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The role of arbuscular mycorrhizal fungi in the bioprotection of ash gourd (*Benincasa hispida*) against damping off disease

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ABSTRACT

This experiment was aimed to determine the capacity of arbuscular mycorrhizal fungi (AMF) inoculation to control the damping off disease of ash gourd (*Benincasa hispida*) seedlings caused by *Sclerotium rolfsii*, *Fusarium oxysporum* and *Rhizoctonia solani*. The virulent strains of *S. rolfsii*, *F. oxysporum* and *R. solani* were isolated before setting the experiment for inoculation of arbuscular mycorrhiza with ash gourd. AMF was collected from the rhizosphere of trap crop sorghum. Seedlings inoculated with AMF had significantly lower incidence (p<0.05) of damping off disease than non-mycorrhizal plants. Growth parameters of ash gourd plants inoculated with arbuscular mycorrhizal fungi significantly (p<0.05) increased than those of non-mycorrhizal plants. Inoculation of pathogenic strain significantly reduced the plant height, root length, root and shoot weight (all p<0.05) than untreated control. Root colonization by arbuscular mycorrhiza in ash gourd plant and mycorrhizal spore density in soil were also higher in AMF inoculated treatment than non-inoculated cases. Inoculation with root infecting pathogen significantly reduced the root colonization and spore density of mycorrhiza. Therefore, arbuscular mycorrhizal fungi can be used in ash gourd against damping off disease.

Keywords: AMF, damping off disease, growth, ash gourd, vegetables

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1 Introduction

Arbuscular mycorrhizal fungi (AMF) associations have been shown to reduce damage caused by soil-borne plant pathogens (Azcon-Aguilar and Barea, 1997). It has suppressive effect on diseases caused by a number of root-infecting fungi (Sharma et al., 1992) while AMF play an important role for plant growth by reducing the susceptibility, or increasing the tolerance of plant pathogens (Padgett and Morrison, 1990; Thiagarajan and Ahmad, 1994). AMF are also known to enhance plant uptake of phosphorus (P) similar to phosphate solubilizing bacteria (Sarkar et al., 2012) but the mechanism is quite dif-

ferent Sarkar et al. (2015b,c,a, 2017). The nutritional superiority of more vigorous AMF plants has been proposed to be a mechanism in reduction of root diseases (Azcon-Aguilar and Barea, 1997; Dar and Reshi, 2017). Alternatively, several mechanisms are also likely to be involved in the interactions between AMF and soil pathogens but the mechanism of the disease suppression is not fully understood.

Seedling damage caused by damping off disease complex (Cook and Baker, 1983; Cook, 2000) generally involves *Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani* and *Sclerotium rolfsii* (Wrather et al., 2001). All these pathogens cause post and pre-emergence damping off by rotting stems at or near the soil surface level. These fungi may also attack germinating seeds before the seedlings emerge from the soil, resulting a poor stand in the seedbed. Pre-emergence mortality was found due to infection by *Pythium* and *Phytophthora* in cool weather with high soil moisture, whereas post-emergence damping off was reported in warmer and drier soil caused by *Rhizoctonia* and *Sclerotium* (Mian and Rodriguez-Kabana, 1982). Information on yield loss of vegetable seedlings are however limited. Many suggestions have been given to control damping off disease of vegetable seedlings using fungicide or chemicals (Kondoh et al., 2001) as fumigant for soil, soil drenching and seed treatment but the success was only partial. Plant disease control is one of the big challenges now. Moreover, chemical control of the pathogen is often expensive and not environment friendly while the presence of AMF in plants roots is known to reduce the disease (Kjøller and Rosendahl, 1997).

Hence, the present study was undertaken to determine the effects of AMF in and controlling damping-off disease of ash gourd seedling caused by the root infecting pathogen.

