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Integrated management of fusarium root rot and wilt disease of soybean caused by *Fusarium oxysporum*

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Abstract

An attempt was taken for the management of Fusarium root rot and wilt disease of soybean caused by *Fusarium oxysporum* using the integration of bio-agent with fungicide and organic amendment. Before going to the field experiments, different *in vitro* trials were conducted to select a virulent isolate of *F. oxysporum*, an effective antagonistic isolate of *Trichoderma harzianum,* suitable fungicide and organic amendment. Among the seven isolates of *F. oxysporum*, FOS-3 isolate was selected as a tested pathogen by the pathogenicity test. On the contrary, among the eighty-seven isolates of *T. harzianum*, ISR-26 isolate showed the highest (78.70%) inhibition of radial growth of test pathogen. In the case of fungicidal evaluation trial, Provax 200WP was found the most effective fungicide at the lowest conc. (75 ppm) for inhibiting the radial growth of *F. oxysporum* isolate FOS-3. Additionally, *in vitro* trial of different organic amendments, mustard oil cake was found the most effective organic amendment for reducing the growth and development of test pathogen at 3% concentration level. In the field trial, integrated use of *T. harzianum* with Provax 200WP and mustard oil cake under the treatment T_9 was appeared the best treatment in reducing seedling mortality (77.67%), disease incidence (81.88%) as well as disease severity (87.51%) caused by the test pathogen. Moreover, treatment T_9 was not only the best treatment for the management of soybean disease but also increased the significant quantity of yield (2.25 tha^{-1}).

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Introduction

Soybean (*Glycine max* L.) is one of the most important oilseeds crop all over the world. Nowadays, it's becoming a popular winter crop in Bangladesh. In 2017, the total soybean production in Bangladesh was about 97000 tons (Anon, 2018). There are several factors attributed to the low production of soybean such as climatic conditions, differences in rainfall patterns, an outbreak of diseases and pests etc. Among these factors, plant diseases play a major role in the yield reduction of soybean. More than hundreds of pathogens are known to affect soybean where sixty-six fungi, six bacteria, eight viruses and seven nematodes (Sinclair, 1978).

F. oxysporum is one of the most destructive seed-borne as well as soil-borne fungus which can cause root rot and wilt disease of soybean. When *F. oxysporum* attacks the soybean plant, the lower taproot and lateral roots become brown to black and show cortical decay or prominent vascular discoloration. Finally, the lateral roots may also die and decompose. In the meantime, secondary roots may develop on the upper taproot of the plant. If root rot becomes severe, infected soybeans may develop foliar symptoms including marginal or whole leaf chlorosis, stunting, wilting and finally leaves defoliation. However, the management of this pathogen is difficult because of its long persistence in soil and wide host range. Some chemical fungicides are effective against this fungus but these chemicals are expensive and harmful for living things as well as the environment (Abdel-Monaim *et al.*, 2011). The green revolution has led to intensified agriculture to meet the ever-increasing demands for food and fiber, which is practiced at great cost to the environment, resulting in continuous damage of natural ecosystems, groundwater and food-stuff pollution and other environmental degradation. Indiscriminate use of chemical pesticides and fertilizers in modern agriculture has resulted in the development of several problems such as pesticide resistance in pests, the resurgence of target and non-target pests, destruction of beneficial organisms like honey bees, and chemical residues in food, feed and fodder. However, a few studies have been done on the management of fusarium root rot and wilt disease of soybean but there is no report on integrated management of the above-mentioned disease of soybean in Bangladesh. Considering the aforesaid facts, the present research was undertaken to evaluate the effectiveness of integrated disease management strategies consisted of bio-agent, fungicide and organic amendment against Fusarium root rot and wilt disease of soybean caused by *F. oxysporum*.

Materials and methods

Collection, isolation and preservation of F. oxysporum isolates

Seven isolates of *F. oxysporum* designated as FOS-1 to FOS-7 were isolated from infected root, stem and pod tissues of soybean, bush bean, and pea. The specimens which had typical symptoms of root rot, as well as wilt, were selected from the infected fields. The fungal isolates were isolated according to Mian (1995). Then, the fungal colonies were grown on PDA and identified by following the standard method (Barnett and Hunter, 1972). The isolates were purified following the hyphal tip technique and stored in PDA slants at 10 °C.

Inoculum preparation of test pathogen

Inoculum of the *F. oxysporum* isolates were prepared and stored according to Rubayet & Bhuiyan (2016).

Pathogenicity test

The pathogenicity test of *F. oxysporum* isolates were conducted in pot culture on soybean plant according to the standard method (Rubayet *et al.*, 2017). Nine seeds of a soybean variety 'Shohag' were sown in each earthen pot. Three replications for each treatment were maintained and arranged following Completely Randomized Design on the floor. Plant disease development was observed regularly and recorded at 10, 15, and 21 days after sowing to estimate the effect of the pathogen in causing pre- and post-emergence seedling mortality. The causal agent of pre- and post-emergence seedling mortality was confirmed after re-isolation of the pathogen from un-germinated seeds, infected roots and stems (Liton *et al.*, 2019).

Collection, isolation and preservation of T. harzianum isolates

A total of 87 isolates of *T. harzianum*, whereas 37 isolates were isolated from the different crop fields of Gazipur, Chuadanga and Meherpur districts of Bangladesh following the soil dilution plate technique (Dhingra and Sinclair, 1985). And rest of the 50 isolates were collected directly from the plant pathology laboratory, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. All the isolated *Trichoderma* spp. were identified as *T. harzianum* based on the different morphological characteristics like hyphal growth, spore formation and color. The pure culture of *T. harzianum* was preserved following a regular method for future applications (Das *et al*., 2019).

Screening of T. harzianum isolates against test pathogen

The *in vitro* screening was conducted to evaluate the antagonistic effect of selected 87 isolates of *T. harzianum* against *F. oxysporum* isolate FOS-3 on Potato Dextrose Agar (PDA) medium by dual plate culture technique (Dhingra and Sinclair, 1985). After 7 days of incubation, the inhibition percentage of the radial growth of *F. oxysporum* isolate FOS-3 was calculated using the following formula (Sundar *et al*., 1995).

% Inhibition of growth = (A-B/ A) ×100. Where, A = Mycelial growth of the pathogen in the absence of *T. harzianum* (control) and B= Mycelial growth of the pathogen in the presence of *T. harzianum*.

In vitro evaluation of fungicides and organic amendments against the test pathogen

Effect on radial colony growth

Five fungicides namely, Conza 5%EC, Cabrio*Top 60WP, Provax 200WP, Bavistin 50WP and Dithane M-45 (Table 1) at three different concentrations viz., 75, 150, and 300 ppm were evaluated their effect on radial colony growth following "poison food technique" (Dhingra and Sinclair, 1985). Three replicated plates were used for each dose of each fungicide. The inoculated plates were incubated in the laboratory having an ambient temperature of 28±3 °C (Rubayet *et al*., 2011). Data on radial colony diameter were recorded after 3-days of incubation when the control plate was covered with the growth of the test pathogen. The diameter of colonies on PDA with and without fungicide were measured from the bottom side of the Petri dishes.

