based IPM lab biopesticide.

MATERIALS AND METHODS

The experiment was conducted at Seed Pathology Centre, Agronomy field laboratory, Bangladesh Agricultural University (BAU), Mymensingh and BSRI, Ishurdi during 2014 to 2015. Sclerotium rot disease incidence was recorded by following the formula of Ansari (1995).

Naturally infected sugarbeet grown in the field of the Department of Agronomy, BAU, was collected and washed in fresh water and surface sterilized with 10% Clorox for 1 minute followed by three times washing in distilled water. Inocula were placed on PDA acidified with one drop of 5% lactic acid and incubated at $22^{\circ}\pm 2^{\circ}$ C for 7 days. After incubation, white mycelia and sclerotia were formed. The pathogen was purified and multiplied subsequently through hyphal tip culture on PDA for preparation of inocula (sclerotia). Biopesticide (formulated *Trichoderma*) was collected from IPM Laboratory, Department of Plant Pathology, BAU, Mymensingh. An *in-vitro* study was conducted to find out the antagonistic effect of *Trichoderma* against *Sclerotium rolfisii* on PDA by dual culture technique. There after inhibition percentage of the *Sclerotium rolfisii* were calculated based on the growth of the pathogen on PDA plates in absence of *Trichoderma* following the formula as suggested by Sundar *et al.* (1995). The soil samples were collected from infected sugarbeet field, Department of Agronomy, BAU. Population of *Trichoderma harzianum* in the soil (before and after application of biopesticide) was determined by soil dilution plate techniques as described by Dhingra and Sinclair (1985). Population of individual fungus in each soil sample was estimated following the formula given below: Population of fungi = Average number of total colonies/ml in 4 Petri-dishes x Dilution factor (10^4). Cauvery, a variety of sugarbeet, was collected from BSRI, Ishurdi. A pot experiment was done to select the effective treatment to control Sclerotium rot of sugarbeet. Soil for raising seedlings in earthen pots were prepared as described by Nabi (2010) and Dhasgupta (1988). Five seeds were sown in each pot. Seedlings of sugarbeet were grown with intensive care (Islam 2006).

A total of eight treatments were used where: T_0 = control, T_1 = *S. rolfisii*, T_2 = *S. rolfisii* + 40 g biopesticide/plant, T_3 = *S. rolfisii* + 60 g biopesticide/plant, T_4 = *S. rolfisii* + 80 g biopesticide/plant, T_5 = 40g biopesticide/plant, T_6 = 60g biopesticide/plant

and T_7 = 80 g biopesticide/plant. Biopesticide at different doses as described in the above treatments was used in pot soils at the base of the plant and after 7 days of soil drenching inoculation was done by *S. rolfisii*. Pure culture of *Sclerotium rolfisii* was prepared in PDA plate which was used as inocula for inoculation purpose. For treatment T_1 , T_2 , T_3 and T_4 (total 4 treatments x 3 replications) twelve plants were individually inoculated in each pot at 30 days after germination. Small (2mm diameter) blocks of 7 days old culture of the test pathogen grown on PDA served as the inocula. The base of the stem (about 3 mm above the soil level) was picked with the help of a fine pointed sterile inoculating needle. Then the inoculation block was placed on the injured point and a small piece of sterile cotton soaked with sterile water placed above the inoculum to keep it moist (Purna 2013). After inoculation the pots were kept in green house. Watering was done regularly to maintain adequate humidity. Disease reaction and No. of infected plants were recorded for each treatment.

In case of field experiment, land was prepared as described by Nabi (2010) and cow dung and fertilizers were applied as recommended by BARC (2012). A total of four treatments were used as follows: T_0 = Control (without Bio-pesticide), T_2 = Bio-pesticide 40 kg/ha, T_3 = Bio-pesticide 60 kg/ha and T_4 = Bio-pesticide 80 kg/ha. After final preparation of land, the plots $5m^2$ (2 x 2.5 m) were prepared. The IPM lab Biopesticide was used following the four rates (0, 40, 60 and 80 kg /ha) was mixed with the soil. Sugarbeet seeds were collected from BSRI and sown on November, 2014 after soaking in water (RR x SS =50 cm x 20cm). Two seeds were placed in a hill to protect the germination failure. Field experiments for sugarbeet were set following RCBD with four treatments and three replications. The size of unit plot was $5m^2$. Data on No. of healthy plants, No. of diseased plants, Length (cm) and weight (g) of shoot, Length and girth of beet (cm), Length (cm) and Weight (g) of leaf, Weight of beet (g)/plant and Yield of sugarbeet (t/ha). Data were tabulated and analyzed through a standard computer package statistical procedure by Wasp-2.