2 Materials and Methods

2.1 Experimental site

This experiment was conducted in the net house of Soil Science Division of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh from January to March, 2010. The experimental area was located at 23°59′31.1″N, 90°24′48.9″E under the sub-tropical climatic zone, which is characterized by moderate to high temperature, heavy rainfall, high humidity and relatively long day during Kharif season (April to September) and scanty rainfall and low humidity.

2.2 Plant material

Ash gourd cv. BARI Chalkumra-2 was used as plant material for the experiments. This is a popular ash gourd variety in Bangladesh for its good taste and yield but very susceptible to damping off disease.

2.3 Isolation of pathogenic isolates

Infected plant specimens (ash gourd) were collected from different locations of Jamalpur and Gazipur district. Strains of *Sclerotium rolfsii*, *Fusarium oxysporum* and *Rhizoctonia solani* were isolated from infected stems and roots of ash gourd. Infected specimens were washed with tap water, discolored parts were cut into small pieces (5 mm), sterilized with 0.1% NaOC1 for two minutes and rinsed in sterilized water for 3 times and dried between folds of sterilized filter paper. The sterilized stem and root pieces were transferred to four replicated petri dishes containing sterilized PDA (Potato Dextrose Agar) and incubated in the laboratory at room temperature for 5 days. The fungal isolates were purified following hyphal tip technique (Tuite, 1969), identified using standard key (Domsch et al., 1980). Repeated culture was done from tip of the single hyphae to obtain pure culture of the identified *S. rolfsii*, *F. oxysporum* and *R. solani* and the pure culture were stored in the PDA slants at 10 °C for further use.

2.4 Preparation of mycorrhizal inocula

Rhizospheric soil was collected from the AMF trap crop sorghum cultured in the net house of Soil Science Division of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. This soil contained several genera of mycorrhiza. Soil based AM inocula containing about 100 spores and/or infected root pieces g^{-1} soil was used in this experiment for suppressing the damping off disease of ash gourd.

2.5 Preparation of potting medium

Clay loam soil was collected from the bank of Turag River at Kodda, Gazipur. The collected soil was mixed with cow dung at 5:1 and was used as potting medium. Chemical fertilizers *viz.* urea, TSP, MP and gypsum were applied @ 10.85, 7.5, 6.0 and 3.4 g 100 g^{-1} soil, respectively. The soil was sterilized in autoclave at 121 °C and 15 PSI for 30 min. Five kg of this medium was used per pot (6 L).

2.6 Experimental design

Seedlings infection was caused by *S. rolfsii*, *F. oxysporum*, *R. solani* and control (no pathogen). Again these seedlings were either inoculated with AMF or not inoculated. Therefore the treatment combinations consisted of C×I, C×N, F×I, F×N, R×I, R×N, S×I and S×N where C = control, F = *Fusarium*, R = *Rhizoctonia*, S = *Sclerotium*, I = AMF inoculated, N = AMF non-inoculated. The experiment was laid out

in a completely randomized design (CRD) with three replications.

2.7 Seedling inoculation

Mycorrhizal inocula were used in the pots where necessary according to the treatment at the rate of 15 g kg^{-1} soil at 3 cm depth of the soil surface and then the soil was saturated with water. Inocula of selected *S. rolfsii*, *F. oxysporum* and *R. solani* strain were incorporated with the pot surface soil at the rate of 20 g kg^{-1} soil at 7 days after application of mycorrhizal inocula. Twenty five seeds of ash gourd were sown per pot at 7 days after inoculation of pathogen. The seedlings of the crops emerged out within 9-10 days after sowing. Irrigation and mulching were done as and when needed. Seedlings were kept free from other disease and insect infestation in controlled environment.