The inhibition of radial colony growth in amended plates was calculated based on colony diameter of the control plate following the formula as suggested by Sundar *et al.* (1995) mentioned earlier.

Another an *in-vitro* experiment was conducted to determine the effect of organic amendments such as mustard oil cake, sesame oil cake, soybean oil cake, coconut oil cake, and tea waste at 3 different concentrations viz. 1, 2, and 3% on the growth of *F. oxysporum* isolate FOS-3 following standard technique (Dhingra and Sinclair, 1985). Each oil cake and tea waste about 100 g was amended in 1000 ml of water and preserved in an earthen pot for fermentation. The earthen pots were rapped with the polythene bag for two weeks to preserve the moisture. After proper fermentation, individual oil cake extract was obtained by filtration with cheesecloth.

The requisite quantity of individual extract was added to the 100 ml conical flask with PDA medium for adjustment of the concentration of 1, 2, and 3% (Rubayet *et al*., 2018). Finally, the mixture was autoclaved at 121°C under 1 $kgcm^{-2}$ for 20 minutes. Approximately 20 ml of melted media with extract was poured into each 90 mm Petri dish (Rubayet *et al*., 2011). After solidification, the plates were inoculated by placing a 5 mm disc of 3-days old culture of *F. oxysporum* isolate FOS-3.

Three replicated plates were maintained for the selected dose for every oil cake and control plates contained without oil cake extract. Three days after incubation the inhibition of radial colony growth in the amended plates was computed based on colony diameter of the control plate using the same formula as stated above by Sundar *et al.* (1995).

Table 1. List of fungicides and their active ingredients.

Fungicides	Active ingredients	Mode of actions
Conza 5%EC	Hexaconazole 5% EC	Systemic
Cabrio*Top	Pyroclostribin 5%+ Metirum 55% WP	Systemic and contact
Provax 200WP	Carboxin 37.5% + Thiram 37.5% WP	Systemic and contact
Bavistin 50WP	Carbendazim 50% WP	Systemic
Dithane M-45	Mancozeb 80% WP	Systemic and contact

Effect on the mycelial dry weight

The effect of fungicides (Conza 5%EC, Cabrio*Top 60WP, Provax 200WP, Bavistin 50WP and Dithane M-45) on the mycelial dry weight of *F. oxysporum* isolate FOS-3 was determined by growing in the potato dextrose broth amended with fungicide. A total of 100 ml potato dextrose was poured into each 250 ml Erlenmeyer flask. The requisite quantity of individual fungicide was added to 100 ml potato dextrose broth for adjustment of the concentration of 75, 150, and 300 ppm. After mixing the amended media, Erlenmeyer flasks were autoclaved at 121 °C under 1 $kgcm^{-2}$ for 20 minutes. There were 3 replications for each treatment and maintained without amendment for the control treatment. Then, the 5 mm disc inoculum from 3-days old culture of the test pathogen was placed into the flask aseptically. The conical flasks were incubated in the laboratory having an ambient temperature of 28±3 °C for 14 days. Mycelia were harvested through the Whatman no. 1 filter paper and mycelial mass kept in the incubator under 70 °C for 24 hours. Finally, mycelial dry weight was measured and calculated based on the mycelial dry weight of the control flask following the same formula as suggested by Sundar *et al.* (1995). On the other hand, the effect of organic amendments on the mycelial dry weight of *F. oxysporum* isolate FOS-3 was determined by growing fungi in the potato dextrose broth amended with individual organic amendments at a concentration of 1, 2, and 3% (v/v) following the same technique as described earlier (Dhingra and Sinclair, 1985). Inhibition of mycelial dry weight in the amended broth was calculated based on the dry weight in control treatment following the aforesaid formula.

Compatibility of T. harzianum isolate ISR-26 with fungicides and organic amendments

The five selected fungicides at a different concentration such as 75, 150, and 300 ppm were used to determine their effect on the growth of *T. harzianum* isolate ISR-26 following standard procedures (Dhingra and Sinclair, 1985). The radial diameter of the fungal colony and sporulation were recorded 7-days after incubation when the control plate was covered with the full growth of *T. harzianum* isolate ISR-26 (Rubayet and Bhuiyan, 2012). Percent inhibition of the radial growth and sporulation were computed according to Sundar *et al.* (1995). Similarly, another an *in vitro* experiment was conducted with five selected organic amendments at three different concentrations viz. 1, 2, and 3% were used to determine their effect on the growth of *T. harzianum* isolate ISR-26.

Preparation of bio-agent inoculum

Wheat grain colonized inoculum for the selected *T. harzianum* isolate ISR-26 was also prepared following the standard procedures (Rubayet and Bhuiyan, 2016).

Treatments of the experiment

T_1= Fresh seeds sown in sterilized soil (Control-1), T_2= Soil inoculated with pathogen (SIP) + Fresh seeds (Control-2), T_3= SIP + Fungicide Treated Seeds (FTS), T_4= SIP + Wheat Grains Colonized *T. harzianum* isolate ISR-26 (WGCT) + Fresh seeds, T_5= SIP+ Organic Amendment (OA) + Fresh seeds, T_6= SIP+ WGCT + FTS, T_7= SIP+ WGCT + OA + Fresh seeds, T_8= SIP+ OA + FTS, and T_9= SIP+ WGCT + OA + FTS.

Integrated effect of Trichoderma, fungicide and organic amendment on F. oxysporum isolate FOS-3

A field experiment was conducted to find out the effect of integrated use of *T. harzianum* isolate ISR-26, Provax-200WP and mustard oil cake against the Fusarium root rot and wilt disease of soybean caused by *F. oxysporum* isolate FOS-3 and response on yield production. The test pathogen was artificially inoculated in the respective experimental field before sowing the seeds.

Cultivation of Soybean

Cultivable land was prepared and made the plot according to Rahman *et al.* (2018). Nine different treatments were allotted randomly to nine-unit plots per block. Before sowing, seeds were soaked for 24 hours to facilitate the germination and also dried for avoiding excess water. For the respective treatment of trial, seeds were treated with Provax 200WP @ 0.2 $g100g^{-1}$ seeds. Then, seeds were sown in lines uniformly by hand (45 $kgha^{-1}$) keeping the row to row distance of 25 cm. Weeding, mulching and irrigation were done in the experimental field whenever necessary.

