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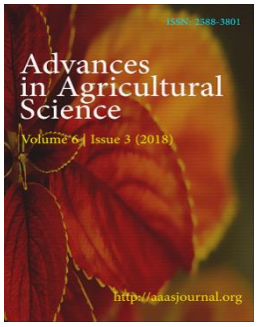

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Effect of biofumigation and soil solarization on stem canker and black scurf diseases of potato (*Solanum tuberosum* L.) caused by *Rhizoctonia solani* isolate PR2

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ABSTRACT

Studies were conducted to evaluate the effect of biofumigation and soil solarization on stem canker and black scurf diseases of potato caused by *Rhizoctonia solani* isolate PR2. A series of *in-vitro* and *in-vivo* assays were laid out to select a virulent isolate of *R. solani* against the susceptible potato variety of cardinal and evaluated the *Brassica* spp. namely cabbage, cauliflower, mustard, and broccoli leaf extracts to select the best biofumigant against *R. solani* isolate PR2 in *in-vitro* condition. Among the different *Brassica* spp., *Brassica nigra* (mustard) was the most effective in inhibiting the radial growth (79.63%) of *R. solani* isolate PR2 at 40% level of concentration followed by broccoli leaf extract (66.67%). At the twice field trials, application of biofumigation and soil solarization in T_5 treatment was appeared to be the most superior in reducing pre and post emergence mortality of potato seedling. The lowest disease incidence (24.44% and 30.67%), and percent disease index (20.37% and 13.89%) also were found in stem canker and black scurf diseases, respectively at treatment T_5 followed by T_3 (where used biofumigation without soil solarization) treatment. The yield of potato (117.13%) was increased in T_5 treatment followed by T_3 treatment (73.01%). The increase of yield was not only because of the reduction of diseases but also it might be due to supplement of the organic materials in the soil, as a result, increases the number of soluble nutrients and secret the growth promoting substances by beneficial soil microorganisms.

Introduction

Potato (*Solanum tuberosum* L.) belongs to the family solanaceae is one of the most important vegetable crops in Bangladesh. In the 2014-15 year, the total potato cultivable land was 471.06 thousand hectares and tuber yield production was 9.25 million tons with an average yield 19.64 t ha^{-1} (Anon., 2016). Although many constraints are present behind the low production of potato, the disease is considered an important one. Hossain *et al*. (2008) were recorded around 57 different kinds of potato diseases. Among all the potato diseases and their pathogens, the most important soil-borne fungi are *Rhizoctonia solani* and *Sclerotium rolfsii* that can cause serious yield

loss of potato. *R. solani* Kühn (telomorph: *Thanaterphorus cucumeris)* was originally observed on potato tubers in 1885 (Wilson *et al.,* 2008). This fungal pathogen that causes two important diseases of potato such as stem canker and black scurf which may lead to reducing the quality and quantity of tubers yield (Brewer and Larkin, 2005). Stem canker results in quantitative losses by sprout, stolon, and root infections, mainly early in the season, affecting tuber size and number, whereas black scurf develops during plant senescence and is associated with the formation of sclerotia on progeny tubers and their malformation (Das *et al.,* 2014; Tsror, 2010). *Rhizoctonia* potato disease accounts for significant marketable yield losses of up to 30% (Carling *et al.,* 1989). *R. solani* is the most common and has wide host range all over Bangladesh (Dey and Ali, 1994). It can survive long periods of time into plant debris, on tuber surfaces and within the soil by forming resting spore i.e sclerotia (El Balkali and Martin, 2006). *R. solani* has been classified into 13 reproductively incompatible groups based on the anastomosis group (AG) concept, numbered from AG1 to AG13 (Carling *et al.,* 2002). The soil-borne sclerotia forming pathogen is really difficult to control through cultural practices or traditional chemical treatment. Although, chemical compounds have been used as seed treating fungicide such as Provax-200 and sometimes effective to control soil-borne pathogens. But excess use of fungicide has favored the development of pathogen-resistant against fungicides and polluted the environment. If the environment is polluted as a result imbalance the ecological harmony among the different animals of the planet.

Consequently, soil solarization by sunlight and biofumigation by *Brassica* spp. may be the effective and eco-friendly substitute to chemicals that are applicable for controlling of soil-borne pests, including phytopathogens and weeds (Smolinska *et al.,* 2003; Kapoor, 2013). Soil solarization also improves soil structure and increases the availability of essential plant nutrients for rapid growth and development of plants (Elmore *et al.,* 1997). Biofumigation works on the principle of exploiting the natural biocide compounds from glucosinolate containing plants to suppress soil microorganisms, such as fungal, bacterial pathogens and nematodes (Smolinska *et al.,* 2003; Matthiessen and Shackleton, 2005). According to Larkin and Griffin (2007), *Brassica* spp. and Barley reduced inoculum levels of *R. solani* by 20-56% in greenhouse tests. On the other hand, radish, rapeseed and Indian mustard reduced 40-83% potato seedling diseases. In Bangladesh, still, no attempt has been made to control the soil-borne fungi especially for *R. solani* by using soil solarization and biofumigation technique. That's why a comprehensive study about to develop a nonchemical management package against the soil-borne fungi is really needed in Bangladesh perspective. This nonchemical management package will be highly effective to control the soil-borne pathogens and promotes the sustainable crop production. Therefore, the present study was undertaken to evaluate the effect of *Brassica* spp. for the control of *R. solani* in *in-vitro* condition, observed the effect of biofumigation and soil solarization alone or in combination in controlling *R. solani* and also the effect on plant growth and tuber yield of potato.