2.8 Pathogenicity test of isolates

A total of 5 isolates were collected for each pathogen for the pathogenicity test. Inocula of the five isolates of *S. rolfsii*, *F. oxysporum* and *R. solani* were prepared on autoclaved moist wheat grains in 500 mL erlenmeyer flask. Before using wheat grain was soaked in water for 12 h. After soaking excess water was drained off and water-soaked grains were poured into 500 mL erlenmeyer flask. Inoculum of each isolate of each pathogen was prepared in a separate flask. Mycelial discs of 5 mm diameter were cut from the edge of three days old PDA culture in petri dishes. Five to seven mycelial discs of each isolate of each pathogen were added to autoclaved wheat grains in the flasks and incubated at 25 °C for 21 days. The flasks were shaken by hand at 23 days interval for even colonization. The colonized wheat grains were air dried for 2 weeks and stored at 4 °C for further study. Selected isolates of *S. rolfsii*, *F. oxysporum* and *R. solani* were evaluated by soil infestation method for their pathogenicity in a pot culture experiment. Inoculum of each isolate of *S. rolfsii*, *F. oxysporum* and *R. solani* were thoroughly mixed with sterilized potting media at the rate of 20 g kg^{-1} potting medium while controls were prepared using only sterilized potting medium. 25 seeds of ash gourd were sown in three replicated pots for each isolate. Pre-emergence and post-emergence seedling mortality was observed regularly and recorded at 5, 10, 15, 20, 25, 30, 35 and 40 days after sowing (DAS). Re-isolation of the pathogen from ungerminated seeds and from post-emergence infected and dead seedling was done to identify the causal agent of seedling infection.

2.9 AMF colonization determination

Pieces of ash gourd roots collected from the experimental pots were thoroughly washed with sterilized water and were stained according to Koske and Gemma (1989). The root pieces were boiled in 2.5% KOH solution for 30 min at 90 °C. Then the root segments were washed in water several times and acidified with 1% HCl solution for 24 h. Heavily pigmented roots were bleached in 10% H_2O_2 for 20 to 60 min and the segments were boiled for 30 min in 0.05% aniline blue at a temperature of 90 °C. Subsequently the roots were destained at room temperature in acidic glycerol. The percentage of AM infection was estimated by root slide technique (Read et al., 1976). The presence or absence of infection in the root pieces was recorded and the present infection was calculated as follows:

$$RI = \frac{RS_{AM}}{RS_T} \times 100 \quad (1)$$

where RI = Root infection (%), RS_{AM} = number of AM positive segments, and RS_T = Total number of root segments observed.

2.10 AMF spore density estimation

Soil samples collected from the rhizospheric zone of the plants were mix thoroughly by breaking up any large lumps. Any large unwanted particles such as stone, roots, twigs etc. were removed. Then 100 g of soil was kept in a bucket (8 L) and the bucket was filled with tap water. The soil with water was agitated by stirring vigorously by hand and washed into the bucket and left to settle for one minute. The suspension was sieved by following the wet sieving and decanting method (Gerdemann and Nicolson, 1963). Two sieves (400 and 100 μm mesh) were used throughout the experiment. The supernatant was poured through a 100 μm sieve into the second bucked (10 L) to avoid the loss of a useful materials. After allowing the suspension to settle for one minute, the supernatant was decanted into the 400 μm sieve, this time water was discarded, and the material was back washed from the sieve into a beaker (250 mL) with a small quantity of water. The solution with spores was distributed in 4 equal size test tubes evenly and balanced up the tubes with water for equal weight. The tubes were plugged properly and then centrifuged for 4 min at 3000 rpm. The supernatant was poured in test tubes and the test tubes were filled with sucrose solution (480 g of sucrose was dissolved in distilled water in 100 mL volumetric flask). The solution was mixed well and volume was made up to the mark and stirred vigorously with the round-ended spatula to re-suspend the precipitate. The test tubes were balanced properly to equal weight and they were plugged. Then, the plugged test tubes were centrifuged for 15 sec at 3000 rpm. After centrifuge, the sucrose supernatant was poured through a 400 μm sieve and rapidly washed with water to remove the sucrose from AM spores by back

washing the materials from the sieve into a watch glass for observation. The extract, with AM spores, was observed under stereo microscope and the numbers of spores were counted. Spore numbers from the three replicates per samples were averaged and the result was expressed as number per 100 g of dry soil basis.

