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Biocontrol of Damping off Disease in Brinjal (*Solanum melongena*) and Tomato (*Solanum lycopersicum*) by Arbuscular Mycorrhiza

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Abstract: Arbuscular Mycorrhiza (AM) fungi are recognized as bioprotectors of plants for their ability to enhance plant health and provide protection against pathogens. This study determines the ability of AM fungi (mixed AM inoculum of *Glomus mosseae*) to suppress the damping off disease of brinjal and tomato seedlings affected by the pathogenic fungi. This experiment consisted of two factors viz. AM fungi inoculation (inoculated and non-inoculated) and pathogen inoculation (*Sclerotium rolfsii*, *Rhizoctonia solani*, *Fusarium oxysporum*, and control). A completely randomized design was used to lay out the treatments, with three replicates per treatment. Preliminary experiments were conducted to select the virulent isolates of *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* against tomato and brinjal seedlings before setting the experiment for integration of AM fungi. The interaction of AM fungi and root-infecting pathogens was investigated under control conditions. Inoculation with pathogenic fungi *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* significantly increased damping off disease and reduced the plant height, root length, root, and shoot weight of brinjal and tomato compared to control. Seedlings inoculated with AM fungi had a lower incidence of damping off disease than AM fungi non-inoculated seedlings in spite of pathogenic fungi inoculation. About 50% of the pre-and post-emergence damping off disease was reduced due to AM fungi inoculation in brinjal and tomato. The root colonization by AM fungi and AM fungi spore density in the rhizosphere of brinjal and tomato was also higher in the AM fungi inoculated seedlings. The plant height, root length, and root and shoot weight of brinjal and tomato were also increased due to AM fungi inoculation. Therefore, the AM fungi can be inoculated to suppress the damping off diseases and vigorous seedling production of brinjal and tomato.

Keywords: Arbuscular Mycorrhiza, Growth, Damping off Disease, Vegetables

Introduction

One of the most common problems encountered worldwide in growing the seedlings of vegetable crops, such as tomato and brinjal, is the attack of various soil-borne fungal diseases (Koike *et al*., 2003). Damping off is a fungal disease occurring in soil, primarily affecting seeds and young seedlings of crops. It typically manifests as rotting of stems and roots below the soil surface. Initially, infected seeds may germinate normally but soon develop water-soaked, mushy tissues. Affected seedlings often collapse at the base and perish within days of emergence. Seedling damage by damping off disease is generally caused by *Pythium* spp., *Phytophthora* spp., *Fusarium oxysporum*, *Rhizoctonia solani*, *and Sclerotium rolfsii* (Grabowski, 2012; Koike *et al*., 2006; Jay *et al*., 2017; Rajendraprasad *et al*., 2017). When seeds are susceptible to rotting before germination, or seedlings may perish prior to emerging from the soil due to damping off, is known as pre-emergence damping-off (Cannon, 2003; Horst, 2013). On

the other hand, young seedlings exhibit rot at the crown, followed by softening and constriction of the tissue, and finally affected plants wilt and collapse, falling over, which is called post-emergence damping-off (Hebbar *et al*., 1998; Horst, 2013). Among the various diseases, the damping-off seedling diseases caused by *Sclerotium rolfsii*, *Rhizoctonia solani,* and *Fusarium oxysporum* are indeed significant constraints in the cultivation of brinjal and tomato in Bangladesh Islam and Faruq (2007). These soil-inhabiting pathogens have a wide host range, making them challenging to control effectively (Rangasami and Mahadevan, 1998; Martin, 1984).

Many strategies are being used to control damping off diseases, particularly, fungicides (Kondoh *et al*., 2001) used to fumigate soil, drench soil, and treat seeds but do not produce the desired results. Azcón-Aguilar and Barea (1997) stated that roots associated with AM fungi showed reduced damage from soil-borne pathogens. The AM fungi exhibited an inhibitory effect on diseases caused by various root-infecting fungi (Sharma *et al*., 1992). These AM fungi also formed symbiotic associations where nutrients flow in bi-directional ways: Carbon to the fungus and minerals to the plant, linking roots and soil (Sarkar *et al*., 2015a-b; 2016). Many mechanisms likely mediate AM fungi and soil pathogen interactions, though precise mechanisms behind disease suppression are still partially understood. The AM fungi reduce the susceptibility of plants to pathogens, thereby increasing their tolerance to infection (Padgett and Morrison, 1990; Thiagarajan and Ahmad, 1994). Furthermore, it boosts plant absorption of phosphorus and other minerals. (Sarkar *et al*., 2016). The nutritional advantages provided by AM fungi to host plants are pivotal in enhancing plant health and resilience to root diseases, thereby contributing to sustainable crop production. (Azcón-Aguilar and Barea, 1997; Linderman, 1994).