Treatments and their application methods

Nine treatments were tested in the open field under artificially inoculated conditions. Control-1 was sterilized with 1% formaldehyde by drenching the soil properly. After treating with formaldehyde the soil was covered with transparent polyethene sheets. Polyethene sheets were removed after 48 hours and exposed to air 7 days before sowing. Inoculum of a selected isolate of *F. oxysporum* was thoroughly mixed with soil according to design and layout @ 90 gm^{-2} soil as suggested by Yuen *et al.* (1994). Water-soaked sterilized and air-dried wheat grains but not colonized by the fungal isolate was inoculated at the same rate in the control plots. Mustard oil cake was mixed with the soil of the concerned treatment plot was used @ 5 tha^{-1}. After 21-days, wheat grains colonized *T. harzianum* isolate ISR-26 was mixed thoroughly with the soil of selected treatments @ 50 gm^{-2} (Ab-El-Khair *et al.*, 2010). Then after three days, soybean seeds were sown in the plots of all treatments. In the case of seeds treatment with fungicide, around 100 g seeds were taken in a conical flask then added 0.2 g Provax 200WP and mixed properly before sowing.

Data recording from open field

The number of emerged seedling was recorded after 15-days of sowing and converted into percent pre- and post-emergence mortality of seedlings. Diseased seedlings were counted every alternate day and continued up to 30 days after sowing (Rahman *et al.*, 2018). Germination and seedling mortality were expressed in percentage based on the total number of seeds planted.

The disease incidence (DI), percent disease index (PDI) and total yield were assessed by the following formulas (Rahman *et al.*, 2013; Razaq *et al.*, 2015).

DI=(No. of infected plants/ Total No. of plants assessed) × 100

PDI=[Summation of all ratings/ {Total No. of rating × Max. disease grade (4)}] × 100

Total pod yield (tha^{-1})=[Yield per plot (kg)/ {Area of plot (m^2) × 1000 (Kg)}] × 10000 m^2

Data analysis

Statistically, data were analyzed using the MSTAT-C computer program after proper transformation whenever it was necessary. The treatment means were compared following Duncan's Multiple Range Test (Gomez and Gomez, 1984).

Results and discussion

Collection, Isolation, and Identification of F. oxysporum isolates

All the isolates were identified based on the morphological characteristics of macroconidia, microconidia, conidiophores, and chlamydospores which produced on PDA medium (Leslie and Summerell, 2006).

Pathogenicity test of F. oxysporum isolates in the pot culture

All the isolates of *F. oxysporum* showed variability in their virulence against soybean seedling in the pot

culture experiment (Table 2). Total seedling mortality was varied from 37.03 to 92.58% depending on the isolate. The highest total seedling mortality was observed by the isolate FOS-3 (92.58%) followed by isolate FOS-5 (81.47%). Based on the present findings isolate FOS-3 was selected for further study. The results of the pathogenicity of *F. oxysporum* in the present study are also agreed with Das *et al.* (2019) who found that plant infection by *Fusarium* can occur from seeds germination to mature stage, depending on the host and *Fusarium* species.

Table 2. Pathogenicity test of *F. oxysporum* isolates in pot culture.

Isolates of *F. oxysporum*	% seedling mortality		
	Pre-emergence	Post-emergence	Total
FOS-1	37.03	14.81	51.84 ^{c} (40.06) *
FOS-2	51.84	22.22	74.06 ^{b**} (59.49)
FOS-3	62.95	29.63	92.58 ^{a} (76.82)
FOS-4	48.14	22.22	70.36 ^{b} (57.11)
FOS-5	40.73	40.74	81.47 ^{b} (62.37)
FOS-6	29.63	18.51	48.14 ^{c} (43.93)
FOS-7	25.92	11.11	37.03 ^{c} (37.44)
Control	0.00	0.00	0.00 ^{d} (1.66)

* Figures within the parentheses are the transformed (arcsine) values. **Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT.

Screening of T. harzianum isolates against F. oxysporum isolate FOS-3

The highest 78.70% reduction of mycelial growth of test pathogen was found with the *T. harzianum* isolate ISR-26 followed by the isolate DT-5 (77.59%) (Fig. 1 and 2).

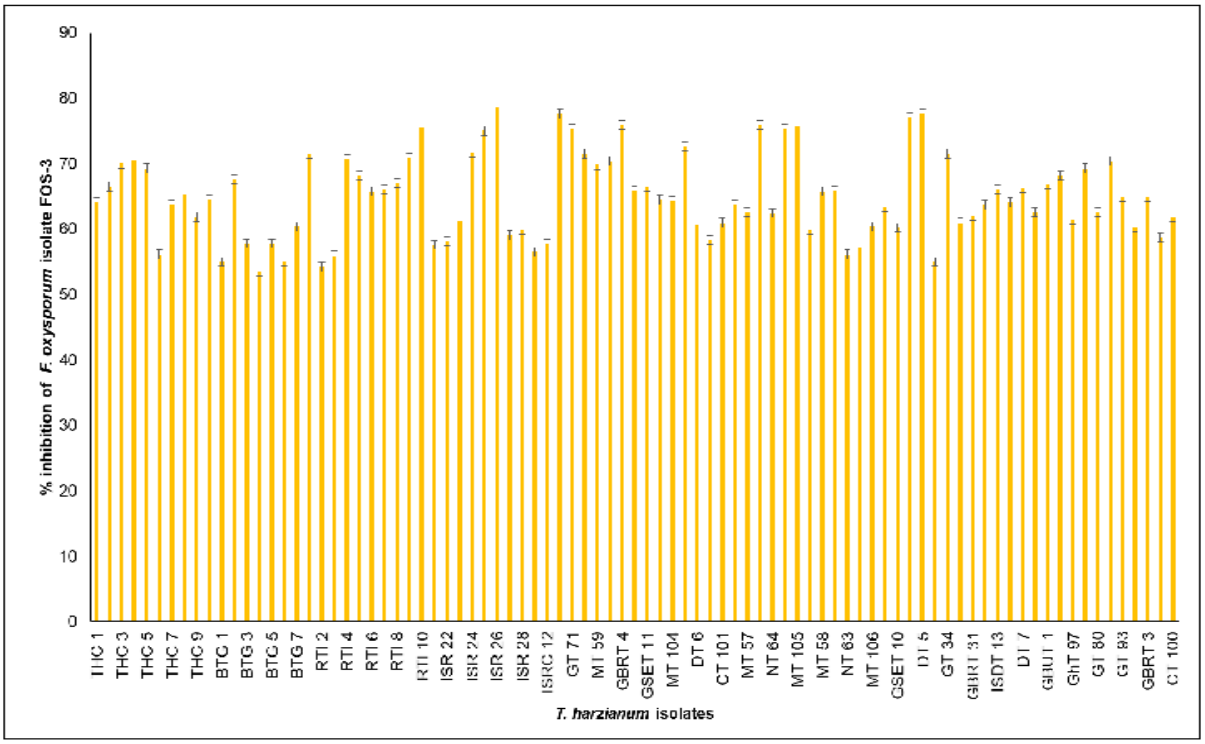

Fig. 1. Percent inhibition of *F. oxysporum* isolate FOS-3 mycelial growth by *T. harzianum* isolates in dual culture on PDA.