2.11 Data collection and analyses

Pre-emergence and post-emergence seedling mortality was recorded similar to pathogenecity test. Data on plant height, number of leaves plant^{-1}, collar diameter and root length were collected at harvest at 50 DAS. Harvested shoot and root was dried at oven and was weighed separately by electrical balance and then shoot and root dry weights were recorded. All data were presented as the mean ± SE (n=3). The effects of mycorrhizal treatment on the plant height, total number of leaves/plant, root length, root and shoot dry weight, collar diameter and also pre- and post-emergence damping off diseases of ash gourd seedlings were analyzed by two-way ANOVA followed by Tukey's *posthoc* test at P=0.05. All statistical analyses were performed using statistical package 'R'.

3 Results

3.1 Pathogenicity of isolates

Results of the pathogenicity test showed that all isolates of all the pathogens were highly pathogenic to ash gourd seedlings (Fig. 1). The isolate AS2, AF3 and AR4 caused 100% seedling mortality of ash gourd seedlings. Therefore, we used these three virulent isolates for the subsequent experiment.

3.2 Effect of AMF on damping off disease

In case of pre- and post-emergence damping off diseases, the AMF inoculation showed the significantly lower damping off disease than AMF non-inoculated condition (Fig. 2). The pre-emergence damping off was observed 8.93, 8.89 and 11.1% due to infection caused by *Sclerotium*, *Fusarium* and *Rhizoctonia*, respectively whereas in presence of AMF inoculation they reduced to 5.63, 4.94 and 3.87%, respectively. Similarly, the post-emergence damping off disease reduced to 24.4% from 44.4% caused by *Sclerotium*; to 24.1% from 42.0% caused by *Fusarium* and to 23.8% from 46.5% caused by *Rhizoctonia* due to AMF inoculation. The total damping off disease was observed 53.3, 50.9 and 57.6% due to infection caused by *Sclerotium*, *Fusarium* and *Rhizoctonia*, respectively. But in presence of AMF inoculation, the total damping off disease was 29.6, 29 and 27.7% caused by *Sclerotium*, *Fusarium* and *Rhizoctonia*.

3.3 Root colonization by AMF

The root colonization of ash gourd was also influenced by the interaction effect of AMF and root infecting pathogen. The highest root colonization was observed in control with AM inoculated plant and without pathogen (56.9%) followed by *Rhizoctonia* (44.4%); *Sclerotium* (43.1%) and *Fusarium* (36.4%). The lowest was observed in control without AM inoculated plant (3.68%) which was statistically similar with *Sclerotium* (5.29%); *Rhizoctonia* (3.70%) and *Fusarium* (4.19%) (Fig. 3).

The highest mycorrhizal spore density in the rhizosphere of ash gourd was observed in control with AMF inoculated plant and without pathogen (36.2 spore 100 g^{-1} soil) followed by *Sclerotium* (27.1 spore 100 g^{-1} soil); *Rhizoctonia* (24.9 spore 100 g^{-1} soil) and *Fusarium* (25.4 spore 100 g^{-1} soil). The lowest was observed in *Sclerotium* without AMF inoculated plant (3.67 spore 100 g^{-1} soil) which was statistically similar with control (5.79 spore 100 g^{-1} soil); *Rhizoctonia* (4.13 spore 100 g^{-1} soil) and *Fusarium* (4.41 spore 100 g^{-1} soil).

3.4 Effect AMF on growth of ash gourd

Mycorrhizal inoculants significantly (p<0.05) influenced the plant height of ash gourd (Fig. 4). AMF inoculated plants showed higher plant height (13.4 cm) than non-inoculated plants (9.02 cm). The highest plant height of ash gourd was observed in control (15.7 cm). Plant height in *Rhizoctonia* inoculated plants was 9.90 cm which was statistically similar to that of *Sclerotium* inoculated plants (9.73 cm) and *Fusarium* inoculated plants (9.51 cm).