Materials and Methods

Laboratory studies

In laboratory studies, different experiments were conducted by using Completely Randomized Design (CRD). An individual experiment was carried out by following three replications for each treatment.

Isolation and preservation of Rhizoctonia solani isolates

The *R. solani* isolates were collected from the rhizosphere and rhizoplane of Potato (*Solanum tuberosum*), Tomato (*Solanum lycopersicon*), Chilli (*Capsicum frutesence*), Carrot (*Daucus carrota),*

Brinjal (*Solanum melongena*), Sweet potato (*Ipomoea batatas*), Cotton (*Gossypium hirsutum*), and Radish (*Raphanus sativus*) crops by root washing method (Hyakumachi, 1994). The specimens which had typical symptoms of stem canker and black scurf were selected from infected potato fields. The fungal isolates were isolated by following standard method (Mian, 1995). The fungal colonies were grown on Potato Dextrose Agar (PDA) and identified by following standard key (Barneet and Hunter, 1972). The pure culture of *R. solani* isolates was named individually with English capital letter and numerical number codes then preserved by using PDA slants at 10 ℃ in the refrigerator as a stock culture for further study.

Cultural characterization of R. solani isolates

The selected isolates PR1 to PR8 were individually inoculated onto three replicated PDA plates using 5 mm diameter mycelial disks which taken from three days old PDA plates. Then, all PDA plates were sealed with parafilm paper and incubated at room temperatures (25±2 ℃). After 72 h of incubation, observation on cultural characteristics such as colony color, colony type, number of zonation, sclerotial population, and sclerotial distribution was recorded. Most of the isolates colony type and colony color were compact and brown in color. The sclerotia population density was measured by eye observation as +++, ++, and +, representing abundant, moderate, and minimum, respectively.

Preparation of inoculum of test pathogen

Inocula of the *R. solani* isolates were prepared individually with autoclaved moist wheat grains by following standard method (Bhuiyan and Sen, 2013). After preparation, the inoculum was stored at 4 ℃ for future use.

Pathogenicity test

Pathogenicity test of the isolated *R. solani* isolates was conducted by soil infestation method in pot culture under the shade condition (Akter *et al.*, 2015). Each earthen pot was filled with 1.0 kg sterilized soil. Inocula of *R. solani* were thoroughly mixed with sterilized soil at the rate of 20 g/kg soil. A control treatment was maintained without adding inoclum in sterilized soil. Three pieces of potato tubers were transplanted in each pot. Disease development was observed regularly and recorded at 15 to 30 days after transplanting to estimate the effect of the pathogen in causing pre-emergence and post-emergence seedling mortality. The causal agents of pre-emergence seedling mortality were confirmed after re-isolation of the pathogen from ungerminated tubers.

Preparation of Brassica spp. leaves extract

Fresh parts of the test plants namely- cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*Brassica oleracea* var. *botrytis*), mustard (*Brassica nigra*), and broccoli (*Brassica oleracea* var. *italica*) were collected and washed thoroughly with distilled water. Hundred grams of each washed samples were ground in mortar and pestle by adding an equal amount (100 ml) of sterilized distilled water (1:1 W/V) and boiled at 80 ℃ for 10 minutes in a hot water bath. The ground material was filtered through muslin cloth followed by filtering through sterilized what man No. 1 filter paper and treated as a standard plant extract (100%). Then, the mixture was diluted 10, 20, 30, and 40% concentrations by adding proper volumes of sterilized water.