RESULTS

The sugarbeet field at BAU farm and BSRI were naturally infected by *Sclerotium rolfisii*. The typical Sclerotium rot symptoms of sugarbeet showed a rot with dry black to brown lesions around the beet. The plants were still alive with pale green leaves. Numbers of round brown to black sclerotia were found (Fig. 1). Sclerotium rot incidence (%) was recorded at two locations at BAU farm and BSRI (Table 1).



Fig. 1. Symptoms of Sclerotium rot of sugarbeet

- A. White mycelial mat grown at the base of the plant
- B. Round brown sclerotia

Table 1. Incidence of sclerotium rot of sugarbeet in two locations during 2014

Months	% Disease incidence	
	BAU farm	BSRI farm
January	15.0	5.0
February	20.0	8.0
March	24.0	10.0
LSD	7.026	NS

The highest disease incidence (24%) was observed in March at BAU farm followed by January and February 15% and 20%, respectively. At BSRI the lowest disease incidence (5 %) was found in January followed by February and March 8% and 10%, respectively. However, Sclerotium rot incidence was

lower in BSRI farm than BAU farm. The fungus *T. harzianum* was tested against *S. rolfsii* on PDA by dual culture method shown in Fig. 2(A and B). *T. harzianum* showed significant growth reduction of *S. rolfsii* on PDA Fig. 2(C).

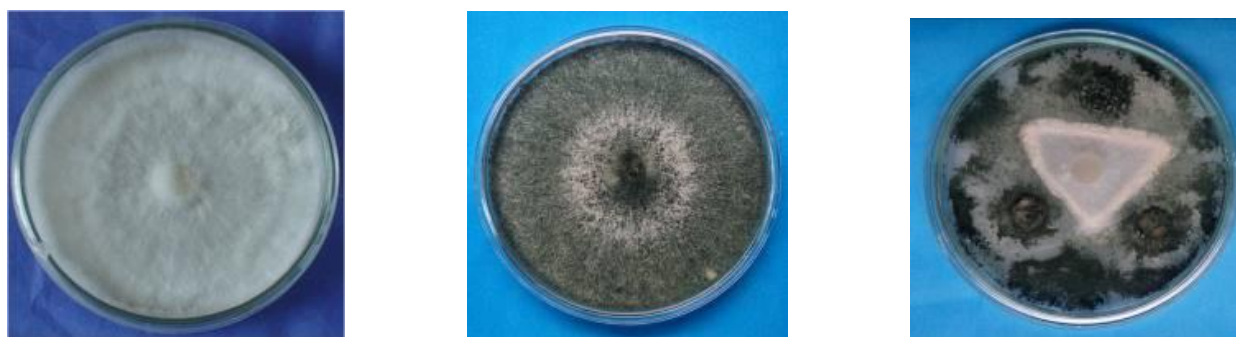


Fig. 2. Antifungal activity of *Trichoderma harzianum* against *S. rolfsii*

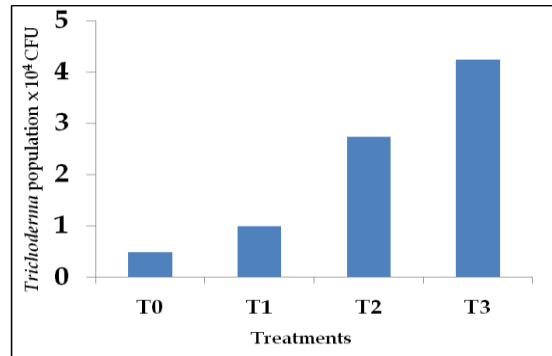
- A: Culture of *Sclerotium rolfsii*
- B: Culture of *Trichoderma harzianum*
- C: Duel culture of *Trichoderma harzianum* and *Sclerotium rolfsii*