Therefore, the current study aimed to evaluate the effectiveness of AM fungi in defeating damping-off disease as well as enhancing the seedling's growth in brinjal and tomato.

Materials and Methods

Description of Study Site

The research was carried out in the net house located at the soil science division of the Bangladesh Agricultural Research Institute in Gazipur, Bangladesh. The study site falls within the subtropical climatic zone. This area is characterized by moderate to high temperatures, heavy rainfall, and high humidity which lead to wet and humid conditions and relatively long days from April to September. On the contrary, scanty rainfall leads to drier conditions, low humidity, sunshine hours, and a short day period from November to March. However, the weather condition of the study period from January to March 2011 is recorded and presented in Table 1.

Isolation of the Pathogenic Fungi, Inoculum Preparation and Pathogenicity Test

Various strains of *Sclerotium rolfsii*, *Rhizoctonia solani,* and *Fusarium oxysporum* were isolated from diseased stem and root tissues of tomato and brinjal. Infected plant specimens were collected from various locations in Jamalpur and Gazipur districts, Bangladesh from January to July 2010. The stains of the pathogenic fungi were isolated following a method described by Talukder *et al.* (2019). The fungal isolates were purified following the hyphal tip technique (Tuite, 1970) and identified with a standard key (Domsch *et al*., 2008). Pure cultures of the particular identified strains were stored for further use in the PDA slants at 10°C. Then, the inocula preparation of these strains and their pathogenicity tests were also done as described by Talukder *et al*., 2019. Finally, the most virulent isolates of *Sclerotium rolfsii*, *Rhizoctonia solani,* and *Fusarium oxysporum* were selected for further experiments.

Experimental Set-Up

The experiment comprised two factors viz; mycorrhizal inoculation (Inoculated and Non-inoculated) and pathogen inoculation (Sclerotium rolfsii, Rhizoctonia solani, Fusarium oxysporum, and control). It was arranged in a completely randomized design with three replicates per treatment. The potting media consisted of silted clay loam soils mixed with cow dung at a ratio of 5:1 and the potting media was sterilized 3 times by autoclaving at 121°C and 15 PSI for 30 min. Five kg of these mixtures was used per pot (vol. 6 liter). Chemical fertilizers like Urea, triple Phosphate, Muriate of Potash, and Gypsum were used in potting media preparation @11.0, 7.6, 6.2, and 3.4 g/100 g soil, respectively. Brinjal cv. BARI Begun-8 and tomato cv. BARI Tomato-14 was used as plant materials for the experiments.

Table 1: The mean monthly air temperature, relative humidity, precipitation, and sunshine hours recorded throughout the study period

	Air temperature (ºC)			Relative humidity		
Month	Maximum	Minimum	Mean	(%)	Rainfall (mm)	Sunshine (hours)
January	25.92	13.46	19.69	85.15	Trace	161.02
February	28.77	15.33	22.05	75.57	Trace	225.04
March	31.00	19.50	25.25	76.16	18.3	210.01

Seeds of the selected varieties and inocula of AM fungi (*Glomus mosseae*) were collected from the Bangladesh Agricultural Research Institute, Gazipur, Bangladesh. Soil-based AM fungi inoculum containing about 100 spores/g soil was used as needed based on the requirement of the treatment @ 15 g/kg soil. It was applied to a depth of 3 cm below the soil surface. Subsequently, the soil was saturated with water. Inocula of selected *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* strains were incorporated with the pot soil surface @ 20 g/kg soil after 7 days of AM fungi application. Seeds of tomato and brinjal, with a germination rate of 100%, were used in the experiment. Fifty seeds from each of tomato and brinjal were sown per pot after 7 days of pathogen inoculation. The seedlings of both crops emerged out within 9-10 days after sowing. Irrigation and other intercultural operations were done properly as needed.