In-vitro screening of Brassica spp. against the R. solani isolate PR2

All the plant extracts were tested at 10, 20, 30, and 40% concentrations under *in-vitro* conditions by using food poison technique (Nene and Thapliyan, 1979) to study the inhibitory effect of these botanicals against the mycelial growth of *R. solani* isolate PR2. PDA medium was separately amended

with the required amount of plant extract after sterilization when it cooled at 40 ℃ under aseptic condition. The control treatment was maintained by pouring PDA medium without plant extract. Five-millimeter circular disc from 3 days old culture of *R. solani* isolate PR2 was cut with the sterilized cork borer and placed in the center of plant extract amended PDA petri-dishes separately. The petri-dishes having PDA solely (control) were inoculated in the same manner. All plates were incubated in the dark for 25 ℃ until the mycelium of *R. solani* isolate PR2 covered the whole control plate individually. The radial growth of the test pathogens per plate was measured by scale and the number of sclerotia per plate counted by haemocytometer. The percent inhibition of the radial growth was calculated as described by Sundar *et al.* (1995).

$$\% \text{ inhibition of growth} = \frac{X - Y}{X} \text{ x } 100$$

Where, X = mycelial growth of pathogen in absence of plant extract

Y = mycelial growth of pathogen in presence of plant extract

Field experiments

A series of experiments were conducted in the research field of Plant Pathology department at Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. The experimental site was located at the center of Madhupur tract (24°09' N latitude and 90°26' E longitude) having an elevation of 8.2 m from the sea level. The soil type of the experimental field belongs to the Shallow red-brown terrace type under Salna series of Madhupur tract (Brammer, 1971; Saheed, 1984). The agroecological zone (AEZ) was 28, which was characterized by silty clay soil with pH value 6.5, less rainfall, almost clear sunshine and moderate temperature. The experiments were conducted during 2015-2016.

The field experiment was designed by using a Randomized Complete Block Design (RCBD) with five treatments and three replications. The unit plot size was 3 m × 2 m where row to row distance 40 cm and plant to plant 25 cm. Tubers were transplanted in rows uniformly at a depth of 2.5 cm.

The treatments are as follows:

T_1 = transplanting healthy potato tubers in field soil (control-1)

T_2 = inoculum of *R. solani* isolate PR2 (IR) + transplanting healthy potato tubers in field soil (control-2)

T_3 = IR + biofumigant + transplanting healthy potato tubers in field soil

T_4 = IR + solarized soil + transplanting healthy potato tubers in field soil

T_5 = IR + biofumigant + solarized soil + transplanting healthy potato tubers in field soil

T_1 = transplanting healthy potato tubers in field soil (control-1)

T_2 = inoculum of *R. solani* isolate PR2 (IR) + transplanting healthy potato tubers in field soil (control-2). Soil was inoculated with Wheat Grain colonized *R. solani* isolate PR2 Inoculum (WGRI) @ 90 g/m^2 before 3 weeks of tuber transplantation.

T_3 = IR + biofumigant + transplanting healthy potato tubers in field soil. Soil was inoculated with WGRI @ 90 g/m^2 and simultaneously mustard seeds were sown. When the mustard crop was about 4 inches height, it was incorporated into the soil for proper decomposition and biofumigation before 4 weeks of tubers transplantation.

T_4 = IR + solarized soil + transplanting healthy potato tubers in field soil. Soil was inoculated with WGRI @ 90 g/m^2 and then it was kept 3 weeks within sufficient soil moisture and without disturbing of the soils for proper growth and development of the test pathogen. After that, it was covered with 100 µm thickness transparent

polyethylene sheet to increase soil temperature for 4 weeks before tubers transplantation.

T_5 = IR + biofumigant + solarized soil + transplanting healthy potato tubers in field soil. Soil was inoculated with WGRI @ 90 g/m^2 and simultaneously mustard seeds were sown. After 4 weeks, when the mustard crop was about 4 inches height, it was incorporated into the soil for proper decomposition and biofumigation. Then, it was covered with 100 µm thickness transparent polyethylene sheet to increase soil temperature for 4 weeks before tubers transplantation.

Data recording

Data were recorded on tuber emergence, a number of healthy and infected plants and tubers during the growing to harvesting period. Disease incidence and severity of stem canker and black scurf were recorded. Total tuber yield was also recorded. Fifteen plants were randomly selected and uprooted from in each individual plot, washed with tap water then checked individually for separation of healthy and infected plants. The disease severity for stem canker was rated (0-6 scale) by following standard method (Dey, 2010).

On the country, fifty tubers were randomly selected after harvesting of tubers from in each individual plot and washed with tap water then checked individually for infection. The disease severity for black scurf was rated (0-5 scale) by following standard method (Zhang *et al.*, 2014).

Rating	Observation
0	no sclerotia present
1	less than 1% of tuber area covered
2	2-10% of tuber area covered
3	11-20% of tuber area covered
4	21-50% of tuber area covered
5	51% or more of tuber area covered

Rating	Observation
0	no symptom on stolon
1	minute brown lesion on stolon or root
2	moderately brown lesion on stolon and curling tendency on central leaf
3	stolon symptom discolored accompanied by brown discoloration on roots
4	brown to black discoloration on underground parts and curling of growing leaves
5	profuse emergence of auxiliary leaves and leaf size reduced with pale green margin
6	production of aerial tubers with green color

