INTEGRATED MANAGEMENT OF STEM ROT OF POTATO CAUSED BY *SCLEROTIUM ROLFSII*

M. T. Rubayet[1] and M. K. A. Bhuiyan[2]

[1] *Lecturer and* [2]*Professor, Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur -1706, Bangladesh*

E-mail of corresponding author: tanbir86plp@gmail.com

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ABSTRACT

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An attempt was taken for management of stem rot of potato caused by *Sclerotium rolfsii* by integration of bio-control agent, organic amendment with fungicide at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur-1706, Bangladesh during 2009-2010. Based on the preliminary laboratory evaluation, Provax-200, *Trichoderma harzianum* isolate T-10 and mustard oil cake were selected as the component of integrated management against stem rot disease of potato in the field experiment. In the field trial, among the different treatments including fungicide, organic amendment and *T. harzianum* either individual or in combination of wheat grain colonized *T. harzianum* @ 90 g/m^2 with Provax-200 @ 0.02% treated seed and mustard oilcake @ 60 g/m^2 was appeared to be significantly superior in controlling the pre and post emergence mortality of potato caused by S. *rolfsii* and also significantly increased the yield of potato. The lowest 5.83% disease incidence and highest 85.12% reduction over control-2 were found in the treatment T_9. Simultaneously, the maximum disease management 85.05% and yield increased 23.61 t/ha over control-2 were recorded in the treatment T_9 and followed by T_1 (Control-1) where seeds were sown in uninoculated field and without any amendment.

Key words: Integrated management, Provax 200, Mustard oil cake, *T. harzianum*, *Sclerotium rolfsii*, Stem rot, Potato.

INTRODUCTION

Potato is susceptible to many diseases, some of them are widespread and some are localized. So far, 55 diseases of potato have been recorded in Bangladesh (Ali and Dey 1994). Among the diseases, stem rot of potato caused by *Sclerotium rolfsii* occurs in almost all potato growing areas of Bangladesh. It can cause up to 60% reduction in tuber yield (Haque and Khan 1977).

Sclerotium rolfsii infects potato plants at the collar region. Grayish brown, slightly sunken spots having 20-30 mm in diameter appear on the stem. The disease occurs mostly in sandy or compact clay soils and is favored by hot moist weather. Under the soil conditions of Bangladesh the disease is destructive in most cultivars. The plants are affected by the pathogen at older age with subsequent yellowing of leaves and stems (Kibria 1971). The pathogen is difficult to control through cultural practices or traditional chemical treatment. Bio-control with *Trichoderma* was found to be effective against different sclerotia forming fungi including *S. rolfsii* (Elad *et al*. 1980). Chemical fungicides such as Provax-200, Rovral 50WP, Dithane M-45, Thiram and Captan sometimes found effective against *S. rolfsii* (Khare 1975). Integrated Pest Management (IPM) strategy is comparatively safe, environment friendly and durable. On the contrary, integration of chemical, cultural and biological approaches to control *S. rolfsii* may be most effective but none of this individual method is very effective. Effective and efficient use of chemicals, bio-control agents and organic amendments therefore may be potential to control the stem rot disease of potato caused by *S. rolfsii*. Several investigators found successful results to control soil borne plant pathogens like *Sclerotium rolfsii* and *Rhizoctonia solani* using *Trichoderma* based integrated with appropriate fungicides and organic amendments. *Trichoderma harzianum* based composts are effective to control soil borne diseases and to enhance plant growth (Khan 2003, Raihan *et al.* 2003, Begum and Bhuiyan 2006, Islam and Bhuiyan 2006, Bhuiyan and Sen 2013). Similarly, integrated management practice may be effective against stem rot of potato.

Keeping this view in mind, the present piece of research was undertaken to evaluate the effectiveness

of integrated disease management strategies consisted of bio-control agent, fungicide and organic amendment to control stem rot disease of potato caused by *Sclerotium rolfsii.*

MATERIALS AND METHODS

An experiment was conducted at the research field of Bangabandhu Sheikh Mujibur Rahman Agricultural University during 2009 to 2010. A series of preliminary experiments were conducted to select virulent isolates of *S. rolfsii*, effective antagonist, fungicide and organic amendment for integrated management of stem rot of potato.