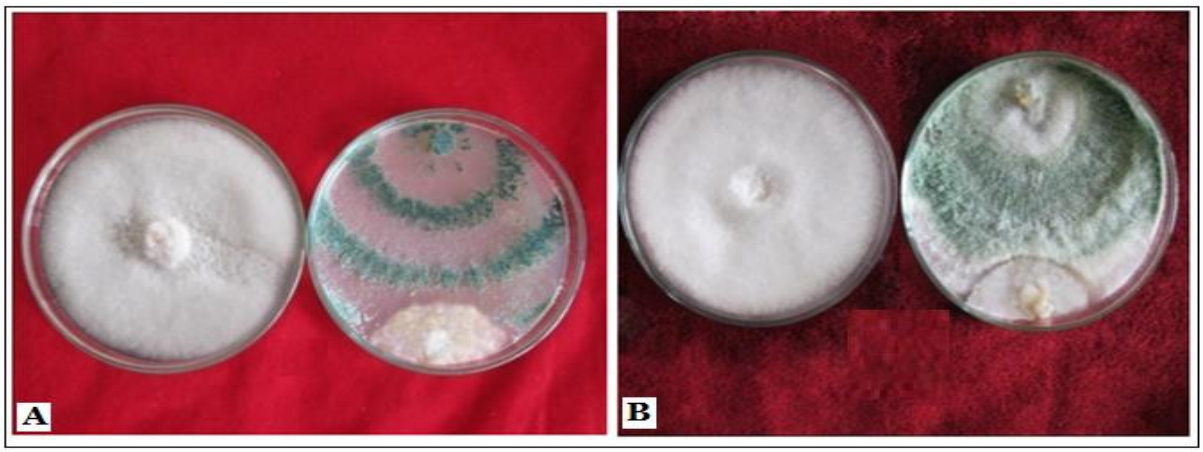

Fig. 2. Antagonism of *T. harzianum* isolates against *F. oxysporum* isolate FOS-3 on PDA (A = ISR-26, B = DT-5).

On the other hands, the lowest inhibition (55%) in radial growth was observed with the isolate GT-20 against test pathogen. Moreover, all *T. harzianum* isolates showed a great variation in their degree of antagonism which differs from isolate to isolate against test pathogen. The degree of antagonism as measured based on the class number on the indexing scale of 1-5 ranged from 1 to 3 against *F. oxysporum* isolate FOS-3. A total of 11 isolates (12.64%) showed antagonism class at 1, 29 isolates (33.34%) class at 2, 47 isolates (54.02%) class at 3, and no isolate was recorded under the antagonism class at 4 or 5 (Table 3). Similar observations were also reported by other investigators (Rahman, 2006; Prodhan, 2007; Das *et al.*, 2019).

Table 3. Antagonism of *T. harzianum* isolates against *F. oxysporum* isolate FOS-3 in dual culture on PDA.

Antagonism Classes	*T. harzianum* isolates	Isolates No.	% isolates
1	ISR-26, DT-5, MYT-75, MT-55, GBRT-4, GT-76, GT-71, MT-105, RTI-10, ISR-25, NT-66	11	12.64
2	ISDT-15,THC-5, THC-3, THC-2, THC-4, THC-8, ISR-24, RTI-1, GT-36, GT-34, RTI-9, RTI-4, GT-44, NT-65, MT -59, TT-112, GT-23, RTI-5, BTG-2, RTI-8, GBUT-1, GSET-11, DT-7, RTI-7, ISDT-13, GT-35, CT-102, RTI-6, MT-58	29	33.34
3	NT-64, GUT-19, MT-53, THC-7, GT-93, BTG-7, MT-104, THC-9, THC-1, GBRT-31, ISR-23, GBRT-3, CT-99, MT-52, GT-74, CT-100, MT-57, MT-106, MT-107, GT-80, THC-10, DT-6, RT-90, ISR-28, GBUT-2, CT-101, GHT-98, GSET-10, MT-51, ISDT-16, ISR-22, GT-77, ISR-27, GHT-97, BTG-5, ISR-21, ISRC-12, NT-63, BTG-3, ISRC-11, THC-6, RTI-3, BTG-6, GT-20, RTI-2, BTG-1, BTG-4	47	54.02
4	–	–	–
5	–	–	–
Total		87	100

Efficacy of fungicides against F. oxysporum isolate FOS-3

In *in vitro* trial, the maximum control of the test pathogen was achieved at all the concentration of Provax 200WP, the higher two conc. of Conza 5%EC and at the highest 300 ppm of Bavistin 50WP. There was no inhibition of mycelial growth observed with the fungicide Cabrio*Top even at the highest concentration. Dithane M-45 showed a low inhibitory effect on the radial growth of the test pathogen which was 5.55, 21.85, and 32.22% at 75, 150, and 300 ppm, respectively (Table 4). Higher inhibition rate of radial growth was also observed with Conza 5% EC at 75 ppm and Bavistin 50WP at 150 ppm. Similarly, complete inhibition was also observed while investigating with mycelial dry weight. Cabrio*Top showed very poor performance in inhibiting mycelial dry weight while Dithane M-45 showed lower inhibition which was 16.11, 42.82, and 53.43%, respectively at all three conc. The results of the present study revealed that Provax 200WP was the most effective fungicide against *F. oxysporum* isolate

FOS-3 which was followed by Conza 5%EC and Bavistin 50WP. Inhibition of colony growth and mycelial dry weight observed in present experiments which are supported by other investigators (Chavan *et al.*, 2003; Singh and Jha, 2003; Rubayet *et al.*, 2011).

Table 4. *In vitro* evaluation of fungicides against the radial growth and mycelial dry weight of test pathogen.

Fungicides	Conc. (ppm)	% inhibition in *F. oxysporum* isolate FOS-3	
		Radial growth	Mycelial dry weight
Conza 5%EC	75	71.11 ^{b} (57.52) *	84.89 ^{c} (67.16)
	150	100 ^{a**} (88.35)	100 ^{a} (88.35)
	300	100 ^{a} (88.35)	100 ^{a} (88.35)
Cabrio*Top	75	0.00 ^{g} (1.654)	4.48 ^{j} (12.14)
	150	0.00 ^{g} (1.654)	9.48 ^{i} (17.92)
	300	0.00 ^{g} (1.654)	13.97 ^{h} (21.95)
Provax 200WP	75	100 ^{a} (88.35)	100 ^{a} (88.35)
	150	100 ^{a} (88.35)	100 ^{a} (88.35)
	300	100 ^{a} (88.35)	100 ^{a} (88.35)
Bavistin 50WP	75	51.85 ^{c} (46.06)	64.21 ^{d} (53.26)
	150	70.37 ^{b} (57.15)	87.85 ^{b} (69.65)
	300	100 ^{a} (88.35)	100 ^{a} (88.35)
Dithane M-45	75	5.55 ^{f} (13.59)	16.11 ^{g} (23.66)
	150	21.85 ^{e} (27.85)	42.82 ^{f} (40.88)
	300	32.22 ^{d} (34.57)	53.43 ^{e} (46.97)
Control		90.00 mm	0.395 g

* Figures within the parentheses are the transformed (arcsine) values. **Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT.