Plant height of ash gourd was also significant affected by the interaction effect of AMF and root infecting pathogen (Table 1).The highest plant height was observed in C×I (18.1 cm) and the lowest was observed in F×N (7.12 cm). AM inoculated plants showed higher root length (9.51 cm) while non-inoculated plants showed (5.80 cm). On the other hand, root infecting pathogen significantly reduced the root length of ash gourd. The highest root length of ash gourd was observed in control (9.87 cm). *Sclerotium* inoculated plants showed root length 7.13 cm which was statistically similar to *Rhizoctonia* inoculated plants (7.12 cm) and *Fusarium* inoculated plants (6.51 cm) (Fig. 4). Ash gourd root length was also significantly influenced by the interaction effect of AMF and root infecting pathogen (Table 1). The highest root length was observed in C×I (11.5 cm) and the lowest was observed in F×N i.e. *Fusarium* inoculated non-mycorrhizal plant (4.73 cm).

AM inoculated plants showed higher root weight (3.07 g) than non-inoculated plants (2.62 g). The highest root weight of ash gourd was observed in control (3.54 g) followed by Rhizoctonia (2.63 g) inoculated plants which was statistically similar to *Scle-*

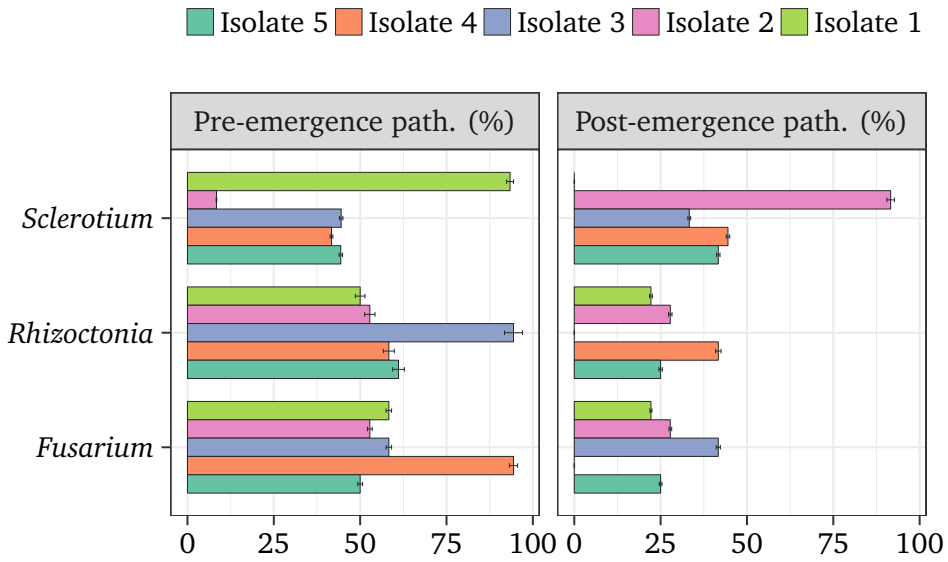

Figure 1. Pre- and post-emergence pathogenicity of different isolates of *S. rolfsii*, *F. oxysporum* and *R. solani* against ash gourd seedlings