Finally, the percent disease incidence and severity were calculated by following formula (Rahman *et al.*, 2013).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants/ tubers}}{\text{Total number of plants/ tubers assessed}} \text{ x } 100$$

$$\text{Percent disease index (PDI)} = \frac{\text{Summation of all ratings}}{\text{Total number of rating x Maximum disease grade}} \text{ x } 100$$

Total tuber yield (tha^{-1}): The tuber yield data were recorded by weighing the whole tubers in each individual plot for each replication and converted into ton per hectare by following formula (Razaq *et al.*, 2015).

$$\text{Total tuber yield } (tha^{-1}) = \frac{\text{Yield per plot (kg)}}{\text{Area of plot } (m^2) \text{ x } 1000 \text{ (Kg)}} \text{ x } 10000\ m^2$$

Statistical analysis

Data were analyzed statistically by using the MSTAT-C and Statistix 10 computer program. The treatment means were compared following the Duncan's Multiple Range Test and data transformed into square root whenever it was necessary (Zaman *et al.*, 1982; Gomez and Gomez, 1984).

Results

Isolation and cultural characterization of R. solani isolates

The eight isolates of *R. solani* were isolated from the different source of crops field at Gazipur district of Bangladesh (Table 1). Most of the isolates colony type was compacted and brown in color. The highest number of distinct zonation (3) in culture plate was observed in isolate PR2 and rest of them was produced two alterations of periodic zonation. On the contrary, abundant (+++) sclerotial population was also observed in isolate PR2, followed by PR6 and others isolates had moderate sclerotial population density on a culture plate.

Pathogenicity test of R. solani isolates against potato seedlings

The results of the pathogenicity test of *R. solani* isolates against potato seedlings are presented in Table 2. All the tested isolates of *R. solani* were found to be pathogenic against potato seedlings causing 25.00 to 100% seedling mortality.

The highest 100% seedling mortality for *R. solani* was observed with the isolate PR2 followed by the isolate PR6 (91.67%), PR4 (83.33%) and PR1 (75.00%). Significantly the lowest 25.00% total seedling mortality was observed by the isolates PR7.

Effect of Brassica spp. on in-vitro radial growth of R. solani isolate PR2

The effect of leaf extract of mustard, cabbage, cauliflower, and broccoli in reducing the radial growth of *R. solani* isolate PR2 is presented in Table 3. Significantly (79.63%) inhibition of mycelial growth of *R. solani* isolate PR2 was observed at the highest 40% concentration of mustard leaf extract followed by 61.48 % inhibition at 30% concentration. On the contrary, cabbage, cauliflower and broccoli leaf extract inhibited 51.85, 54.81, and 66.67% mycelial radial growth of *R. solani* isolate PR2 at 40% conc. level. The lowest inhibition of *R. solani* isolate PR2 was recorded 17.78% at 10% conc. cauliflower leaf extract. Results indicated that mustard leaf extract was significantly superior to all others leaf extract in reducing the radial colony growth of *R. solani* isolate PR2.

Table 1. Cultural characterizations of *R. solani* isolates on culture plate in *in-vitro* condition

Isolates of *R. solani*	Source of isolates	Colony type	Colony color	No. of zonation	Sclerotial population*
PR1	Tomato	compact	brown	2	++
PR2	Potato	compact	brown	3	+++
PR3	Chilli	compact	brown	2	++
PR4	Carrot	compact	Light brown	2	++
PR5	Brinjal	Slightly fluffy	brown	2	++
PR6	Sweet potato	compact	brown	2	+++
PR7	Cotton	Slightly fluffy	Light brown	2	++
PR8	Radish	Slightly fluffy	Light brown	2	+

*Sclerotia of *R. solani* was measured on eye observation as +++, ++, and +, representing abundant, moderate, and minimum, respectively.

Table 2. Pathogenicity test of *R. solani* against potato seedlings in pot soil

Isolates of *R. solani*	% mortality		% total mortality
	Pre-emergence	Post- emergence	
PR1	33.33	41.67	75.00 a-c
PR2	66.67	33.33	100.00 a
PR3	41.67	8.33	50.00 cd
PR4	58.33	25.00	83.33 a-c
PR5	25.00	8.33	33.33 de
PR6	50.00	41.67	91.67 ab
PR7	16.67	8.33	25.00 de
PR8	41.67	16.67	58.33 b-d
Control	0.00	0.00	0.00 e

Means within same column followed by common letter(s) are not significantly different (P=0.05).

Table 3. Effect of *Brassica* spp. leaf extract in inhibition of radial growth of *R. solani* isolate PR2 *in-vitro*

Brassica spp.	Conc. %	% inhibition radial growth of *R. solani* isolate PR2
Mustard	10	31.48 f
	20	43.33 d
	30	61.48 b
	40	79.63 a
Cabbage	10	21.48 g
	20	30.00 f
	30	40.37 de
	40	51.85 c
Cauliflower	10	17.78 g
	20	29.63 f
	30	35.93 ef
	40	54.81 c
Broccoli	10	22.22 g
	20	30.74 f
	30	51.48 c
	40	66.67 b
Control		90.00 mm

Means within same column followed by common letter(s) are not significantly different (P=0.05).