Compatibility of T. harzianum isolate ISR-26 with fungicides

Another *in vitro* test was conducted to evaluate the compatibility of *T. harzianum* isolate ISR-26 with selected fungicides. Provax 200WP appeared the most compatible with all concentrations followed by Bavistin 50WP at the lowest concentration (Table 5).

Table 5. Compatibility of fungicides with *T. harzianum* isolate ISR-26 in *in vitro* test.

Fungicides	Conc. (ppm)	% inhibition in *T. harzianum* isolate ISR-26	
		Radial growth	Mycelial dry weight
Conza 5%EC	75	100 ^{a} (88.35) *	100 ^{a} (88.35)
	150	100 ^{a**} (88.35)	100 ^{a} (88.35)
	300	100 ^{a} (88.35)	100 ^{a} (88.35)
Cabrio*Top	75	100 ^{a} (88.35)	100 ^{a} (88.35)
	150	100 ^{a} (88.35)	100 ^{a} (88.35)
	300	100 ^{a} (88.35)	100 ^{a} (88.35)
Provax 200WP	75	0.00 ^{g} (1.65)	0.00 ^{h} (1.65)
	150	0.00 ^{g} (1.65)	0.00 ^{h} (1.65)
	300	0.00 ^{g} (1.65)	0.00 ^{h} (1.65)
Bavistin 50WP	75	0.00 ^{g} (1.65)	3.95 ^{g} (11.44)
	150	24.40 ^{e} (31.00)	31.11 ^{e} (33.90)
	300	49.25 ^{b} (44.22)	58.22 ^{b} (49.78)
Dithane M-45	75	22.59 ^{f} (28.45)	27.55 ^{f} (31.65)
	150	27.40 ^{d} (31.96)	35.44 ^{d} (36.54)
	300	31.48 ^{c} (34.43)	49.56 ^{c} (44.75)
Control		90.00 mm	0.422 g

* Figures within the parentheses are the transformed (arcsine) values. **Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT.

On the contrary, Dithane M-45 and Bavistin 50WP at 150 ppm showed moderate compatibility with the selected *T. harzianum* isolate ISR-26. In case the of Conza 5%EC and Cabrio*Top were found the incompatibility with *T. harzianum* isolate ISR-26 even at the lowest conc. which was also observed by Mclean *et al.* (2001) and Sarkar *et al.* (2010). Several investigators also showed that Provax 200WP (Vitavax 200) is very compatible with *T. harzianum* for radial growth and sporulation (Begum and Bhuiyan, 2004; Muniruzzam, 2004; Rubayet and Bhuiyan 2012). The findings of the present study are in agreement with the above-mentioned investigators.

In-vitro evaluation of organic amendments against F. oxysporum isolate FOS-3

The maximum 54.44% inhibition of the radial growth of *F. oxysporum* isolate FOS-3 was observed with mustard oil cake at the highest concentration (3%) which was significantly superior to all other amendments (Table 6). Mustard oil cake at 2% and sesame oil cake at 3% concentration produced a considerable 44.81 and 42.59% inhibition of mycelial growth. Lower rate of mycelial inhibition was obtained with soybean oil cake, coconut oil cake and tea waste, while the minimum inhibition (13.70%) appeared from tea waste at 1% conc. In the case of mycelial dry weight, 3% mustard oil cake showed significantly the highest (67.69%) reduction followed by 2% mustard oil cake (50.95%), and 3% til oil cake (50.14%). Tea waste at 1% concentration showed the lowest inhibition of mycelial dry weight. The results of the present study revealed that mustard oil cake was the most effective in inhibiting radial growth and mycelial dry weight of *F. oxysporum* isolate FOS-3 which is supported by other authors (Prodhan, 2007; Rubayet and Bhuiyan, 2012).

Compatibility of T. harzianum isolate ISR-26 with organic amendments

The compatibility of *T. harzianum* isolate ISR-26 with the organic amendments was observed *in vitro* assay. The inhibitory effect of the lowest concentration (1%) of mustard oil cake and coconut oil cake were found statistically identical and significantly showed the highest compatibility with the *T. harzianum* isolate ISR-26. In the case of tea waste at the highest concentration (3%) was found the moderate inhibitory effect on selected *T. harzianum* isolate. Moreover, the minimum compatibility was also observed with soybean and sesame oil cake (Table 7). The result of the experiment revealed that mustard oil cake was significantly inferior to all other

Table 6. *In vitro* evaluation of organic amendments against *F. oxysporum* isolate FOS-3.

Organic amendments	Conc. (%)	% inhibition in *F. oxysporum* isolate FOS-3	
		Radial growth	Mycelial dry weight
Mustard oil cake	1	35.55 ^{c} (36.60)*	45.58 ^{c} (42.47)
	2	44.81 ^{b**} (42.02)	50.95 ^{b} (45.55)
	3	54.44 ^{a} (47.55)	67.69 ^{a} (55.37)
Sesame oil cake	1	28.88 ^{de} (32.50)	34.11 ^{e} (35.73)
	2	35.18 ^{c} (36.38)	40.75 ^{d} (39.67)
	3	42.59 ^{b} (40.74)	50.14 ^{b} (45.08)
Soybean oil cake	1	18.88 ^{gh} (25.75)	20.08 ^{ij} (26.62)
	2	22.22 ^{f} (28.11)	24.04 ^{h} (29.35)
	3	29.62 ^{d} (32.98)	36.21 ^{e} (37.00)
Coconut oil cake	1	16.66 ^{h} (24.09)	18.32 ^{j} (25.34)
	2	21.11 ^{fg} (27.35)	26.52 ^{g} (30.99)
	3	26.66 ^{de} (31.08)	29.73 ^{f} (33.05)
Tea waste	1	13.70 ^{i} (21.70)	19.07 ^{j} (25.89)
	2	18.51 ^{gh} (25.49)	22.25 ^{hi} (28.15)
	3	25.92 ^{e} (30.60)	26.96 ^{g} (31.28)
Control		90.00 mm	0.397 g

* Figures within the parentheses are the transformed (arcsine) values. **Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT.

amendments in reducing the radial colony growth and spore formation of *T. harzianum* isolate ISR-26. Mustard oil cake was selected for further study due to its inhibitory effect of the test pathogen and good compatibility with *T. harzianum*. Compatibility of mustard oil cake with *Trichoderma* and fungicides in controlling *Rhizoctonia solani*, *Sclerotium rolfsii, F. oxysporum* and *Macrophomina phaseolina* were also observed by other investigators (Begum and Bhuiyan, 2004; Anis *et al*., 2010; Rubayet and Bhuiyan, 2012).

