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Effectiveness of *Glomus mosseae* Inoculation in Enhancing *Fusarium* Wilt Tolerance in Eggplants

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ABSTRACT

Effectiveness of endotrophic mycorrhizal fungus (*Glomus mosseae*) inoculation in eggplants was evaluated in pot experiments under net-house for management of wilt disease of brinjal caused by *Fusarium oxysporum* f. sp. *melongenae*. The ability of *G. mosseae* in mycorrhization of eggplant roots and spore abundance in soil were assayed in co-inoculated condition by *F. oxysporum* f. sp. *melongenae* and compared with untreated control plants. Significant variation in plant growth parameters *viz.* plant height, shoot length, root length, fresh weight and dry weight were recorded at 30, 60 and 90 Days After Transplanting (DAT). *Glomus mosseae* inoculated plants showed 58.90%, 39.00% and 41.97% higher plant height when co-inoculated with *F. oxysporum* f. sp. *melongenae* and *G. mosseae* compared to *F. oxysporum* f. sp. *melongenae* inoculated plants alone at 30, 60 and 90 DAT. Wilt incidence was reduced 74.47, 84.88, and 95.86% in co-inoculated plants compared to pathogen-inoculated plants at 30, 60 and 90 DAT. Wilt disease suppressing ability of *G. mosseae* is statistically comparable with positive control (Chemical fungicide- Carbendazim) from 50 DAT to 90 DAT. The sole application of *G. mosseae* resulted in significant increase in vegetative growth and mycorrhization of roots compared to untreated control. Mycorrhizal spore abundance in soil was significantly increased in sole application of *G. mosseae* than co-inoculation of *F. oxysporum* f. sp. *melongenae* and *G. mosseae*. Present findings have provided the evidence of ability of *G. mosseae* to enhance tolerance of eggplants against wilt pathogen and ensure an excellent symbiotic association with eggplant plants.

Introduction

Eggplant (*Solanum melongena* L.) belongs to the family Solanaceae and is the most popular, inexpensive and economically important vegetable all over the world. It is widely grown in the tropics and sub-tropics (Roychowdhury and Tah, 2011), especially in Asia, Europe, Africa and America (Demir *et al.,* 2010). Eggplant has nutritional value due to its composition, which includes minerals like potassium, calcium, sodium and iron (Raigón *et al.,* 2008) as well as dietary fiber (USDA and FAS, 2014). It is positioned second in acreage, production, yield, and in consumption next to potato. In 2016-2017, total 125,860 acres land was under eggplant cultivation, total production was 507,432 MT (BBS, 2017). Eggplant is prone to be attacked by numerous bacterial, fungal, viral and nemic diseases as well as physiological disorders. However, *Fusarium* wilt is considered as one of the devastative and destructive diseases affecting production of eggplant around the globe (Altinnok, 2005; Altinnok *et al.,* 2013; Patil *et al.,* 2017). In Bangladesh, eggplant suffers from 12 diseases. Among the diseases wilt caused by *Fusarium oxysporum* f. sp. *melongenae* cause 100% of the plants death in Kitchen gardens in Bangladesh (Rahman *et al.,* 2011). *Fusarium oxysporum* f. sp. *melongenae* is a ubiquitous pathogen which can survive in soil, plant debris and seed for long time and can enters through natural openings of plants as well as wounds in the roots. The fungus can infect the stem, where profusely growth of this fungus can develop blockade in the vascular system and interfere the supply of nutrients and water to the upper parts of plants. Wilting of eggplant plants in the sunny afternoon is the first indication (Meah, 1997). About 60-90% production loss may occur due to wilt disease of eggplant (Begum, 2007).

Arbuscular Mycorrhizal Fungi (AMF), a common and very important soil-borne fungus, has gained considerable recognition as biological agent. AMF are key components of soil microbiota and obviously interact with other

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microorganisms in the rhizosphere which is the zone of influence of plant roots on microbial populations and other soil constituents (Brundrett, 2004). Among the mycorrhizal fungi, *Glomus mosseae* can suppress root pathogens by morphological, physiological and histochemical alterations in the host plant (Jothi and Sundarababu, 2001). Exclusion of pathogens, variation in P uptake, cell wall lignifications, releasing low molecular weight compounds have been attributed to manoeuvring suppression of plant pathogens by mycorrhizal application (Sati *et al.,* 2020). The farmers are completely dependent on chemical pesticides for crop disease management in Bangladesh. On the other hand, developing pesticide resistance, environment pollution, destroying biodiversity, hazardous effect on human health by residues in the vegetables are becoming alarming due to indiscriminate pesticides application in the developing countries like Bangladesh (Aktar *et al*., 2009). The consumers are more conscience regarding pesticide residues and keeping safe environment around the world. Using endotrophic mycorrhizae for controlling wilt disease in eggplant would be an alternative to chemical approach. Therefore, the objective of the research was to know the effect of inoculation of eggplants with arbuscular mycorrhizae in enhancing tolerance for management of *Fusarium* wilt.