Determination of Root Colonization by AM Fungi

After 45 days of sowing, five seedlings were uprooted carefully and percent root colonization was determined (Koske and Gemma, 1989). The collected root sections were prepared as described in our previous article (Talukder *et al*., 2019). The AM infection percentage was determined using root slide methodology (Read *et al*., 1976). The % root colonization was calculated as follows:

$$Root\ colonization\ by\ AM\ fungi\ (\%) = \frac{Number\ of\ AM\ positive\ segments}{Total\ number\ of\ segments\ scored} \times 100$$

Measurement of Growth Parameters of Brinjal and Tomato Plant

The height of the plant was assessed starting from its bottom base to the uppermost point of the tallest leaf. and was recorded just before harvesting time at 45 days after sowing. Then, the mean values were calculated. The harvesting was done by uprooting the entire plants. The soil clinging to the roots was eliminated through a gentle shaking motion followed by rinsing with water. Subsequently, root length was measured. The harvested shoot and root were dried and weighed separately and mean values were recorded.

Calculation of the spore density of AM fungi in the rhizosphere of both brinjal and tomato plants.

Soil samples were collected from the rhizosphere of the respective plant species for the AM fungi spore density assessment. AM fungal spores were obtained from the soil using a technique involving wet sieving followed by decanting (Gerdemann and Nicolson, 1963). The extract, with AM spores, was observed under a stereomicroscope and the number of spores was counted. The detailed procedure was described in our previous article (Talukder *et al*., 2019). Spore numbers from the three replicates were averaged and the result was expressed as a number per 100 g of dry soil.

Detection of AM Fungi

The initial identification of AM fungi genera was done under the stereomicroscope. Then, it was further confirmed under the compound microscope with different magnifications (Talukder *et al*., 2019). The spores were categorized into various genera based on their morphological characteristics, including spore size, color, spore walls, hyphal attachments, and other relevant traits. Schenck and Perez (1990); Morton and Benny (1990). Thus, the AM fungi from all the genera were identified, counted, and put together as per treatment.

Statistical Analyses

Before starting the statistical analyses, we ensured that all data met the assumptions of normality and equal variance through Levene's test. All data are presented as the mean ± SE (n = 3). The effects of mycorrhizal treatment on the plant height, root length, and root and shoot dry weights were analyzed by two-way ANOVA followed by Tukey's post-hoc test at a 0.05 significant level. We evaluated correlations between the parameters using Pearson's correlation coefficient at the 0.05 significance level. All statistical analyses were conducted using SPSS for Windows (version 13.0, SPSS, Inc., Chicago, IL, USA).

Results

Pathogenicity Tests of Different Isolates

The outcomes of the pathogenicity assessment indicated that every isolate of the pathogens exhibited a high level of pathogenicity towards both brinjal and tomato seedlings (Fig. 1). In tomato, isolates TS_2 of *Sclerotium rolfsii*, TF_3 of *Fusarium oxysporum* and TR_3 of *Rhizoctonia solani* resulted in complete seedling mortality, encompassing both pre- and post-emergence damping off, with a 100% mortality rate. The isolate TS_1, TS_3, TS_4, and TS_5 of *Sclerotium rolfsii* caused 91.6, 77.8, 86.1 and 94.5% seedling mortality, respectively while the isolate TF_1, TF_2, TF_4, and TF_5 of *Fusarium oxysporum* caused 83.3, 80.5, 94.4 and 75.0% seedling mortality, respectively. On the other hand, the isolates TR_1, TR_2, TR4 and TR_5 of *Rhizoctonia solani* caused 85.3, 82.6, 93.4, and 76.0% seedling mortality, respectively. Therefore, the most virulent pathogenic isolates TS_2, TF_3, and TR_3 were included in the subsequent experiments for tomatoes.

Similarly, in brinjal, the isolates BS_2 of *Sclerotium rolfsii*, BF_2 of *Fusarium oxysporum*, and BR_3 of *Rhizoctonia solani* were selected as the most virulent pathogenic isolates since they caused 100% seedling mortality (pre and post-emergence damping off) of brinjal. These BS_2, BF_2, and BR_3 isolates were also included in further brinjal experiments.