Population of *T. harzianum* in the soils of different treatments were weekly monitored for one month and

the results are presented in Fig. 3. From dilution plate technique, it was observed that *Trichoderma* population differed due to different treatments applied in the soil. The total number of colony gives the

understanding about the multiplication of *Trichoderma* itself in soil. One month after treatments application, the highest number of *Trichoderma* colony (4.25×10^4 CFU) was recorded in treatment T₃ and the lowest (0.50×10^4 CFU) was found in treatment T₀. In treatment T₁ and T₂ the total number

of colony was 1.00×10^4 and 2.75×10^4 CFU respectively. The total number of colony is an indicator of the *Trichoderma* population in the soil. It gives the evidence that with the increase of rate of biopesticide the number of population was increased.



T₀ = Control
T₁ = Biopesticide 40kg/ ha

T₂ = Biopesticide 60kg/ha
T₃ = Biopesticide 80kg/ha

Fig 3. *Trichoderma* population of field soil under different treatments

A total of eight treatments were used in the pot experiment to observe the disease reaction of sugarbeet *Sclerotium* rot against *Sclerotium rolfsii*. In treatment T₁ where only *S. rolfsii* was used, three days after inoculation of plants with the pathogen, a white mycelial growth was observed near the inoculated plant base. White mycelial mat was formed rapidly day by day. Immature white rounded sclerotia were also observed on soil surface near plant base, which frequently turned brown to black and started to

germinate producing white mycelia (Photo was not shown). Symptoms as expressed by the plants due to *Sclerotium* rot were exhibited through development of lesions resulting characteristics *Sclerotium* rot of the plants, thus enhancing wilting, yellowing and leaf fall, ultimately killed plants. Sugarbeet plants treated with other treatments did not show any infection may be due to the antagonistic effect of *T. harzianum* (Table 2).

Table 2. Effect of treatments on *Sclerotium* rot incidence of sugarbeet in the pot experiment

Treatments	Disease reaction
T ₀	+
T ₁	-
T ₂	+
T ₃	+
T ₄	+
T ₅	+
T ₆	+
T ₇	+

(+) = resistant, (-) = susceptible, T₀ = non treated, T₁ = *S. rolfsii*,
T₂ = *S. rolfsii* + 40g biopesticide/plant, T₃ = *S. rolfsii* + 60g biopesticide/plant
T₄ = *S. rolfsii* + 80g biopesticide/plant, T₅ = 40g biopesticide/plant, T₆ = 60g biopesticide/plant and T₇ = 80g biopesticide/plant

The lowest infected plants (10.67%) were found in the treatment T₃ (biopesticide 80 kg/ha) followed by the treatments T₂ and T₁ (26.67% and 42.00%) respectively. The highest (56.67%) infection was

found with control treatment T₀ (Table 3). The highest significant length of shoot (72.76 cm) was observed in the treatment T₃ with biopesticide (80 kg/ha) followed by the treatments T₂ and T₁ (66.99 and 58.83 cm)

respectively and . the lower shoot length (50.09 cm) was found in treatment T₀. The highest significant length (9.08 cm) of beet was observed in the treatment T₃ with biopesticide (80 kg/ha) followed by the treatments T₂ and T₁ (8.13 and 6.03 cm) respectively. Maximum fresh weight (393.21g) of shoot was recorded in the treatment T₃ followed by the treatments T₂, T₁ and T₀ (252.05, 225.17 and

200.00g) respectively. Significantly the highest weight (703.17 g) of beet was observed in the treatment T₃ followed by the treatments T₂, T₁ and T₀ (643.07, 599.27 and 553.23 g) respectively. Significantly the highest beet girth (23.66 cm) was found in the treatment T₃ followed by the treatments T₂, T₁ and T₀ (20.99, 17.73 and 15.80 cm) respectively (Table 3).