Preparation of Inoculum of *S. rolfsii*

Five isolates of *Sclerotium rolfsii* designated as PS-3, TS-4, CS-2, CS-1 and BS-5 were collected from the rhizosphere and rhizoplane of various crop by root washing method (Hyakumachi 1994) and isolated and identified by following the standard key (Barnett and Hunter 1980). Then, inoculum of the *S. rolfsii* isolate was prepared on autoclaved moist wheat grains in 500 ml Erlenmeyer flask. Before using, wheat grains were soaked in water for 12 hours. After soaking excess water was drained out and water soaked grains were poured into 500 ml Erlenmeyer flask. Five-millimeter diameter mycelial discs were cut from the edge of three days old PDA cultures in petridishes. Five to seven mycelial discs of pathogen were added to autoclaved wheat grains in the flasks and incubated at 25 C for 21 days. It was shaken by hand at 2-3 days interval for proper colonization. The colonized wheat grains were air dried for two days and stored at 10 C for further use.

Pathogenicity Test

Isolates of *S. rolfsii* was evaluated by soil infestation method for their pathogenicity in a pot culture experiment (Bhuiyan *et al.* 2012) under the shade condition in front of the Plant Pathology laboratory at BSMRAU. Each earthen pot was filled with 1.0 kg sterilized soil. Inoculum of *S. rolfsii* was thoroughly mixed with sterilized soil at the rate of 20 g /kg soil. Control was prepared by using sterilized soil only. Three pieces of tuber variety cardinal (BARI Alu-8) were sown in each pot. Disease development was observed regularly and recorded at 15 to 30 days after sowing to estimate the effect of pathogen in causing pre-emergence and post-emergence seedling mortality. The causal agent of pre-emergence seedling mortality was confirmed after re-isolation of the pathogen from un-germinated seeds.

Selections of *T. harzianum* isolate, fungicide and organic amendment

T. harzianum isolate was collected from rhizosphere and rhizoplane of the various crops. Then it was isolated from individual samples following the soil dilution plate technique (Dhingra and Sinclair 1985). A total of 20 fungal isolates were identified as *T. harzianum* on the basis of growth, colony and morphological characters following the standard key (Barnett and Hunter 1980). Among the 20 isolates of *T. harzianum* the best isolate *T. harzianum* T-10 was selected by screening test following dual culture technique against *S. rolfsii* (Dhingra and Sinclair 1985). Then, the inoculum was prepared as like as the test pathogen which was described earlier. Provax 200 was selected based on the preliminary laboratory evaluation of Provax-200, Rovral 50% WP and Bavistin 50% WP at four different concentrations viz. 50, 100, 250 and 500 ppm against the radial growth and sclerotia formation of a selected virulent *S. rolfsii* isolate PS-3 by "Poison food technique" on PDA medium (Dhingra and Sinclair 1985). Similarly, Mustard oil cake was also selected as one of the component of integrated management among the five organic amendments namely Chickpea meal, Mustard oil cake, Rice bran, Tea waste, and Wheat meal at three different concentrations viz. 10, 20 and 30% based on the *in vitro* evaluation against the radial growth and sclerotia formation of the same isolate of *S. rolfsii* as used in case of fungicides evaluation.

Integrated use of fungicide, *T. harzianum* and organic amendment for management of stem rot of potato

A field experiment was conducted to evaluate the effectiveness of *T. harzianum* isolate T-10 integration with selected fungicide Provax-200 and mustard oil-cake on seedling mortality, stem rot and yield of potato. The wheat grains substrate was prepared and inoculated with the isolate T-10 following the procedure as described earlier. The substrate was allowed to colonize by the antagonist for 21 days. After 21 days the colonized wheat grains substrate was air dried, ground with a warring blender and applied in the selected plots @ 90 g/m^2. The organic amendment (Mustard oilcake) was soaked in water for 24 hours and then kept into the hole for adjustment with the soil temperature. After, 3 days mustard oilcake was incorporated into the selected plots @ 60 g/m^2. Provax-200 @ 0.02% was used as a seed treating agent for controlling of seedling mortality, stem rot caused and increasing yield of potato. For seed treatment with Provax-200, 0.2 g fungicide was taken in a bucket and added 1000 ml sterilized water. The solution was mixed properly and before 24 h of sowing each potato tuber was longitudinally divided and submerged for 30 minutes (Khandakar 2004). Finally, tuber was air dried and sown.