Effect on pre and post emergence mortality

Significantly the highest total seedling mortality of 44.17% was recorded in control-2 treatment (T_2) where potato tubers were transplanted in the *R. solani* isolate PR2 inoculated soil without any other amendment followed by treatment T_1 (control-1) where tubers were transplanted in uninoculated field soil. On the contrary, significantly lower total seedling mortality was observed at treatment T_5 (14.17%) followed by T_3 (18.33%), and T_4 (24.17%), respectively. Among the different treatments including biofumigant and solarized soil either individually or in combination, treatment T_5 was appeared to be most superior in reducing the pre and post emergence mortality of potato caused by *R. solani* isolate PR2 (Table 4).

Table 4. Effect of biofumigation and soil solarization on potato seedling mortality caused by *R. solani* isolate PR2 in field soil.

Treatments	% mortality		
	Pre emergence	Post emergence	Total
T_1	15.00	12.50	27.50 b
T_2	22.50	21.67	44.17 a
T_3	10.83	7.50	18.33 cd
T_4	12.50	11.67	24.17 bc
T_5	8.33	5.83	14.17d
CV			16.78

Means within same column followed by common letter(s) are not significantly different (P=0.05).

The experiment was conducted at two times. Significant treatment effects were identical in the two trials. Data shown are from one trial.

Here,

T_1 = transplanting healthy potato tubers in field soil (control-1)

T_2 = inoculum of *R. solani* isolate PR2 (IR) + transplanting healthy potato tubers in field soil (control-2)

T_3 = IR + biofumigant + transplanting healthy potato tubers in field soil

T_4 = IR + solarized soil + transplanting healthy potato tubers in field soil

T_5 = IR + biofumigant + solarized soil + transplanting healthy potato tubers in field soil

Effect of biofumigant and solarized soil on disease incidence and severity

Disease incidence and severity of stem canker and black scurf disease of potato were significantly influenced by a single component or combined application of biofumigant and solarized soil (Table 5, and 6). In stem canker disease the lowest disease incidence (24.44%) and severity (20.37%), and in black scurf disease the lowest disease incidence (30.67%) and severity (13.89%) were found in T_5 where biofumigant and solarized soil were used in integration (Plate 1). On the contrary, significantly in stem canker disease the highest disease incidence (73.33%) and severity (71.48%), and in black scurf disease the highest disease incidence (95.33%) and severity (77.68%) were observed in the T_2 (control-2) treatment where potato tubers were transplanted in the *R. solani* isolate PR2 inoculated soil without any other amendment. The highest disease incidence and severity was observed in T_2 treatment because of the extreme inoculum pressure of test pathogen and existing soil-borne pathogens. The highest percent reduction of stem canker and black scurf diseases of potato were found in T_5 treatment over control-1. Results indicated that biofumigation and soil solarization alone or in combination were effective in reducing stem canker and black scurf

diseases incidence and severity of potato in field soil.