Materials and Methods

The experiments were conducted in the Laboratory of Bioactive compounds, Bio-formulation and Bio-signaling, Department of Plant Pathology and Professor Golam Ali Fakir Seed Pathology Centre (SPC), Bangladesh Agricultural University, Mymensigh-2202 during the period from October, 2017 to November, 2018. Eggplant seeds were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. Germination test was carried out according to ISTA (ISTA, 2010) to know the quality of the eggplant seeds. Four hundred seeds were placed into petridishes having three moistened blotter papers and incubated at room temperature (25 ± 2 °C). After 7 days of incubation germination percentage was calculated. The initial inoculum of *G. mosseae* was collected from Soil Science Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. Initial inocula were preserved in zip-lock bag in room condition (20±2 °C) before multiplication. Multiplication of initial inoculum was done by using sorghum plants following trap culture. After multiplication, the secondary inoculum of *G. mosseae* (mycorrhized sorghum roots and spores in soil) was mixed with soil @ 10 g inocula/kg soil for experimental pot.

Fusarium oxysporum f. sp. *melongenae* was isolated from wilted eggplant plants following tissue planting method on Potato Dextrose Agar (PDA) medium. The fungi were purified by successive culture following hyphal tip method. Identification of the fungus was done by preparing temporary slide of the pathogen. Conidial suspension of *F. oxysporum* f. sp. *melongenae* @ 1 x 10^7 spores/mL standardized by haemocytometer collected from nine days old culture were considered for artificial inoculation. The responses of the treatments were compared with positive chemical control 0.2% Knowin 50 WP (carbendazim). One month old eggplant seedlings were transplanted in the pots for further experimentation. Inocula of *G. mosseae* were mixed with pot soil ten days before transplanting eggplant seedlings in the pots. During transplantation seedlings were inoculated by dipping the seedlings in *F. oxysporum* f. sp. *melongenae* suspension and soil was drenched by 50 mL *F. oxysporum* f. sp. *melongenae* suspension containing 1x10^7 spores/mL. Regular and uniform care of cultivation was maintained as and when necessary (Rashid and Singh, 2000). Abundance of spores in soil were measured estimated by wet sieving and decanting (Rajesh *et al.,* 2011). Histochemical staining of roots of eggplant was done by following modified technique of Rajesh *et al.* (2011) to assess the percent mycorrhized roots. Data recording started from 30 days after transplanting and continued up to harvest. The following parameters were considered during data collection: root length, shoot length, fresh weight, shoot dry weight, root dry weight, disease incidence, percent root colonization and abundance of spores.

The experiment was carried out using a completely randomized design (CRD) maintaining three replications and means were compared by Duncan's multiple range test ($p \leq 0.05$). The data were analyzed by statistical software (S-PLUS) (Huet *et al.,* 2006).

Results

Effect of G. mosseae inoculation on vegetative growth and reproductive parameters of eggplant

All growth parameters *viz.* plant height, shoot length, root length, shoot weight and root weight at 30 DAT showed significant variation among the treatments while the chemical treatment was superior (Table 1). The highest plant height (35.11 cm) was recorded in chemical treatment (T_4 = *F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL + 0.2% Knowin 50WP). On the contrary, the lowest plant height (23.00 cm) was found in T_2 (*F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL) where only pathogen was applied. Other parameters like shoot length (63.66 cm), root length (15.33 cm), shoot weight (30.00 g) and root weight (2.98 g) were recorded highest in chemical treatment T_4 (*F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL + 0.2% Knowin 50WP) and lowest in T_2 (*F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL) where only pathogen was applied.

However, inoculation of *G. mosseae* did not show promising effect up to 30 DAT as the mycorrhization of eggplant roots might not establish intricate relationship with eggplant root system for up taking more nutrients from soil.

Vegetative growth of eggplant plants significantly varied in different treatments at 60 DAT. The highest plant height (61.0 cm) was recorded in T_1 where only *G. mosseae* was applied (Table 2). In contrast, the lowest plant height (32.33 cm) was found in T_2 (*F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL) where only pathogen was applied. Similar trends were found in case of shoot length (46.73 cm), root length (20.66 cm), shoot weight (55.00 g), root weight (7.91 g), and fruit weight (35.00 g) when *G. mosseae* applied alone (Table 2). However, co-inoculation of *G. mosseae* and *F. oxysporum* f. sp. *melongenae* significantly increased all parameters compared to untreated control and inoculation with *F. oxysporum* f. sp. *melongenae*. During co-inoculation, *G. mosseae* can suppress the pathogenic effect of *F. oxysporum* f. sp. *melongenae.* It is speculated that enhanced uptake of minerals by mycorrhized roots enhanced growth parameters of eggplant and suppress growth of *F. oxysporum* f. sp. *melongenae*.

Variation in vegetative and reproductive parameters was found significant among the treatments at mature stage (90 DAT). The highest plant height (70.60 cm) was recorded in T_1 where *G. mosseae* was applied alone, whereas the lowest plant height was found in T_2 (*F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL) where only pathogen was applied (Table 3). Other parameters like shoot length (60.00 cm), root length (22.30 cm), shoot weight (70.00 g), root weight (9.40 g) and fruit weight (68.00 g) showed similar trends of responses when *G. mosseae* applied alone (Table 3). However, significant increase in vegetative and reproductive parameters was recorded in plants co-inoculated with *G. mosseae* and *F. oxysporum* f. sp. *melongenae* compared to untreated control and inoculated with pathogen.