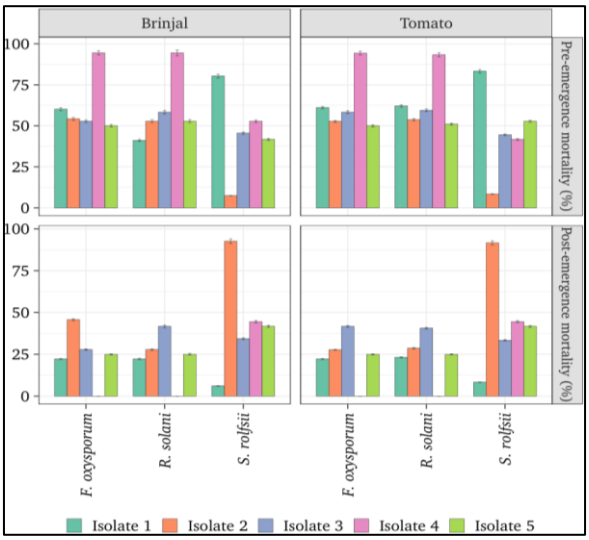

Fig. 1: Pathogenicity tests of different isolates of sclerotium rolfsii, Rhizoctonia solani, and fusarium oxysporum against brinjal and tomato seedlings. Error bars indicate ± SE of mean. A column having no error bar indicates that the SE value of the respective mean was too small or zero

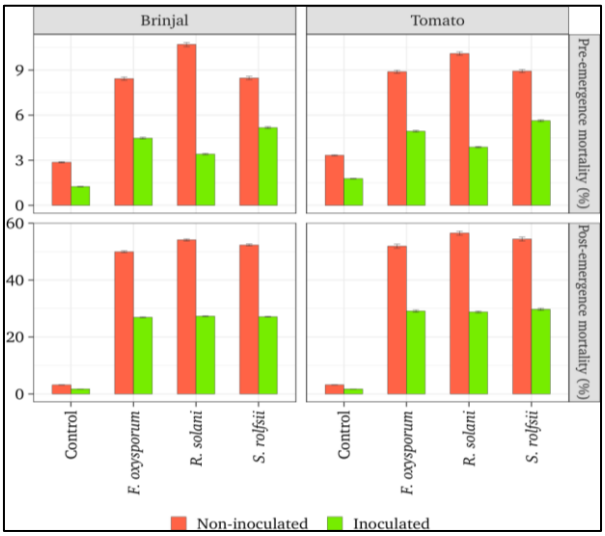

Fig. 2: Effect of AM fungi inoculation in suppressing the damping off disease (pre-emergence and post-emergence) of brinjal and tomato. Error bars indicate ± SE of mean. A column having no error bar indicates that the SE value of the respective mean was too small or zero

Inoculation of AM Fungi in Suppressing Damping-off Disease

The interaction effects of AM and pathogenic fungi causing pre-emergence and post-emergence damping off disease were significant (Fig. 2). Results showed that pre-emergence seedlings mortality of brinjal due *to Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* were 8.47, 10.7 and 8.43%, respectively in AM non-inoculated plants. But when AM fungi were inoculated, the pre-emergence seedlings mortality due to *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* were reduced to 5.17, 3.41, and 4.47%, respectively. Results indicated that about 39, 68, and 47% of pre-emergence seedling mortality due to *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum*, respectively were reduced by the influence of AM fungi. Similarly, the post-emergence mortality of brinjal seedlings due to *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* were 52.7, 54.5 and 50.3%, respectively in AM non-inoculated plants. But when AM fungi were inoculated, the post-emergence seedlings mortality due to *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* were reduced to 27.3, 27.5, and 27.1, respectively. These results also pointed out that about 48, 49, and 46% of post-emergence seedling mortality due to *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum*, respectively were reduced by the influence of AM fungi. Finally, we could conclude that more or less 50% of pre- and post-emergent seedling mortality in brinjal caused by different pathogenic fungi were inhibited by the influence of AM fungi.

A similar trend of results was also observed in the case of pre-and post-emergence damping off disease of tomatoes where AM-inoculated plants showed significantly lower damping off disease compared to AM-non-inoculated plants in cases of each pathogen (Fig. 2). The pre-emergence seedlings mortality of tomato due to *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* were 8.93, 10.1 and 8.89%, respectively in AM non-inoculated plants. But when AM fungi were inoculated, the pre-emergence seedlings mortality due to *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* were reduced to 5.63, 3.87 and 4.93%, respectively. Results indicated that about 37, 62, and 45% of pre-emergence seedling mortality due to *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum*, respectively were reduced by the influence of AM fungi. Similarly, the post-emergence mortality of tomato seedlings due to *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* were 54.4, 56.5 and 51.9%, respectively in AM non-inoculated plants. However, when fungi AM were inoculated, the post-emergence seedlings mortality due to *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* were reduced to 29.7, 28.8 and 29.1%, respectively. These results also showed that about 45, 49, and 44% of post-emergence seedlings mortality due to *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum*, respectively were reduced by the influence of AM fungi. At this time, we could also accomplish that approximately 50% of pre-and post-emergence seedling mortality in tomatoes caused by different pathogenic fungi were inhibited by the influence of AM fungi.