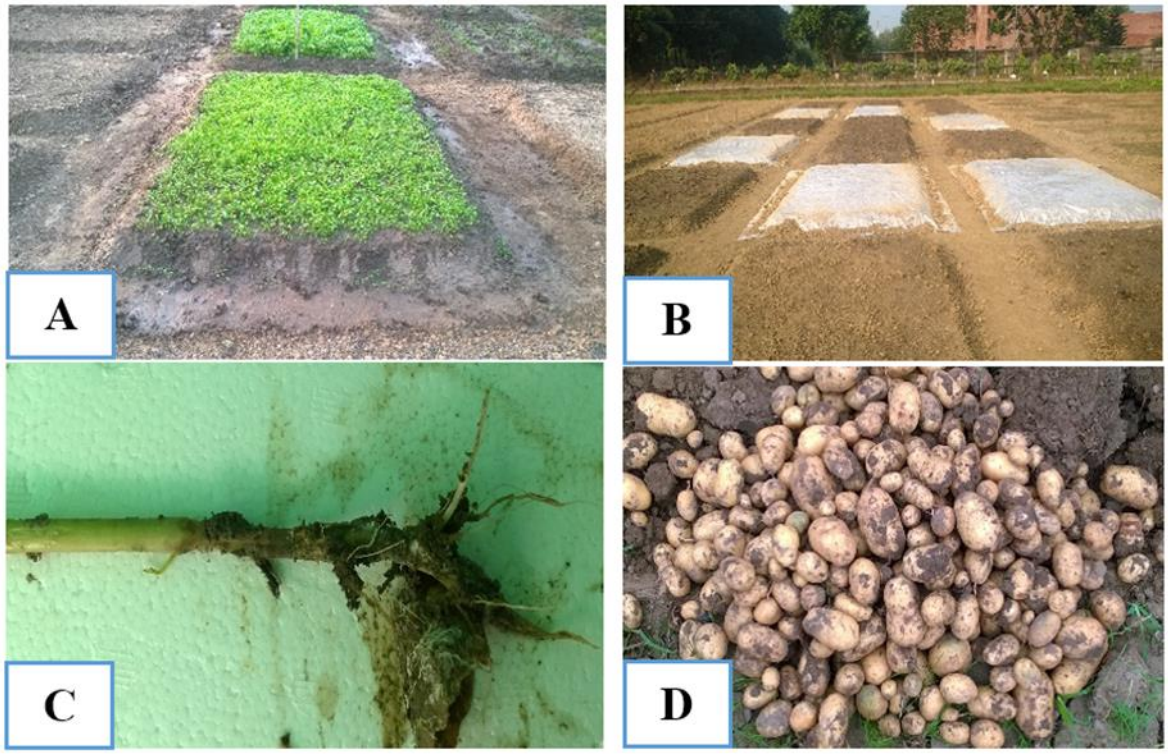

Plate 1. Biofumigation by musatrd (**A**), Soil Solarization by transparent polythen sheet (**B**), Stem canker disease on potato stolon/ root (**C**), and Black scurf disease on potato tuber (**D**)

Table 5. Effect of the biofumigation and soil solarization on incidence and severity of stem canker disease of potato plants in field soil

Treatments	Disease incidence (%)	Disease severity (%)
T1	53.33 b	50.37 b
	(7.30)	(7.10)
T2	73.33 a	71.48 a
	(8.56)	(8.45)
T3	33.33 cd	29.26 cd
	(5.77)	(5.41)
T4	42.22 bc	40.37bc
	(6.50)	(6.35)
T5	24.44 d	20.37 d
	(4.94)	(5.41)
CV	17.81	14.62

Means within same column followed by common letter(s) are not significantly different (P=0.05).

Figures within the parentheses are represented square root transformed (X+0.5) values. The experiment was conducted at two times. Significant treatment effects were identical in the two trials. Data shown are from one trial.

Here,

T_1 = transplanting healthy potato tubers in field soil (control-1)

T_2 = inoculum of *R. solani* isolate PR2 (IR) + transplanting healthy potato tubers in field soil (control-2)

T_3 = IR + biofumigant + transplanting healthy potato tubers in field soil

T_4 = IR + solarized soil + transplanting healthy potato tubers in field soil

T_5 = IR + biofumigant + solarized soil + transplanting healthy potato tubers in field soil

Effect of biofumigant with soil solarization on yield of potato

The highest yield (14.17 t ha^{-1}) was significantly recorded in the plot where *B. nigra* (mustard) used as biofumigant and soil solarized by polythene mulch in the treatment T_5 (IR + biofumigant + solarized soil + transplanting healthy potato tubers in field soil) followed by T_3 (IR+ biofumigant + transplanting healthy potato tubers in field soil), and T_4 (IR + solarized soil + transplanting healthy potato tubers in field soil), respectively (Table 7). On the contrary, significantly the lowest yield (3.33 t ha^{-1}) was recorded in the treatment T_2 (control-2) where tubers sown in *R. solani* isolate PR2 inoculated field without any other amendment followed by control-1 (6.52 t ha^{-1}), where inoculation of *R. solani* isolate PR2 or other amendments of soil were not performed. The (%) disease reduction or increase (-) over control-1 was recorded 67.79 to -53.30% in treatment T_5 and T_2, respectively and (%) yield increase or reduction (-) over control-1 was 117.33 to -48.93% in also treatment T_5 and T_2, respectively. In the treatment T_2, the result was always negative against the control-1 due to existing pathogens

Table 6. Effect of the biofumigation and soil solarization on incidence and severity of black scurf disease of potato tubers in field soil

Treatments	Disease incidence (%)	Disease severity (%)
T1	78.67 b	60.33 b
	(8.87)	(7.77)
T2	95.33 a	77.68 a
	(9.76)	(8.82)
T3	43.33 c	27.65 d
	(6.58)	(5.26)
T4	52.67 c	38.11 c
	(7.26)	(6.17)
T5	30.67 d	13.89 e
	(5.54)	(3.73)
CV	8.67	6.97

Means within same column followed by common letter(s) are not significantly different (P=0.05).

Figures within the parentheses are represented square root transformed (X+0.5) values. The experiment was conducted at two times. Significant treatment effects were identical in the two trials. Data shown are from one trial.

Here,

T_1 = transplanting healthy potato tubers in field soil (control-1)

T_2 = inoculum of *R. solani* isolate PR2 (IR) + transplanting healthy potato tubers in field soil (control-2)

T_3 = IR + biofumigant + transplanting healthy potato tubers in field soil

T_4 = IR + solarized soil + transplanting healthy potato tubers in field soil

T_5 = IR + biofumigant + solarized soil + transplanting healthy potato tubers in field soil

population density in the soil without any kind of amendment. All treatments were compared with control-1 for measurement of the test pathogen disease incidence and severity rate in the potato cultivated field soil.