Treatments of the Experiment:

T_1= Seeds sown in uninoculated field (Control-1)

T_2= Seeds sown *in S. rolfsii* inoculated field (Control-2)

T_3= Provax 200 treated seeds sown in *S. rolfsii* inoculated field

T_4= Seeds sown in colonized *T. harzianum* and *S. rolfsii* inoculated field

T_5= Seeds sown in mustard oil cake and *S. rolfsii* inoculated field

T_6= Provax 200 treated seeds sown in colonized *T. harzianum* and *S. rolfsii* inoculated field

T_7= Seeds sown in mustard oil cake, colonized *T. harzianum* and *S. rolfsii* inoculated field

T_8= Provax 200 treated seeds sown in mustard oil cake and *S. rolfsii* inoculated field

T_9 = Provax 200 treated seeds sown in mustard oil cake, colonized *T. harzianum* and *S. rolfsii* inoculated field

Data recording and analysis: Data were recorded on germination, number of healthy plants and number of infected plants. Seedling mortality, Plant with stem rot symptoms were observed during the growing period and at harvest. Stem rot was recorded up to the harvest of potato. Stem rot disease severity was rated as 0-4 scale in which 0= no symptoms, 1=1-25%, 2=26-50%, 3 = 51-75% and 4=76-100% of potato stolon covered with lesions (Fenille *et al*. 2003). Disease incidence and disease severity were assessed by the following formula.

$$Disease inc idence(\%) = \frac{No.\ of\ \text{inf}\ ected plants}{Total\ plants\ \text{int}\ he\ plot} \times 100$$

$$\text{Percent disease index (PDI)} = \frac{\sum \text{Disease ratings}}{\text{Total plants observed x Maximum rating}} \times 100$$

Experimental design and Data Analysis

The experiments were conducted with three replications of each treatment following Randomized Complete Block Design (RCBD). Data were analyzed by using MSTAT- C programme. The significant difference, if any, among the means were compared by Duncan's Multiple Range Test (DMRT). Whenever necessary the data were transformed before statistical analysis following appropriate method.

RESULTS AND DISCUSSION

Pathogenicity test of the isolates of *Sclerotium rolfsii*

Five selected isolates of *S. rolfsii* were evaluated in the pot culture experiment to select the most virulent isolate causing seedlings mortality potato. The results of the pathogenicity test of *S. rolfsii* against potato seedlings are presented in the Table 1. All the tested isolates of the pathogen were found to be highly pathogenic against potato seedlings causing 75 to 100 % seedling mortality. The highest 100% seedling mortality was observed with the isolate PS-3 followed by the isolate TS-4 (91.67%) but identical in disease development. Significantly the lowest 75% total seedling mortality was observed by the isolate CS-2. The identical total seedling mortality was recorded with the isolates TS-4, CS-1 and BS-5. Pre and Post-emergence mortality of seedling caused by *S. rolfsii* was also reported by several investigators (Khan 2003, Islam and Bhuiyan 2006, Bhuiyan and Sen 2013) and the results of the present investigation is also in agreement with the above mentioned investigators.

Table 1. Pathogenicity test of *S. rolfsii* against seedling mortality of potato under inoculated conditions

Isolates of	Mortality (%)		
S. rolfsii	Pre-emergence	Post- emergence	Total
CS-1	75.00	8.33	83.33 b*
	(60.00)	(16.78)	(65.91)**
CS-2	58.33	16.67	75.00 c
	(49.80)	(24.09)	(60.00)
PS-3	83.33	16.67	100.00 a
	(65.91)	(24.09)	(90.00)
TS-4	66.67	25.00	91.67 ab
	(54.74)	(30.00)	(73.22)
BS-5	50.00	33.33	83.33 b
	(45.00)	(35.26)	(65.91)

* Values within the last column with a common letter (s) do not differ significantly (P=0.05)