Table 7. *In vitro* evaluation of organic amendments on *T. harzianum* isolate ISR-26.

Organic amendments	Conc. (%)	% inhibition in *T. harzianum*	
		Radial growth	Mycelial dry weight
Mustard oil cake	1	0.00 ^{g**} (1.65) *	0.00 ^{j} (1.65)
	2	0.00 ^{g} (1.65)	0.00 ^{j} (1.65)
	3	0.00 ^{g} (1.65)	4.90 ^{i} (12.78)
Sesame oil cake	1	13.70 ^{f} (21.70)	15.52 ^{g} (23.20)
	2	20.00 ^{e} (26.56)	24.75 ^{f} (29.84)
	3	26.66 ^{d} (31.08)	29.79 ^{d} (33.08)
Soybean oil cake	1	25.18 ^{d} (30.11)	27.11 ^{e} (31.38)
	2	32.22 ^{c} (34.58)	33.49 ^{c} (35.36)
	3	36.66 ^{b} (37.26)	39.89 ^{b} (39.17)
Coconut oil cake	1	0.00 ^{g} (1.65)	0.00 ^{j} (1.65)
	2	0.00 ^{g} (1.65)	0.00 ^{j} (1.65)
	3	0.00 ^{g} (1.65)	0.00 ^{j} (1.65)
Tea waste	1	11.85 ^{f} (20.10)	12.81 ^{h} (20.97)
	2	37.03 ^{b} (37.48)	40.58 ^{b} (39.58)
	3	41.85 ^{a} (40.31)	43.69 ^{a} (41.38)
Control		90.00 mm	0.417 g

* Figures within the parentheses are the transformed (arcsine) values. **Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT.

Integrated effect of bio-agent, fungicide and organic amendment on the disease of soybean

Management of soybean Fusarium root rot and wilt disease

The lowest pre- and post-emergence as well as total seedling mortality was found in the Treatment T_1 (4.82, 2.19, and 7.01%) where no pathogen was inoculated. But, the highest 77.67% reduction of seedling mortality was recorded in the treatments T_9. On the contrary, significantly the lowest reduction of seedling mortality was observed in the treatment T_5 but identical with T_3 (Table 8). Moreover, disease incidence and severity of Fusarium root rot and wilt of soybean were also influenced by the application of bio-agent, fungicide and organic amendment either alone or in combination.

Table 8. Effect of bio-agent, fungicide and organic amendment on seedling mortality of soybean.

Treatments Ø	% seedling mortality			% reduction
	Pre-emergence	Post-emergence	Total	
T_1	4.82 (12.68)	2.19 (8.51)	7.01 ^{f} (15.36)*	-
T_2	31.57 (34.19)	17.54 (24.76)	49.12 ^{a} (44.49)	-
T_3	21.49 (27.61)	12.28 (20.51)	33.77 ^{b**} (35.53)	31.25
T_4	18.42 (25.41)	11.40 (19.73)	29.82 ^{bc} (33.10)	39.29
T_5	26.75 (31.14)	15.78 (23.41)	39.54 ^{b} (40.71)	19.50
T_6	12.28 (20.51)	5.70 (13.81)	17.98 ^{de} (25.09)	63.39
T_7	14.91 (22.71)	7.45 (15.84)	22.36 ^{cd} (28.22)	54.48
T_8	18.42 (25.41)	8.77 (17.22)	27.19 ^{bc} (31.43)	44.65
T_9	7.01 (15.36)	3.94 (11.45)	10.96 ^{ef} (19.33)	77.67

* Figures within the parentheses are the transformed (arcsine) values. **Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT.

The maximum reduction of disease incidence 81.88% and severity 87.51% were observed in the treatment T_9 followed by the treatments T_6 and T_7 (Table 9 and Fig. 3). The more or less same result also found in vegetable crops disease management which is caused by *Alternaria*, *R. solani*, *S. rolfsii* and *F. oxysporum* (Rubayet and Bhuiyan, 2016; Arefin *et al.*, 2019; Das *et al.*, 2019).

Table 9. Effect of bio-agent, fungicide and organic amendment on Fusarium root rot and wilt disease of soybean.

Treatments Ø	% disease incidence	PDI	% disease reduction	
			Incidence	PDI
T_1	0.00 ^{h} (1.28) *	0.00 ^{g} (1.28) *	100	100
T_2	30.08 ^{a} (33.26)	34.67 ^{a} (36.06)	-	-
T_3	19.27 ^{c} (26.03)	26.67 ^{b**} (31.08)	35.93	23.07
T_4	17.70 ^{d} (24.88)	22.67 ^{c} (28.41)	41.15	34.61
T_5	25.92 ^{b} (30.60)	29.33 ^{b} (32.78)	13.82	15.40
T_6	10.95 ^{f} (19.32)	13.33 ^{e} (21.37)	63.63	61.55
T_7	13.48 ^{e} (21.54)	14.67 ^{e} (22.47)	55.18	57.68
T_8	16.57 ^{d} (24.62)	18.67 ^{d} (25.57)	44.91	46.14
T_9	5.45 ^{g} (13.50)	4.33 ^{f} (13.12)	81.88	87.51

* Figures within the parentheses are the transformed (arcsine) values. **Means within same column followed by common letter(s) are not significantly different (*P=0.05*) by DMRT.