Table 7. Effect of the biofumigation and soil solarization on tuber yield of potato in field soil

Treatments	Tuber Production (t ha^{-1})			% tuber infestation	% disease reduction/ increase (-) over control-1	% yield increase/ reduction (-) over control-1
	Fresh	Infected	Total			
T1	2.42	4.10	6.52 c	62.88	--	--
T2	0.12	3.21	3.33 d	96.40	-53.30	-48.93
T3	8.15	3.13	11.28 b	27.75	55.87	73.01
T4	6.75	3.42	10.17 b	33.63	46.52	55.98
T5	11.3	2.87	14.17 a	20.25	67.79	117.33
CV			7.29			

Means within same column followed by common letter(s) are not significantly different (P=0.05).

The experiment was conducted at two times. Significant treatment effects were identical in the two trials. Data shown are from one trial.

Here,

T_1 = transplanting healthy potato tubers in field soil (control-1)

T_2 = inoculum of *R. solani* isolate PR2 (IR) + transplanting healthy potato tubers in field soil (control-2)

T_3 = IR + biofumigant + transplanting healthy potato tubers in field soil

T_4 = IR + solarized soil + transplanting healthy potato tubers in field soil

T_5 = IR + biofumigant + solarized soil + transplanting healthy potato tubers in field soil

Discussion

In Bangladesh, this is the first comprehensive study that aimed to develop a sustainable nonchemical management package against the stem canker and black scurf diseases of potato and increase the tuber production in field soil.

Diversification of *R. solani* fungi is very high which is confirmed by its *in-vitro* characterization such as colony appearance and pattern of sclerotia formation (Dubey *et al.*, 2014). The brown color vegetative is the diagnostic character of *R. solani* (Butler and Bracker, 1970). Alteration of periodic zonation has been found to vary among *R. solani* isolates (Ui, 1979). The consequence of different characteristics of the *R. solani* isolates was found by their *in-vitro* culture and pathogenicity test. Many researchers reported on morphological and pathological

characters for distinguishing between strains of *Rhizoctonia* (Butler and Bracker, 1970). Black scurf is caused by *R. solani* which is a complex species, at least 13 related but distinct genetically anastomosis groups (AGs). AG-3 is the main cause of black scurf, on the tubers, there is brown to black sclerotia that develop late in the growing season (Kumar *et al*., 2017a). PR1 to PR8, total eight number of isolates were collected and isolated from the different source of crops field. Among the eight isolates PR2 was produced the highest number of distinct zonation with compact, brown colony and abundant sclerotial population density on culture media. In pot soil trial, isolate PR2 showed maximum number of seedling mortality of potato and was selected for further study. Faruk and Rahman (2015); Goswami *et al.* (2010), reported that seedling mortality of various crops is hugely caused by a mixture of patho-morphological contrasting groups of *R. solani* in field condition of Bangladesh. Similar results were also observed by Sen, 2010. These results are highly imperious for controlling of potato seedling mortality disease in Bangladesh.

The fungal radial growth inhibition was clearly found by *in-vitro* studies. Among the different *Brassica* spp., *B. nigra* plant leaf extract was highly inhibited the radial growth of virulent isolate of *R. solani* isolate PR2. Although the other *Brassica* spp. plant leaf extract was also inhibited the radial growth of the same isolate at varying levels. Plant remains natural chemicals compound which has the potentiality to control soil-borne pathogens. In *Brassica* spp. has been recognized two pesticidal compounds i.e the enzyme myrosinase and secondary metabolites glucosinolates. Myrosinase has thioglucoside glucohydrolase which is situated in myrosin cells. On the contrary, secondary metabolites glucosinolates which is retained in cell vacuoles (Adreasson *et al.,* 2001). Due to plant tissue interruption, the myrosinase catalyzes a hydrolysis reaction which ultimately converts glucosinolates into isothiocyanates. This isothiocyanates natural chemical compound is primarily responsible for the plant's pesticidal properties. It can easily break down the eukaryotic cell components such as glucose, nitriles, thiocyanates, oxazolidine-2-thiones, hydroxynitriles, and epithionitriles (Al-Turkib and Dick, 2003; Morra and Kirkegaard, 2002). Soil-borne pathogens such as *R. solani* are highly sensitive to isothiocyanates (Kirkegaard *et al.,* 1996). The reduction of radial growth of *R. solani* using various plant extracts was reported by Mithen *et al.* (1986), Adandonon *et al.* (2006), Dwivedi and Prasad (2016). Aparna and Girija (2018), reported that mustard oil cake at 10% concentration was evaluated for their antifungal action by poisoned food technique and highest inhibition (100%) was obtained on the incorporation of mustard oil cake into the PDA medium. This indicates that using *B. nigra* (mustard) as a biofumigant which can play a vital role to protect the potato seedling mortality in the field soil and the result of the present investigation are in full agreement with the above-mentioned investigators. Thus *B. nigra* (mustard) was selected as biofumigant producer in the field experiment for management of *R. solani* isolate PR2.