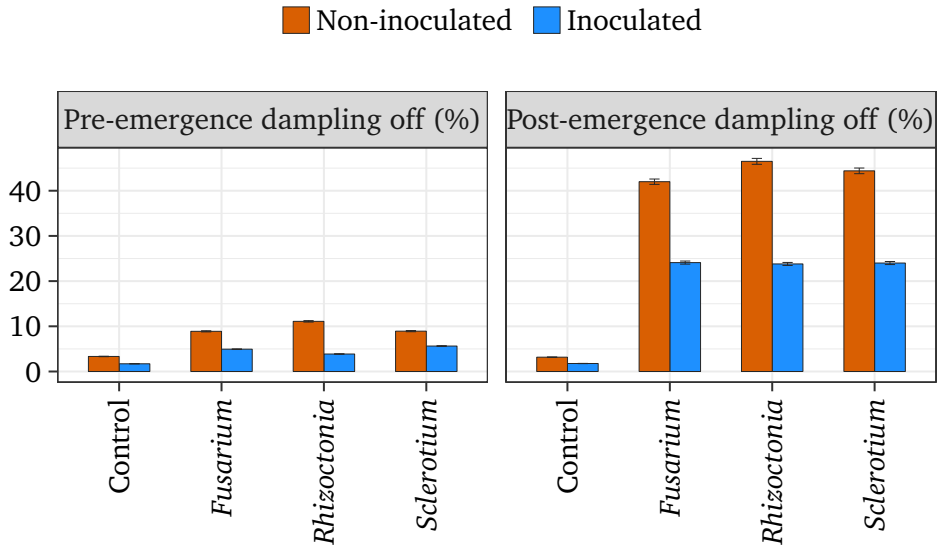

Figure 2. Inoculation of AM in suppressing damping off disease (pre-emergence and post-emergence). Error bars indicate ± standard deviation (SD) of mean. A column having no error bar indicates that the SD value of the respective mean was too small or zero.

rotium (2.61 g) and *Fusarium* (2.62 g) inoculated plants (Fig. 4). The highest root weight was observed in C×I (3.79 g) and the lowest was observed in S×N (2.24 g), however, the interaction of the two factors did not affect root dry weight significantly. AM inoculated plants showed greater shoot weight (3.07 g) than non-inoculated plants (2.62 g). The highest shoot weight of ash gourd was observed in control (3.54 g) due to the absence of root infecting pathogen differing from *Rhizoctonia* inoculated plants (2.63 g) which was statistically like *Sclerotium* inoculated plants (2.61 g) and *Fusarium* inoculated plants (2.62 g) (Fig. 4). Interaction effect of AMF and root infecting pathogen on shoot weight of ash gourd was also significant (Table 1). The highest shoot weight was observed in C×I (3.87 g) and the lowest was observed in F×N (2.42 g). AM inoculated plants showed the significantly higher values compare to their non- mycorrhizal plant in all cases (Table 1).

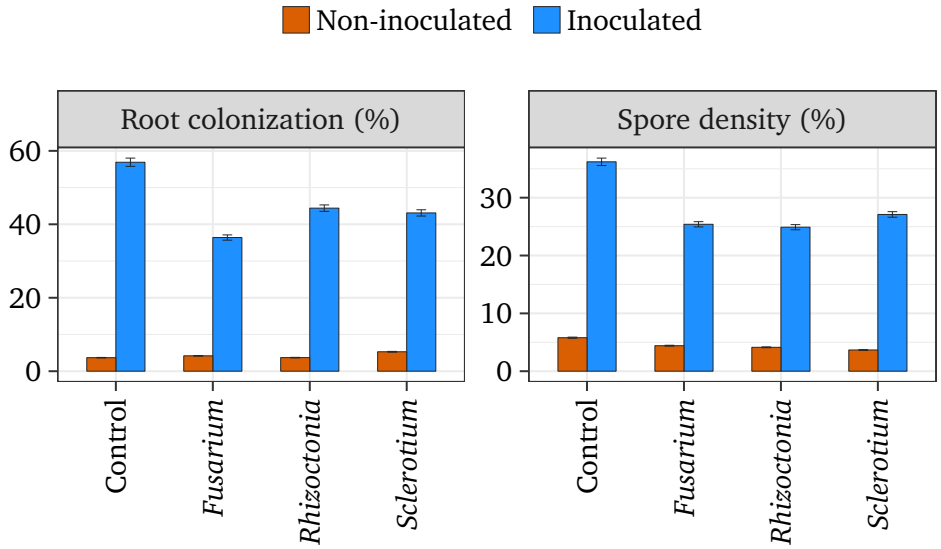

Figure 3. Root colonization and spore density of ash gourd as affected by AMF inoculation.