Root Colonization by AM Fungi and AM Fungi Spore Density in the Rhizosphere of Brinjal and Tomato Seedlings

Interaction effects of AM fungi and root infecting pathogens on the root colonization by AM fungi in brinjal and tomato seedlings were significant (Fig. 3). In the case of AM-inoculated brinjal seedlings, 45.5, 49.4 and 42.7% root colonization by AM fungi, respectively were observed *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium oxysporum* were inoculated. However, without AM inoculation, 5.29, 3.70, and 4.19% root colonization by AM fungi, respectively were observed while *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* were inoculated.

A similar trend was also observed in the case of tomato seedlings where AM-inoculated plants showed significantly higher root colonization by AM fungi compared to AM non-inoculated plants in spite of *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium oxysporum* (Fig. 3).

The AM fungi spore density in the rhizosphere of brinjal and tomato seedlings was significantly influenced by the interaction of AM fungi and root-infecting pathogens inoculation (Fig. 3).

In brinjal, due to the AM fungi inoculation, the root infecting pathogens *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* produced AM fungi spore density 35.2, 33.0, and 33.5 spore 100^{-1} g soil, respectively in the rhizosphere soil. Alternatively, without the AM fungi inoculation, root infecting pathogens *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* showed AM fungi spore densities 2.78, 3.24 and 3.52 spore 100^{-1} g soil, respectively. Results indicated that the application of AM fungi in the rhizosphere of brinjal seedlings had higher AM fungi spore density while inoculated with *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* than AM fungi non-inoculated brinjal seedlings.

Effects on Plant Growth Parameters

Plant Height, Root Length, Root and Shoot Weight of Tomato

The plant height, root length, and root and shoot weight of tomato seedlings were significantly influenced by AM fungi and root-infecting pathogen inoculation (Table 2). In the case of AM inoculation, the plant height was higher in AM Inoculated (I) seedlings (15.0 cm) than in AM Non-inoculated (N) seedlings (10.6 cm). On the other hand, due to pathogenic inoculation, the highest plant height (17.2 cm) was observed in Control (C) treatment. The *Rhizoctonia solani* (R), *Sclerotium rofsii* (S), and *Fusarium oxysporum* (F) inoculated seedlings had plant heights of 11.5, 11.3, and 11.1 cm, respectively and they were statistically similar (Table 2). In the interaction between AM and root infecting pathogen, the highest plant height (19.7 cm) was observed in C × I, and the lowest (8.7 cm) was observed in F × N. In the case of pathogens infection, AM-inoculated seedlings (S × I, F × I, and R × I) showed significantly higher plant heights compared to the AM non-inoculated seedlings (S × N, F × N and R × N).

A similar trend of result was also observed in tomato seedlings where the inoculation of AM fungi in the rhizosphere soil though inoculated with *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* had higher spore density than AM fungi non-inoculated seedlings.

The root lengths of tomato seedlings in S × I, F × I and R × I were 9.5, 8.83, and 9.83 cm, respectively while the root length in S × N, F × N, and R × N were 5.83, 5.26 and 5.50 cm, respectively (Table 2). These results showed that in case of pathogens infection AM inoculated seedlings showed significantly higher root length AM non-inoculated seedlings.

The root dry weight of tomato seedlings in S × I, F × I, and R × I were 1.74, 1.77, and 1.78 g/plant, respectively while the dry root weight in S × N, F × N, and R × N were 1.24, 1.25 and 1.25 g/plant, respectively (Table 2). The shoot dry weight of tomato seedlings in S x I, F x I, and R x I were 2.13, 2.15, and 2.17 g/plant, respectively while the dry shoot weight in S × N, F × N, and R × N were 1.78, 1.77 and 1.79 g/plant, respectively (Table 2). These results showed that in case of pathogens infection, AM inoculated seedlings showed significantly higher root and shoot weight compared to the AM non-inoculated seedlings.