Effect of biofumigation with soil solarization on potato seedling mortality in the field soil was clearly differentiated. The highest seeding mortality was appeared at treatment T_2, followed by T_1. Because, treatment T_2 plot soil (control-2) was inoculated with test pathogen without any amendment. Existing inocula in soil and artificially incorporated test pathogen inoculum was made high population density. As a result, the highest seedling mortality occurred. The experiment was the pathogenicity test case that's why test pathogen inoculum was added. But, T_1 treatment (control-1) was designed without incorporation of test pathogen inoculum and amendment into the soil. All treatments were compared with control-1 to determine the rate of infestation occurred by test pathogen into the field soil. The lowest seedling mortality appeared at treatment T_5 where used biofumigant and soil solarization. Fayzalla *et al*., (2009) explained the

bio-fumigant effect of mustard seed meal in the management of soil-borne pathogens such as *R. solani, Macrophomina phaseolina* and *S. rolfsii* causing damping off, root rot and wilt diseases of soybean under laboratory conditions and recorded 92.2% suppression of *R. solani* at a concentration of 25 mg/petri-dish. Addition of organic materials such as *Cannabis sativa*, *Toona ciliata*, and *Eucalyptus* sp. together with soil solarization further reduced black scurf disease incidence (Arora and Jyosana, 2007). Soil incorporated with aerobic compost tea (ACT) and the combination of ACT with a mixture of beneficial microorganisms have been observed to reduce stem canker, black scurf, and common scab on potato tubers (Larkin, 2008). The current investigations are completely agreed with the aforesaid researchers.

In the field experiment, biofumigation with soil solarization effect was found significantly more superior than either alone biofumigation or solarization effect in reducing disease incidence and severity of stem canker and black scurf diseases, respectively. Its effect not only minimized the potato diseases but also improved fresh tuber yield of potato. Biofumigant is used as means to control many soil-borne diseases by biocidal compounds (mainly isothiocyanates) released from glucosinolates in mustard seed meal which is hydrolyzed during degradation in soil (Shaban *et al.,* 2011). Additionally, soil solarization practice can raise soil temperature which may kill many pathogens such as nematodes, fungi, bacteria and weed seeds and seedlings. It also speeds up the breakdown of the organic materials and increases the amount of soluble nutrients such as nitrate, ammonium, calcium, magnesium and potassium in the soil which improved plant growth and yield over control (non-solarized soil) by 25-432% in broad Beans, Onions, Tomatoes and Clover in various types of soils (Abdel-Rahim *et al.,* 1988). Seedling mortality and other seedling diseases of different crops were controlled through the integration of antagonist with diverse organic amendments by different investigators (Adandonon *et al.,* 2006; Rahman *et al.,* 2012). According to Rahman *et al.* (2013), Chandel and Sharma (2014), biofumigation and soil solarization can be reduced population density of sclerotia forming fungi and their diseases of different crops which support the findings of the present study.

Several volatile bio-toxic compounds are released when mustard decomposed. Mustard is heated and they may augment the biocidal activity of the soil. It has been observed that plants grow faster when grown in solarized soil in comparison to non-solarized soils (Kumar *et al.,* 2017b). Combined biofumigation with solarization and compost amendments promote re-introduction of biocontrol agents such as *Trichoderma* spp. and *Bacillus* spp. etc. Populations of these two microbial antagonists increase relatively higher than other microorganisms in solarized soil (Stapleton and De Vay, 1986). Beneficial microorganism *T. harzianum* produces a large number of chemicals to solubilize rock phosphate, Zn, Mn^{4+}, Fe^{3+}, and Cu^{2+} and increase iron availability and enhance iron uptake which might be contributed in increasing yield of potato (Altomare *et al.,* 1999). The solubilization and chelating abilities of *T. harzianum* may also be influenced in increasing yield of potato (Harman, 2000). The findings of the present investigation are in agreement with the findings of other researchers Chellemi et al. (1994), Kirkegaard and Sarwar (1998), Charron and Sams (1999), Harvey *et al.* (2002).

Conclusion

The study revealed that mustard leaf extract appeared to be highly effective in inhibiting the radial growth of *R. solani* isolate PR2 in *in-vitro* trial. The combined use of biofumigation and solarization provided the best control measure for stem canker and black scurf diseases of potato caused by *R. solani* isolate PR2. It may be an eco-friendly alternate way to suppress the growth and development of the hazardous soil-borne pathogens

particularly *R. solani* and also directly or indirectly intensification the growth and yield of potato tubers.

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