**Figures within the parentheses are arcsine transformed value

Effect on pre and post emergence mortality

Pre-emergence and post-emergence seedling mortality of potato is documented in the Table 2 and Plate I. Significantly the lowest pre-emergence mortality and no post- emergence mortality were observed in the control 1 where seeds were sown in soil without pathogen (T_1). On the contrary, significantly the highest pre and post emergence mortality 52.5 and 19.07% was recorded in the control 2 treatment (T_2) where untreated seeds were sown in the *S. rolfsii* inoculated soil without any other amendment. Significantly lower seedling mortality was observed with all other treatments in comparison to the control-2. Among the different treatments including fungicide, organic amendment and *T. harzianum* either individual or in combination, integration of wheat grain colonized *T. harzianum* with Provax-200 treated

seed and mustard oilcake in the treatment T_9 was appeared to be most superior in reducing the pre and post emergence mortality of potato caused by S. *rolfsii*. The performance of the treatments T_3, T_4 and T_5 were identical with T_9 in reducing the total seedling mortality and significantly superior to the treatments T_6, T_7 and T_8. The results of the current study suggest the superiority of integrated approach for management of *S. rolfsii* in comparison to the individual treatment either by antagonist or by fungicides. Control of seedling mortality and other seedling diseases of different crops were achieved through the integration of antagonist with chemical by different investigators (Raihan *et al.* 2003, Begum and Bhuiyan 2006, Nahar *et al.* 2007, Bhuiyan and Sen 2013) which support the findings of the present study of controlling seedling mortality caused by *S. rolfsii* of potato by integration of antagonist with fungicide and organic amendment.

Table 2. Effect of integrated use of bio-control agent, fungicide and organic amendment on potato seedling mortality caused by *S. rolfsii* in the field

Treatments	Mortality (%)			% Reduction over control-2
	Pre- emergence	Post-emergence	Total	
T_1= Seeds sown in uninoculated field (Control-1)	9.17	0.00	9.17 e* (17.62)**	87.21
T_2= Seeds sown *in S. rolfsii* inoculated field (Control-2)	52.50	19.17	71.67 a (57.84)	-------
T_3= Provax 200 treated seeds sown in *S. rolfsii* inoculated field	35.83	7.50	43.33 b (41.67)	39.54
T_4= Seeds sown in colonized *T.harzianum* and *S. rolfsii* inoculated field	35.00	5.83	40.83 b (39.72)	43.03
T_5= Seeds sown in mustard oil cake and *S. rolfsii* inoculated field	40.00	8.33	48.33 b (44.04)	32.57
T_6= Provax 200 treated seeds sown in colonized *T. harzianum* and *S. rolfsii* inoculated field	17.50	3.33	20.83 d (27.16)	70.94
T_7= Seeds sown in mustard oil cake, colonized *T. harzianum* and *S. rolfsii* inoculated field	20.00	4.17	24.17 cd (29.44)	66.28
T_8= Provax 200 treated seeds sown in mustard oil cake and *S. rolfsii* inoculated field	25.83	5.83	31.67 c (34.25)	55.81
T_9 = Provax 200 treated seeds sown in mustard oil cake, colonized *T. harzianum* and *S. rolfsii* inoculated field	13.33	1.67	15.00 de (22.78)	79.07

*Values within in a column with a common letters do not differ significantly (P=0.05)
** Figures within the parentheses are arcsine transformed value.