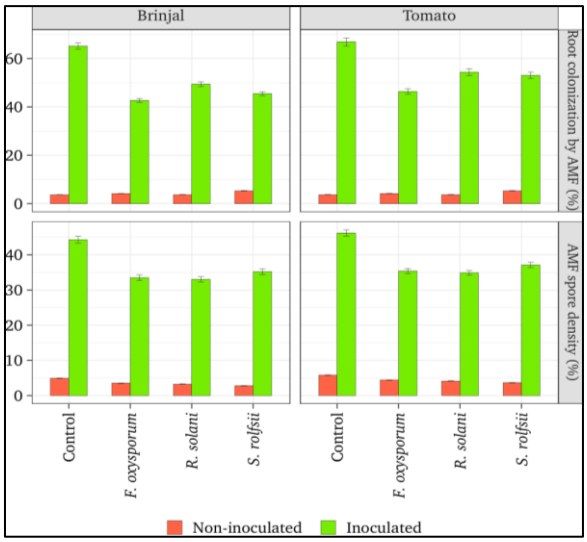

Fig. 3: Root colonization by AM Fungi (AMF) and AM Fungi (AMF) spore density in the rhizosphere of brinjal and tomato seedlings. Error bars indicate ± SE of mean. The values represent the means of three replicates and 10 root segments per replicate were selected for observation. A column having no error bar indicates that the SE value of the respective mean was too small or zero

Table 2: Inoculation effects of AM fungi and root infecting pathogens on the plant's growth parameters. The values represent the means of three replicates

	Tomato				Brinjal			
	Plant height (cm)	Root length (cm)	Root dry weight (g/plant)	Shoot dry weight (g/plant)	Plant height (cm)	Root (cm)	Root dry weight (g/plant)	Shoot dry weight (g/plant)
AM inoculation effect								
Non-inoculated (N)	10.6^{b}	6.34^{b}	1.49^{b}	1.97^{b}	9.62^{b}	5.14^{b}	1.43^{b}	1.87^{b}
Inoculated (I)	15.0^{a}	10.1^{a}	2.01^{a}	2.42^{a}	13.08^{a}	8.86^{a}	1.95^{a}	2.32^{a}
p-value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Root infecting pathogen effect								
Sclerotium rofsii (S)	11.3^{b}	7.66^{b}	1.48^{b}	1.96^{b}	10.3^{b}	6.47^{b}	1.42^{b}	1.86^{b}
Fusarium oxysporum (F)	11.1^{b}	7.05^{b}	1.50^{b}	1.95^{b}	10.0^{b}	5.85^{b}	1.45^{b}	1.87^{b}
Rhizoctonia solani (R)	11.5^{b}	7.67^{b}	1.52^{b}	1.97^{b}	10.4^{b}	6.48^{b}	1.46^{b}	1.89^{b}
Control (C)	17.2^{a}	10.4^{a}	2.52^{a}	2.88^{a}	16.2^{a}	9.21^{a}	2.45^{a}	2.79^{a}
p-value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Interaction effect of AM fungi and root infecting pathogen inoculation								
S x N	9.50^{c}	5.83^{c}	1.24^{d}	1.78^{c}	8.50^{c}	4.64^{c}	1.18^{d}	1.68^{c}
F x N	8.72^{c}	5.26^{c}	1.25^{cd}	1.77^{c}	7.72^{c}	4.08^{c}	1.29^{cd}	1.67^{c}
R x N	9.50^{c}	5.50^{c}	1.25^{cd}	1.79^{c}	8.50^{c}	4.30^{c}	1.09^{cd}	1.69^{c}
C x N	4.5^{b}	8.77^{b}	2.23^{b}	2.55^{ab}	13.8^{b}	7.57^{b}	2.17^{b}	2.44^{ab}
S x I	13.2^{b}	9.50^{b}	1.74^{bc}	2.13^{bc}	12.0^{b}	8.30^{b}	1.65^{bc}	2.03^{bc}
F x I	13.5^{b}	8.83^{b}	1.77^{bc}	2.15^{bc}	12.4^{b}	7.63^{b}	1.71^{bc}	2.04^{bc}
R x I	13.5^{b}	9.83^{ab}	1.78^{b}	2.17^{bc}	12.4^{b}	8.63^{ab}	1.91^{b}	2.07^{bc}
C x I	19.7^{a}	12.1^{a}	2.79^{a}	3.23^{a}	18.6^{a}	10.9^{a}	2.73^{a}	3.13^{a}
p-value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Legend: S × N = *Sclerotium rofsii* inoculated seedlings without AM fungi inoculation; F × N = *Fusarium oxysporum* inoculated seedlings without AM fungi inoculation; R × N = *Rhizoctonia solani* inoculated seedlings without AM fungi inoculation; C × N = Seedlings without both pathogen and AM fungi inoculation; S × I = *Sclerotium rofsii* inoculated seedlings with AM fungi inoculation; F × I = *Fusarium oxysporum* inoculated seedlings with AM fungi inoculation; R × I = *Rhizoctonia solani* inoculated seedlings with AM fungi inoculation; C × I = Seedlings with AM fungi inoculation only