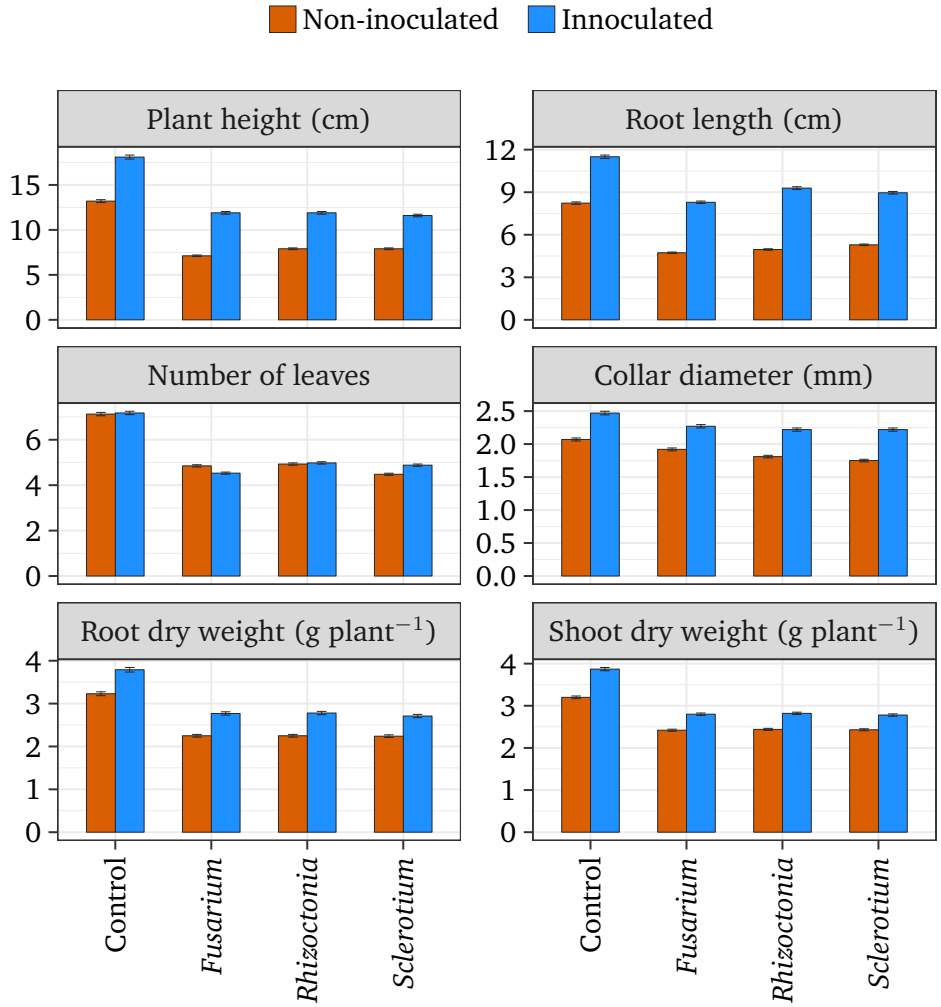

Figure 4. Growth of ash gourd as affected by AMF inoculation. Error bars indicate ± standard deviation (SD) of mean. A column having no error bar indicates that the SD value of the respective mean was too small or zero.

Table 1. Effect of interaction between fungi and arbuscular mycorrhizal fungi inoculation on the growth parameters of ash gourd