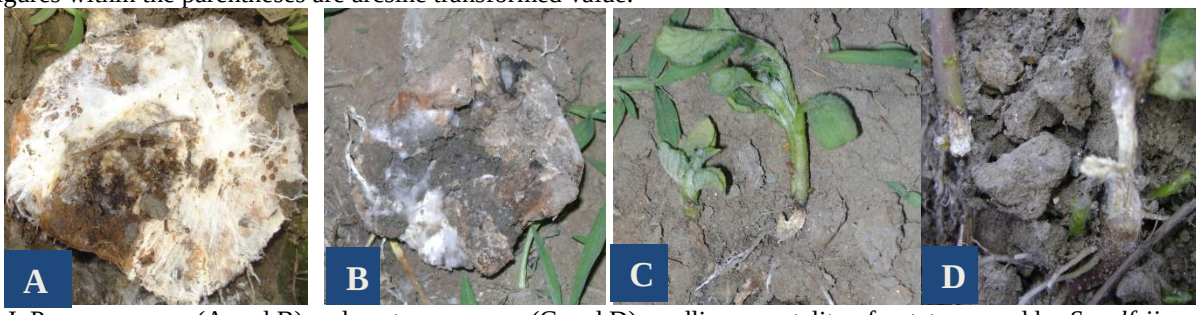

Plate I. Pre-emergence (A and B) and post-emergence (C and D) seedlings mortality of potato caused by *S. rolfsii*

Effect on stem rot disease of potato

Disease Incidence and severity of stem rot was significantly influenced by single component or combine application of *T. harzianum*, fungicides and organic amendments (Table 3 and Plate II). No disease incidence was observed when seeds (tuber) were sown in the soil without pathogen in the control-1 treatment (T_1). Except the control-1 (T_1), the lowest disease incidence (5.83%) and severity (2.5%) was observed in treatment T_9 where Provax-200 treated

seed were used in the colonized *T. harzianum*, and mustard oilcake inoculated amended in the pathogen inoculated soil. On the contrary, significantly the highest disease incidence (39.17%) and severity (30.0%) was observed in the T_2 treatment where untreated seeds were sown in the *S. rolfsii* inoculated soil (control-2) without any other amendments. The highest disease incidence reduction percent over control-2 was recorded in the treatment T_9 and followed by T_9, T_6, T_7 and T_4 respectively. Results indicated that all the treatments were significantly effective in reducing disease incidence and severity of potato seedlings. The findings of the present investigation were found to be similar to the findings of many other researchers (Begum and Bhuiyan 2006, Nahar *et al.* 2007, Bhuiyan and Sen 2013).

Table 3. Effect of integrated use of *T. harzianum*, fungicide and organic amendment on stem rot disease incidence and severity of potato in the field

Treatments	% Disease incidence	% Reduction over control-2	Severity(PDI)*
T_1= seeds sown in uninoculated field (Control-1)	0.00 h (0.00)**	100.00	0.0 d (0.00)**
T_2= Seeds sown *in S. rolfsii* inoculated field (Control-2)	39.17 a (6.26)	0.00	30.0 a (5.48)
T_3= Provax 200 treated seeds sown in *S. rolfsii* inoculated field	23.33 bc (4.83)	40.44	15.0 bc (3.87)
T_4= Seeds sown in colonized *T. harzianum* and *S. rolfsii* inoculated field	20.00 cd (4.47)	48.94	15.0 bc (3.87)
T_5= Seeds sown in mustard oil cake and *S. rolfsii* inoculated field	29.00 b (5.39)	25.96	20.0 ab (4.47)
T_6= Provax 200 treated seeds sown in colonized *T. harzianum* and *S. rolfsii* inoculated field	10.83 f (3.29)	72.35	5.0 cd (2.24)
T_7= Seeds sown in mustard oil cake, colonized *T. harzianum* and *S. rolfsii* inoculated field	14.17 ef (3.76)	63.82	7.5 bcd (2.74)
T_8= Provax 200 treated seeds sown in mustard oil cake and *S. rolfsii* inoculated field	17.50 de (4.18)	55.32	10.0 bcd (3.16)
T_9 = Provax 200 treated seeds sown in mustard oil cake, colonized *T. harzianum* and *S. rolfsii* inoculated field	5.83 g (2.41)	85.12	2.5 cd (1.58)

*Mean disease severity of three replicates, rated 0-4, in which 0= no symptoms, 1=1-25%, 2= 26-50%, 3= 51-75% and 4=76-100% of the potato stolon covered with lesions

** Figures within the parentheses are squire root (X+0.5) transformed value

Values within in a column with a common letters do not differ significantly (P=0.05)

Plate II. Stem rot disease of potato caused by *S. rolfsii* at different stages in the control 2 (T_2) plot (A. Initiation of rotting at the base of the stem. B. Stem base is rotten with appearance of white mycelium of *S. rolfsii*, C. Severe rotting with appearance of young sclerotia D. Severely rotten with enormous visible young and mature sclerotia of *S. rolfsii*).