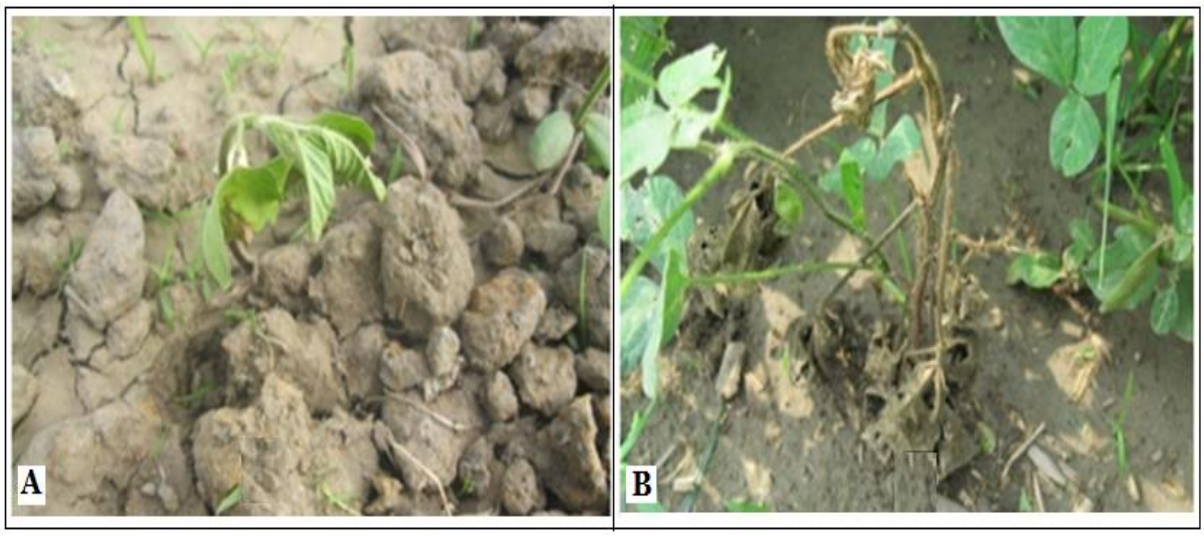

Fig. 3. Fusarium root rot and wilt disease symptom of soybean in the field (A-B).

Effect on yield and yield components of soybean

The highest plant height, number of pod per plant and seed weight were observed in the treatment T_9 followed by T_7. Growth promotion in the treatment T_3 has appeared significantly the most inferior in comparison to the other treatments and identical to the control-2 treatment. In the case of disease reduction, the treatment T_5 was significantly inferior to the treatments T_3 and T_4 but significantly higher in improving the growth promotion parameter. Different treatments also showed significant variation in total yield over control-2. The highest (2.25 tha^{-1}) seed yield was obtained from the treatment T_9, where colonized *T. harzianum*, Provax 200WP treated seed and mustard oil cake were used. Which was higher than the other treatment except for control-1. Considerably higher and statistically similar seed yield over control-2 was obtained in the treatment T_6, T_7, T_8 and T_4, respectively. On the other hand, the lowest yield (1.15 tha^{-1}) was obtained in the treatment T_2 where fresh seeds were sown in *F. oxysporum* isolate FOS-3 inoculated soil without application of any means of control (Table 10).

Table 10. Effect of different treatments on yield and yield components of soybean.

Treatments[Ø]	Plant height (cm)	No. of pod plant^{-1}	100 seed weight (g)	Yield plot^{-1} (g)	Yield (tha^{-1})
T_1	76.77 ^{bcd}	51.78 ^{cd}	10.75 ^{cd}	629.90 ^{a}	2.10 ^{a*}
T_2	70.23 ^{e}	44.78 ^{e}	9.23 ^{e}	344.73 ^{d}	1.15 ^{d}
T_3	71.67 ^{e}	47.33 ^{e}	9.78 ^{de}	418.87 ^{c}	1.39 ^{c}
T_4	72.93 ^{de}	50.44 ^{d}	10.99 ^{c}	504.39 ^{b}	1.68 ^{b}
T_5	78.00 ^{bc}	53.11 ^{bcd}	10.67 ^{cd}	436.17 ^{c}	1.45 ^{c}
T_6	74.13 ^{cde}	51.67 ^{cd}	11.10 ^{bc}	568.02 ^{b}	1.89 ^{b}
T_7	80.20 ^{ab}	55.67 ^{ab}	12.02 ^{ab}	555.10 ^{b}	1.85 ^{b}
T_8	79.77 ^{ab}	54.00 ^{bc}	10.88 ^{c}	520.59 ^{b}	1.74 ^{b}
T_9	83.70 ^{a}	58.00 ^{a}	12.75 ^{a}	674.06 ^{a}	2.25 ^{a}

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

[Ø]T_1= Fresh seeds sown in sterilized soil (Control-1), T_2= Soil inoculated with pathogen (SIP) + Fresh seeds (Control-2), T_3= SIP + Fungicide Treated Seeds (FTS), T_4= SIP + Wheat Grains Colonized *T. harzianum* isolate ISR-26 (WGCT) + Fresh seeds, T_5= SIP+ Organic Amendment (OA) + Fresh seeds, T_6= SIP+ WGCT + FTS, T_7= SIP+ WGCT + OA + Fresh seeds, T_8= SIP+ OA + FTS, and T_9= SIP+ WGCT + OA + FTS.

Results of the present study indicated that the application of different treatments in the field seed yield and yield contributing components were significantly increased by all the treatments over treatment T_2. Statistically, the highest yield was found with the treatment T_9. The yield increased not only because of declining plant disease but also might be due to the secretion of different growth-promoting substances in the soil by bio-agent *T. harzianum*. Altomare *et al.* (1999) reported that *Trichoderma* produced a plethora of chemical substances that are accelerated to solubilize minerals, for instance, rock phosphate, Zn, Mn^{4+}, Fe^{3+}, Cu^{2+} etc. and increased iron availability. These nutrient substances might be subsidized in increasing crop yield. The present study is supported by many researchers (Bhuiyan and Sen, 2013; Ahmed *et al.*, 2019; Simi *et al.*, 2019).

Conclusion

The result of the present study revealed that integrated use of bio-agent (*T. harzianum* isolate ISR-26), fungicide (Provax 200WP), and organic amendment (Mustard oil cake) delivered the effective control measure against Fusarium root rot and wilt disease of soybean caused by *F. oxysporum* isolate FOS-3. Moreover, this technique may be an ecofriendly alternative to diminish the population density of seed- and soil-borne pathogens as well as increasing the soybean yield at field conditions.

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References

Abd-El-Khair, Khalifa H, Karima RKM, Haggag HE. 2010. Effect of *Trichoderma* species on damping off diseases incidence, some plant enzymes activity and nutritional status of bean plants. Journal of American Science **6(9),** 486-497.

Abdel-Monaim MF, Ismail ME, Morsy KM. 2011. Induction of systemic resistance in soybean plants against Fusarium wilt disease by seed treatment with benzothiadiazole and humic acid. African Journal of Agricultural Research **6(26),** 5747-5756.

Ahmed MU, Bhuiyan MKA, Hossain MM, Rubayet MT, Khaliq QA. 2019. Efficacy of chitosan and bio-agent in controlling southern blight disease of carrot caused by *Sclerotium rolfsii* and improvement the crop production. Research in: Agricultural & Veterinary Sciences **3(3),** 113-125.

Altomare C, Norvell WA, Bjorkmanand T, Harman GC. 1999. Solubilization of phosphates and micronutrients by the plant-growth promoting and

biocontrol fungus *Trichoderma harzianum* Rifai. Applied and Environmental Microbiology **65,** 2926-2933.

Anis M, Zaki MJ, Dawar S. 2010. Effect of oilseed cakes alone or in combination with *Trichoderma* species for the control of charcoal rot of sunflower (*Helianthus annus*). Pakistan Journal of Botany **42(6),** 4329-4333.