Interactions	Plant height (cm)	Root length (cm)	Leaf number	Collar diameter (mm)	Root dry weight (g plant^{-1})	Shoot dry wt. (g plant^{-1})
C×I	18.1±0.2	11.5±0.12	7.18±0.07	2.47±0.03	3.79±0.05	3.87±0.04
C×N	13.2±0.2	8.23±0.08	7.13±0.07	2.07±0.02	3.23±0.04	3.2±0.03
F×I	11.9±0.1	8.29±0.09	4.53±0.05	2.27±0.03	2.77±0.03	2.8±0.03
F×N	7.1±0.1	4.73±0.05	4.85±0.05	1.92±0.02	2.25±0.03	2.42±0.02
R×I	11.9±0.1	9.29±0.10	4.98±0.04	2.22±0.02	2.78±0.03	2.82±0.03
R×N	7.9±0.1	4.96±0.05	4.93±0.05	1.81±0.02	2.25±0.03	2.44±0.02
S×I	11.6±0.1	8.96±0.09	4.88±0.05	2.22±0.03	2.71±0.04	2.78±0.03
S×N	7.9±0.1	5.29±0.05	4.48±0.04	1.75±0.02	2.24±0.03	2.43±0.02
Sig. level	<0.05	<0.05	<0.05	<0.05	NS	<0.05

C = control (no fungi infection), F = *Fusarium*, R = *Rhizoctonia*, S = *Sclerotium*, I = AMF inoculated, N = AMF non-inoculated.

4 Discussion

Our results revealed that AM inoculation significantly suppressed the root infecting pathogen causing damping off disease. Findings of this experiment was simillarin agreement with Harrier and Watson (2004) experiments. They found that AM fungi can effectively reduce root rot diseases caused by a number of soil-borne pathogens, such as *Fusarium* spp., *Aphanomyceseuteiches* and *Phytophthora* spp. These results also supported the findings of other researchers (ichi Matsubara et al., 1995, 2004) who found that application of AM fungi alone or combined with rhizobia reduced disease incidence of some soil-borne fungal disease of many vegetables. These experiment results are also in agreement with the findings of Srivastava et al. (2010) who stated that damage and infection caused by *Fusarium oxysporum* f. sp. *lycopersici* was decreased due to AM inoculation in tomato. Kapoor (2008) reported that AMF were capable of imparting disease tolerance in tomato plants pre-infected with *Fusarium oxysporum* f. sp. *lycopersici* (FOL). Inoculation of tomato seedlings with *Glomus macrocarpum* or *Glomus fasciculatum* at 20 days after infection with FOL reduced pathogen spread and disease severity by 75% and 78%, respectively (Kapoor, 2008).

The mycorrhizal root colonization of ash gourd in our study was significantly influenced by AMF and pathogens. Similar result was noted by previous researchers who reported augmented mycorrhizal root colonization due to AM inoculation (Sarkar et al., 2015b,c,a, 2017). In case of AMF inoculated plant, the highest mycorrhizal root colonization of ash gourd was observed in control but inoculation of pathogens decrease the colonization level. It proved that pathogen inhibit the colonization of AMF. Fitter and Garbaye (1994) also showed that interactions among AMF and other organisms in soil might be inhibitory and were clearly competitive sometimes. AMF inoculated plants showed higher mycorrhizal spore density while non-inoculated plants showed statistically lower spore density.

The results of interaction between fungal infection and AMF inoculation revealed that root infecting pathogen reduced the plant growth but it can be minimized by inoculation of AMF. Similar results were also found in previous research where tomato root and shoot dry mass yield increased by AMF (Subramanian et al., 2006; Al-Karaki, 2006; Gamalero et al., 2003). In addition, Ojala and Jarrell (1980) also proved that inoculated roots of tomato plants became highly infected with mycorrhizal fungi, and yield parameters were significantly increased with inoculation of mycorrhizal fungi over non-inoculated control plants. Karagiannidis et al. (2002) also found that growth of tomato and brinjal was increased due to inoculation of AMF. However, Turrini et al. (2004) showed that brinjal released organic acids through root exudates in presence of pathogenic fungi and mycorrhizal symbiosis which reduced the growth of soil borne pathogenic fungus.

5 Conclusions

AMF can suppress about 40-50% damping off disease in ash gourd. In addition, AMF also improved the seedling growth. Therefore, based on the above findings, we recommend the application of AMF in order to suppress damping off disease as well as to obtain good quality seedling of ash gourd in eco-friendly approach.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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