Effect on plant growth and Yield of Potato

The application of *T. harzianum*, mustard oilcake and fungicide not only reduced the disease development caused by *S. rolfsii* but also significantly increased the yield of potato (Table 4 and Plate III). Significantly the highest 28.61 t/ha total yield was recorded in the plot where colonized *T. harzianum* was integrated with Provax-200 treated seed and mustard oilcake in the treatment T_9 followed by the control-1 treatment (T_1) where untreated healthy seeds sown in soils without pathogen. On the contrary, significantly the lowest total yield 5.00 t/ha was recorded in the treatment T_2 where untreated seeds were sown in the *S. rolfsii* inoculated soil (control-2) without any other amendment. The 100% tuber infestation was found in the treatment T_2 and zero percent in the treatment T_1 and followed by T_9, T_6, T_7 and T_4 respectively. Simultaneously, highest disease management over control-2 (T_2) was recoded 85.05% and also yield increased 23.61% in the treatment T_9 and followed by T_1 (Control-1) where seeds were sown in uninoculated field and without any amendment. The increase of yield not only because of the reduction of diseases but also might be due to the secretion of growth promoting substances in the soil by *T. harzianum*. Altomare *et al*. (1999) stated that *T. harzianum* produced a large number of chemicals to solubilize rock phosphate, Zn, Mn^{4+}, Fe^{3+}, and Cu^{2+} and increased iron availability and enhanced iron uptake, which might be contributed in increasing yield of potato. The solubilization and chelating abilities of *T. harzianum* may also be influenced in increasing yield of potato in the present study as supported by Harman *et al*. (2004), Wilson *et al*. (2008), Hermosa *et al*. (2012) and Bhuiyan and Sen (2013).

Table 4. Effect of integrated use of *T. harzianum*, fungicide and organic amendment on stem rot disease management and increase of yield of potato in the field

Treatments	Tuber Production (t/ha)			Tuber Infestation (%)	Disease management over control-2 (%)	Yield increase over control-2 (t/ha)
	Fresh	Infected	Total			
T_1= Seeds sown in uninoculated field (Control-1)	26.67	0.00	26.67 a	0.00 (0.00)*	0.00	21.67
T_2= Seeds sown *in S. rolfsii* inoculated field (Control-2)	0.00	5.00	5.00 h	100.00 (10.00)	-----	-----
T_3= Provax 200 treated seeds sown in *S. rolfsii* inoculated field	7.56	4.56	12.11 f	37.61 (6.13)	62.39	7.11
T_4= Seeds sown in colonized *T. harzianum* and *S. rolfsii* inoculated field	9.72	5.17	14.89 e	34.70 (5.89)	65.30	9.89
T_5= Seeds sown in mustard oil cake and *S. rolfsii* inoculated field	5.00	3.61	8.61 g	41.94 (6.48)	58.06	3.61
T_6= Provax 200 treated seeds sown in colonized *T. harzianum* and *S. rolfsii* inoculated field	20.00	4.44	24.44 b	18.18 (4.26)	81.82	19.44
T_7= Seeds sown in mustard oil cake, colonized *T. harzianum* and *S. rolfsii* inoculated field	15.28	5.00	20.28 c	24.66 (4.97)	75.34	15.28
T_8= Provax 200 treated seeds sown in mustard oil cake and *S. rolfsii* inoculated field	11.89	5.06	16.94 d	29.84 (5.46)	70.16	11.94
T_9 = Provax 200 treated seeds sown in mustard oil cake, colonized *T. harzianum* and *S. rolfsii* inoculated field	24.33	4.28	28.61 a	14.95 (3.87)	85.05	23.61

* Figures within the parentheses are squire root (X+0.5) transformed value

Values within in a column with a common letters do not differ significantly (P=0.05)

Plate III. Plant growth and development and yield of potato in the field (A and C. Control 2 plot only pathogen inoculated, B and D. Provax 200 treated seeds sown in the colonized *Trichoderma* and Mustard oil cake amended in pathogen inoculated soil (T_9).

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