Table 3. Effect of Biopesticide at different doses on the growth and yield of sugarbeet

Treatments	% infected plants	Length of shoot (cm)	Weight of shoot (g)	Length of beet (cm)	Weight of beet (g/plant)	Beet girth (cm)	Yield (t/ha)
T ₀	56.67	50.09	200.00	5.13	553.23	15.80	38.96
T ₁	42.00	58.83	225.17	6.03	599.23	17.73	43.14
T ₂	26.67	66.99	252.05	8.13	643.07	20.99	53.13
T ₃	10.67	72.76	393.21	9.08	703.17	23.66	65.00
LSD(0.05)	4.465	1.079	7.129	0.420	1.788	0.385	3.480

T₀ = Control, T₁ = Biopesticide 40kg/ha, T₂ = Biopesticide 60kg/ha, T₃ = Biopesticide 80kg/ha

It was revealed that *Trichoderma* based biopesticide gave positive response in increasing yield due to the improvement of growth characters of sugarbeet by suppressing disease incidence. The effects of different treatments on the % reduction of disease over control are presented in Table 4. The highest % reduction of Sclerotium rot of sugarbeet over control was observed in plots treated with treatment T₃ (81.17%) followed by treatments T₂ and T₁ (52.94% and 25.89%) respectively. The highest significant yield (65.00 t/ha)

of beet was observed in the treatment T₃ with biopesticide (80 kg/ha) followed by the treatments T₂, T₁ and T₀ (53.13, 43.14 and 38.96 t/ha) respectively (Table 4). The effects of different treatments on the % increase of yield over control are presented in (Table 4). The highest % increased of sugarbeet yield over control was observed in plots treated with treatment T₃ (66.84%) followed by treatments T₂ and T₁ (36.37% and 10.73%), respectively.

Table 4. Effects of treatments on % reduction of Sclerotium rot and % increase of yield of sugarbeet

Treatments	%DI	% Diseases reduction over control	Yield of beet (t/ha)	% Increase of yield over control
T ₀	56.67	-	38.96	-
T ₁	42.00	25.89	43.14	10.73
T ₂	26.67	52.94	53.13	36.37
T ₃	10.67	81.17	65.00	66.84

T₀ = Control, T₁ = Biopesticide 40kg/ha, T₂ = Biopesticide 60kg/ha, T₃ = Biopesticide 80kg/ha

DISCUSSION

In this experiment, the incidence of Sclerotium rot of sugarbeet and their control by using of *Trichoderma* based biopesticide was investigated in different growth periods of sugarbeet plants. Symptoms of Sclerotium rot of sugarbeet as observed in the present investigation conformed to those reported by Yaqub and Shahzad (2005), Abada (1994) and Waraitch *et al.* (1986). The causal organism of Sclerotium rot of sugarbeet identified as *Sclerotium rolfsii* has earlier been reported by many workers (Yaqub and Shahzad, 2005; Abada,1994; Upadhyay and Mukhopadhyay,

1986; Chaluat, *et al.* 1981;).Incidence of Sclerotium rot of sugarbeet was observed at two locations of BAU farm and BSRI in 2014. The highest disease incidence (24%) was observed in March at BAU farm (Table 1). The lowest incidence was found at BSRI (5%) in January. However, these findings are in agreement with the statement of Waraitch *et al.* (1986) who showed Sclerotium rot incidence in sugarbeet upto 50% at 25-30°C with high soil moisture. The present study indicated that IPM Lab biopesticide (*Trichoderma* based) has great significant effect in controlling Sclerotium rot of sugarbeet. To observe antagonistic effect the fungus *Trichoderma*