Results indicated that all growth parameters such as the plant height, root length, root and shoot dry weight of tomato seedlings were significantly higher in AM fungi inoculated seedlings in case of each root infecting pathogen inoculation.

Plant Height, Root Length, Root and Shoot Weight of Brinjal

Due to the interaction of AM fungi and root infecting pathogen, the AM-inoculated brinjal seedlings showed all growth parameters significantly higher than the AM non-inoculated seedlings (Table 2). The plant height in AM inoculated seedlings in the presence of pathogens in S × I, F × I, and R × I were 12.0, 12.4, and 12.4 cm, respectively in contrast AM non-inoculated seedlings in S × N, F × N, and R × N were 8.5, 7.72 and 8.50 cm, respectively (Table 2). Similarly, the root length in AM inoculated seedlings in the presence of pathogens in S × I, F × I, and R × I were 7.57, 8.30, and 7.63 cm, respectively whereas AM non-inoculated seedlings in S × N, F × N and R × N were 4.64, 4.08 and 4.30 cm, respectively (Table 2). The root dry weight in AM inoculated seedlings in S × I, F × I, and R × I were 1.65, 1.71, and 1.91 g/plant, respectively whereas AM non-inoculated seedlings in S × N, F × N and R × N were 1.18, 1.29 and 1.09 g/plant, respectively (Table 2). Correspondingly, the shoot dry weight in AM inoculated seedlings in the presence of pathogens in S × I, F × I, and R × I were 2.03, 2.04, and 2.07 g/plant, respectively in contrast AM non-inoculated seedlings in S × N, F × N and R × N were 1.68, 1.67 and 1.69 g/plant, respectively (Table 2).

Discussion

Damping off seedling diseases caused by *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* has been treated as the major problem of brinjal and tomato cultivation in Bangladesh (Islam and Faruq, 2007). Controlling these soil-inhabiting pathogens poses significant challenges. (Martin, 1984; Rangasami, 1998). Many strategies are being used to control damping off diseases, particularly, fungicides (Kondoh *et al*., 2001) but do not produce the desired results. The AM fungi exhibit a suppressive effect on diseases caused by various root-infecting fungi. (Azcón-Aguilar and Barea, 1997; Sharma *et*

al., 1992). In this study, the damping off disease of brinjal and tomato was significantly suppressed by AM inoculant (Fig. 2). The AM-inoculated plants showed lower pre-emergence damping off disease than non-inoculated plants. Similarly, the AM-inoculated plants showed lower post-emergence damping off disease than non-inoculated plants. Significant interaction effect of AM and root infecting pathogen was also observed in brinjal and tomato causing pre-emergence and post-emergence damping off disease (Fig. 2). These findings indicated that inoculation with AM fungi notably suppressed the root-infecting pathogen responsible for damping off disease. These results supported the findings of Harrier and Watson (2004); Mazen *et al*. (2008); Talukder *et al.* (2019). These results are consistent with the observations made by Dehne and Schönbeck (1979), who reported a reduction in damage and infection caused by *Fusarium oxysporum f.* sp. *lycopersici* in tomato plants as a result of AM inoculation. Additionally, Kapoor (2008) reported that AM fungi have the ability to confer disease tolerance in tomato plants that are already infected with *Fusarium oxysporum f.* sp. *lycopersici*.