Anonymous. 2018. Statistical Year Book of Bangladesh. Bangladesh Statistics Division, Ministry of Planning, Govt. of the People's Republic of Bangladesh, p 318-319.

Arefin MN, Bhuiyan MKA, Rubayet MT. 2019. Integrated use of fungicide, plant extract and bio-agent for management of Alternaria blight disease of Radish (*Raphanus sativus* L.) and quality seed production. Research in: Agricultural & Veterinary Sciences **3(1),** 10-21.

Barnett HL, Hunter BB. 1972. Illustrated genera of imperfect fungi. New York, USA: American Phytopathol. Society Press, p 218.

Begum JA, Bhuiyan KA. 2004. Effect of temperature and pH on *Trichoderma harzianum* and its sensitivity to fungicides. Bangladesh Journal of Plant Pathology **20,** 21-26.

Bhuiyan KA, Sen BC. 2013. Integrated management of southern blight disease of carrot caused by *Sclerotium rolfsii*. Acta Phytopathologica Sinica **43,** 515-523.

Chavan RA, Dhoke PK, Bharose AA, Jadhav VT. 2003. *In vitro* efficacy of fungicides in controlling *Fusarium oxysporum* f. sp. *carthami*. Journal of Soils and Crops **13(1),** 98-100.

Das IR, Bhuiyan MKA, Jannat R, Kayesh E, Rubayet MT, Arefin MN. 2019. Effect of bio-fortified compost in controlling soil-borne diseases of lentil (*Lens culinaris* L.) and enhance the crop growth and yield. Advances in Biology & Earth Sciences **4(2),** 93-106.

Dhingra OD, Sinclair JB. 1985. Basic plant pathology methods. CRC Press. Inc. Boca Raton Florida. p 13-44.

Gomez KA, Gomez AA. 1984. Statistical Procedures for Agricultural Research. 2nd ed. NY, USA: John, Wiley and Sons.

Leslie JF, Summerell BA. 2006. The *Fusarium* Laboratory Manual," Blackwell Publishing, Oxford, UK. P 388.

Liton MJA, Bhuiyan MKA, Jannat R, Ahmed JU, Rahman MT, Rubayet MT. 2019. Efficacy of Trichoderma-fortified compost in controlling soil-borne diseases of bush bean (*Phaseolus vulgaris* L.) and sustainable crop production. Advances in Agricultural Science **7(2),** 23-136.

Maniruzzaman MK. 2004. Stem canker and Black scraf diseases of potato in Bangladesh: its severity, characteristics of the casual organisum and management practices. Ph.D thesis paper, Department of Botany, Jahangirnagar University, Dhaka, Bangladesh.

Mclean KL, Hunt J, Stewart A. 2001. Compatibility of the biocontrol agent *Trichoderma harzianum* C52 with selected fungicides. New Zealand Plant Protection **54,** 84-88.

Mian IH. 1995. Methods in plant pathology. *IPSA*-JICA project publication number twenty-four. Institute of Post Graduate Studies in Agriculture, Bangladesh. p 51-52.

Prodhan MSUR. 2007. Integrated control of seedling mortality of tomato caused by *Fusarium oxysporum* f. sp. *lycopercisi*. MS thesis paper, Department of Plant Pathology, BSMRAU, Gazipur-1706. Bangladesh.

Rahman MM, Ali MA, Ahmad MU, Dey TK. 2013. Effect of tuber-borne inoculum of *Rhizoctonia solani* on the development of stem canker and black scurf of potato. Bangladesh Journal of Plant Pathology **29**, 29-32.

Rahman MT, Bhuiyan MKA, Karim MA, Rubayet MT. 2018. Screening of soybean resistance genotypes against *Fusarium oxysporum*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Sclerotium rolfsii*. Research in: Agricultural & Veterinary Sciences **2(3),** 139-156.

Rahman MT. 2006. Investigation on blossom blight (*Fusarium equisetti*) disease of tube rose and their integrated management. MS thesis, Department of plant pathology, BSMRAU, Gazipur-1706, Bangladesh.

Razaq M, Rab A, Alam H, Salahuddin S, Saud, Ahmad Z. 2015. Effect of potash levels and plant density on potato yield. Journal of Biology, Agriculture and Healthcare **5**, 55-62.

Rubayet MT, Bhuiyan MKA, Akanda MAM. 2011. Effect of fungicides, organic amendments and antagonist on in vitro growth of *Sclerotium rolfsii*. Bangladesh Journal of Plant Pathology **27(1-2),** 47-52.

Rubayet MT, Bhuiyan MKA, Jannat R, Masum MMI, Hossain MM. 2018. Effect of biofumigation and soil solarization on stem canker and black scurf diseases of potato (*Solanum tuberosum* L.) caused by *Rhizoctonia solani* isolate PR2. Advances in Agricultural Science **6(3),** 33-48.

Rubayet MT, Bhuiyan MKA. 2016. Integrated management of stem rot of potato caused by *Sclerotium rolfsii*. Bangladesh Journal of Plant Pathology **32(1-2),** 7-14.

Rubayet MT, Bhuiyan MKA. Hossain MM. 2017. Effect of soil solarization and biofumigation on stem rot disease of potato caused by *Sclerotium rolfsii*. Annals of Bangladesh Agriculture **21(1-2),** 49-59.

Rubayet T, Bhuiyan MKA. 2012. Compatibility of an Isolate of *Trichoderma harzianum* with fungicides and organic amendments. Bangladesh Journal of Plant Pathology **28(1&2),** 63-66.

Sarkar S, Narayanan P, Divakaran A. 2008. The *in vitro* effect of certain fungicides, insecticides, and biopesticides on mycelial growth in the biocontrol fungus *Trichoderma harzianum*. Turkish Journal of Biology **34(2010),** 399-403.

Simi SA, Jannat R, Rubayet MT, Bhuiyan MKA. 2019. Efficacy of bio-fortified compost in controlling anthracnose disease of chilli caused by *Colletotrichum capsici* and improvement the crop production. Scholars Academic Journal of Biosciences **7(12),** 482-489.

Sinclair JB. 1978. The seed borne nature of some soybean pathogens. The effect of phomopests and *Bacillus subtilis* on germination and their occurrence in soybean produced in Illinois. Seed Science and Technology **(6),** 957-964.

Singh DK, Jha MM. 2003. Effect of fungicidal treatment against chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri*. Journal of Applied Biology & Biotechnology **13(1-2),** 41-45.

Sundar AR, Das ND, Krishnaveni D. 1995. *In-vitro* antagonism of *Trichoderma* spp. against two fungal pathogens of castor. Indian Journal of Plant Protection **23(2),** 152-155.

Yuen GY, Craig MI, Geisler LJ. 1994. Biological control of *Rhizoctonia solani* on tall fescue using fungal antagonists. Plant Disease **78,** 118-123.