harzianum was tested against *Sclerotium rolfsii* on PDA by dual culture method. *Trichoderma harzianum* showed significant growth reduction (100%) of *Sclerotium rolfsii* on PDA. The findings are supported by the report of Das *et al.* (2000), Begum (1997), Benhamou and Chet (1996). IPM Lab biopesticide was formulated by *Trichoderma harzianum*. Therefore, it is necessary to determine *Trichoderma* population in the treated soil. From the experiment it was seen that the initial population of *Trichoderma* is very low. When biopesticide was supplemented the *Trichoderma* population was found high in the soil. The highest *Trichoderma* population (4.25×10^4 CFU) in soil was observed in T₃ (@80kg/ha) treatment and the lowest population (0.50×10^4 CFU) were observed in T₀ treatment (Fig. 1). This is supported by the report of Upadhyay and Mukhopadhyay (1986) who reported that degree of control of *Sclerotium* rot of sugarbeet increased with increasing amount of *Trichoderma* population. After inoculation, plants those treated with *Trichoderma* based biopesticide showed resistant reaction against *S. rolfsii* and others died due to pathogenic infection. These findings are supported by the report of Singh *et al.* (2007), Anand and Singh (2004). Agrawal *et al.* (1978) who reported that in pot experiment, the antagonist *Trichoderma harzianum* controlled the plant death caused by *Sclerotium rolfsii*. Field experiment was done to determine the efficacy of IPM Lab Biopesticide in controlling *Sclerotium* rot of sugarbeet. These findings are in agreement with the statement of Nabi (2010) and Islam (2010) who reported the potential of Biopesticide in controlling *Sclerotium* rot of eggplant, Indian spinach, tomato. The biopesticide was applied to soil at four doses-0, 40, 60, 80 kg/ha which is supported by Ali and Meah (2007) and Elad *et al.* (1980) who reported that for controlling *Sclerotium rolfsii* infection of bean, soil inoculation with *Trichoderma harzianum* reduced *S. rolfsii* and yield was increased by 20%.

Significant effect of bio-pesticide was recorded on growth parameters of sugarbeet like plant height, plant weight, beet length, and yield and yield contributing characters.

From the experiment it was observed that the application of biopesticide @ 80 kg/ha reduced *Sclerotium* rot disease incidence (10.67%) supported by the report of Xu *et al.* (1993) and increased growth parameters such as 80kg/ha biopesticide yielded highest plant height (79.68 cm), leaf weight (340.05 g/plant), beet length (9.08 cm) and yield of beet (65.00 ton/ha) (Table 3). These findings are supported by the report of Patale and Mukadam (2007) who reported that additional advantage for *Trichoderma* is that it increased growth in various plants and Pranab *et al.* (2002) who reported that *T. harzianum* was the

inhibitor to *S. rolfsii* which showed 61.5% inhibition in mycelial growth of the pathogen. Soil application of *Trichoderma* spp. inoculum at the time of transplanting, reduced disease incidence and increased growth parameters. On the other hand, the highest reduction (81.17%) of *Sclerotium* rot of sugarbeet over control was observed in plots treated with treatment T₃ (Table 4). Upadhyay and Mukhopadhyay (1986) reported that application of *T. harzianum* gave up to 88% disease control in sugarbeet seedlings, infected with *Sclerotium rolfsii*. Sultana and Hossain (1999) reported that *T. harzianum* contributed 47.85 to 112.49% reduction of *Sclerotium* rot of lentil over control. The highest significant length of shoot (72.76 cm) and maximum fresh weight (393.21g) of shoot was observed in the treatment T₃ with biopesticide 80 kg/ha (Table 3) supported by the report of Pranab *et al.* (2002) who found increased shoot weight of tomato and Inbar *et al.* (1994) who found 23.8% increase in seedlings height of cucumber due to application of *T. harzianum*. The highest significant length (9.08 cm) and highest weight (703.17 g) of beet was observed in the treatment T₃ (biopesticide 80 kg/ha) (Table 3). These findings are in agreement with the statement of Pranab *et al.* (2002) and Shafiq (2008) who reported that application of *T. harzianum* reduced disease incidence of collar rot of tomato, caused by *S. rolfsii* and increased dry mass of root. Inbar *et al.* (1994) found that *T. harzianum* increased 96.1% leaf area in cucumber seedlings. In this study, the highest significant yield (65.00 t/ha) and % increase of sugarbeet yield over control was observed in plots treated with treatment T₃ (66.84%) supported by the report of Anand and Singh (2004), Pranab *et al.* (2002), Das *et al.* (2000) and Sultana and Hossain (1999). They reported that *T. harzianum* resulted in *Sclerotium* rot disease control and increased yield per plant. Elad *et al.* (1980) also reported that soil inoculation with *T. harzianum* reduced *S. rolfsii* infection and increased 20% yield of bean.

IPM Lab Biopesticide is effective against *Sclerotium rolfsii* and its antagonistic action is strong enough to control *Sclerotium* rot of sugarbeet that also increased the plant growth and yield. So, IPM lab biopesticide can successfully be used for controlling *Sclerotium* rot of sugarbeet in Bangladesh.

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