The root colonization of brinjal and tomato by AM fungi was significantly influenced by AM and pathogenic fungi (Fig. 3). The AM-inoculated plants showed higher root colonization by AM fungi while AM non-inoculated plants showed lower root colonization in the presence of each pathogen (Fig. 3). The above result indicated that AM inoculation increased the root colonization of selective vegetables by AM fungi. Karagiannidis *et al*. (2002) have also found better root colonization by AM in brinjal and tomato due to AM inoculation. A similar result was found by many researchers who obtained increased root colonization by AM fungi of mycorrhizal-associated plants (Sarkar *et al*., 2015a-b; 2016; 2017). In plants inoculated with AM fungi, the highest level of root colonization by AM fungi in both brinjal and tomato was observed in the control group. However, inoculation with pathogens decreased the colonization level in plants for each fungal species. It proved that pathogens inhibit the root colonization by AM fungi. The AM spore density in the rhizosphere of all selective vegetables was influenced by AM fungi (Fig. 3). The plants inoculated with AM fungi exhibited significantly higher spore density compared to plants that were not inoculated with AM fungi. Spore density was also significantly reduced by pathogenic fungi in all cases (Fig. 3). Results of these studies indicated that the AM fungi inoculation increased the AM fungal structures by augmented root colonization and spore density in the rhizosphere of the selected vegetables. As a result, the abundance of pathogenic fungi in the roots of brinjal and tomato seedlings is reduced. Fitter and Garbaye (1994) also demonstrated that interactions between AM fungi and other soil organisms were inhibitory and exhibited clear competitiveness. Several studies have reported also the negative correlations between the abundance of AM fungal structures and pathogenic microorganisms in roots and soil (Filion *et al*., 2003; Bødker *et al*., 2002; St-Arnaud *et al*., 1994), as well as on growth medium (St-Arnaud *et al*., 1995). It is presumed that pathogenic and AM fungi compete for common resources within the root, such as infection sites, space, and photosynthate (Whipps, 2004). Interference competition may occur if AM fungal colonization reduces the number of infection loci within the root system (Vigo *et al*., 2000).

It is well known that AM fungi can improve the growth of their host plants (Smith and Read, 2010). In our present study, the effect of AM fungi, root infecting pathogen and their interaction effect on plant height, root length, root and shoot weight of tomato and brinjal were significant (Table 2). Pathogen inoculation reduced the plant growth significantly though there were no significant differences among pathogen species (Table 2). However, the plant height, root length, and root and shoot weight of tomato and brinjal plants were significantly improved by the inoculation of AM fungi (Table 2). Results revealed that root-infecting pathogens reduced the plant growth, but it can be minimized by the inoculation of AM fungi. Similar results were also found in some previous research where tomato root and shoot dry mass yield increased by AM fungi (Al-Karaki, 2006; Gamalero *et al*., 2004; Subramanian *et al*., 2006). In addition, Ojala and Jarrell (1980) also proved that the AM-inoculated roots of tomato plants became highly infected with AM fungi, and yield parameters were significantly increased with inoculation over non-inoculated plants. Karagiannidis *et al*. (2002) have also found that the growth of tomato and brinjal was increased due to inoculation of AM fungi. Studies unrelated to pathogen protection have also shown that increased AM fungal richness resulted in improved plant growth via superior nutrient supply to their host plants (Jakobsen *et al*., 1992; Van Der Heijden *et al*., 1998, 2003; Smith *et al*., 2000; Van Aarle *et al*., 2002).

Our above discussion revealed that the AM fungi inoculation confers protection of host plants brinjal and tomato from root pathogenic fungi and also improves the seedling's growth.

Conclusion

The AM fungi have a big influence on plant growth and damping off disease control. The AM fungi can reduce about 50% of the pre-and post-emergence damping off diseases of brinjal and tomato. Therefore, the application of AM fungi may be advised to the farmers for the management of damping off disease of brinjal and tomato seedlings as well as increasing seed germination and seedling growth. However, more work should be needed to develop a suitable model for controlling damping off disease of brinjal and tomato.

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Author's Contributions

Md. Raihan Talukder: Conception, design, execution, analysis and interpretation of the data, prepared of drafted manuscript.

Animesh Sarkar: Analysis and interpreted the data, prepared of drafted manuscript.

Md. Harun Rashid: Prepared of draft manuscript, revised the manuscript for intellectual content and approved the manuscript to be published.

Md. Motaher Hossain: Analysis and interpretation of the data, participated in drafted, reviewed the manuscript for intellectual content and approved the manuscript to be published.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

Conflict of Interest

The authors assert that the study was carried out without any commercial or financial associations that could be interpreted as a possible conflict of interest.

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