Effect of co-inoculation of G. mosseae and F. oxysporum f. sp. melongenae on eggplant root mycorrhization and mycorrhizal spore production in soil

An increasing tendency of mycorrhizal spore production and root mycorrization by *G. mosseae* was recorded at every stage of plant growth. Significantly higher amount of spores (166 spores/100g soil) and mycorrhized roots (74%) were developed at 90 DAT when *G. mosseae* was inoculated alone. On the contrary, the spore number (110 spores/100g soil) and mycorrhized roots (60%) were decreased when *G. mosseae* was co-inoculated with *F. oxysporum* f. sp. *melongenae* (Table 4). The number of spores of *G. mosseae* in soil and mycorrhized roots reduced in co-inoculated condition. The spores of *G. mosseae* and mycorrhized roots are still available in considerable number in co-inoculated application of *G. mosseae* and *F. oxysporum* f. sp. *melongenae*. It indicates that mycorrhizae can grow and multiply in the presence of plant pathogen and can enhance the growth and development of plant.

Effect of co-inoculation of G. mosseae and F. oxysporum f. sp. melongenae on the incidence of wilt disease of eggplant

At 20 DAT, disease incidence was highest in the pots where *F. oxysporum* f. sp. *melongenae* was applied alone (Table 5). Co-inoculation of *G. mosseae* and *F. oxysporum* f. sp. *melongenae* reduced wilt disease (7.54) compared to pathogen inoculation alone which rendered the plants more tolerant to *F. oxysporum.* Up to 40 DAT, the disease incidence was lowest in co-inoculation of mycorrhizae and *F. oxysporum* indicating that *G. mosseae* has already developed an intimate relation by invading into the root system of eggplant. On the other hand, significant but gradual reduction (16.64) in wilt disease incidence was recorded in chemical treatment at 20 DAT and continued up to 90 DAT (2.65) compared to pathogen inoculation alone (Table 5).

Table 1. Effect of different treatments on vegetative growth of eggplant at seedling stage (30 DAT)

Treatment	Plant height(cm)		Shoot length (cm)	Root length (cm)	Shoot weight (g)		Root weight (g)	
	15 DAT	30 DAT			Fresh	Dry	Fresh	Dry
T_0	12.64	31.66bc	20.83bc	15.16a	30.18a	3.66	2.39ab	0.30b
T_1	12.21	33.66ab	22.50ab	15.50a	25.99a	2.61	2.32ab	0.24b
T_2	13.25	23.00d	12.33d	10.33c	18.71b	2.06	1.46c	0.22b
T_3	13.62	30.00c	20.00c	12.66b	25.21a	2.35	1.94bc	0.18b
T_4	14.46	35.11a	23.66a	15.33a	30.00a	3.96	2.98a	0.54a
LSD (0.05)	NS	3.18	2.15	2.04	5.12	NS	0.84	0.21
CV	9.04	5.71	5.95	8.15	10.80	27.26	21.02	39.84

T_0 = Control, T_1 = *G. mosseae* 10 g/kg soil, T_2 = *F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL, T_3 = *G. mosseae* 10 g/kg soil + *F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL, T_4= *F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL + 0.2% Knowin (carbendazim) 50 WP; column having similar letter(s) do not differ significantly

Table 2. Effect of different treatments on vegetative growth and yield parameter of eggplant at maximum growth stage (60 DAT)

Treatment	Plant height (cm)	Shoot length (cm)	Root length (cm)	Shoot weight (g)		Root weight (g)		Fruit Weight (g)
				Fresh	Dry	Fresh	Dry	
T_0	51.00c	41.00b	18.33bc	50.33bc	10.43bc	6.00 abc	1.41	22.16d
T_1	61.00a	46.73a	20.66a	55.00ab	11.65ab	7.91 a	2.22	35.00b
T_2	32.33d	15.33c	12.66d	26.66d	6.54d	4.33 c	1.15	0.00e
T_3	53.00bc	40.33b	16.66c	47.66c	10.01c	5.60 bc	1.51	38.66a
T_4	58.00ab	46.33a	19.33ab	59.33a	12.37a	6.66 ab	2.09	31.00c
LSD (0.05)	6.21	4.38	1.93	5.75	1.06	2.00	NS	56
CV	6.68	6.33	6.58	7.14	7.24	18.05	29.93	5.55

T_0 = Control, T_1 = *G. mosseae* 10 g/kg soil, T_2 = *F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL, T_3 = *G. mosseae* 10 g/kg soil + *F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL,T_4= *F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL + 0.2% Knowin (carbendazim) 50 WP; column having similar letter(s) do not differ significantly

Table 3. Effect of different treatments on vegetative growth and yield parameter of eggplant at mature stage (90 DAT)

Treatment	Plant height (cm)	Shoot length (cm)	Root length (cm)	Shoot weight (g)		Root weight (g)		Fruit weight (g)
				Fresh	Dry	Fresh	Dry	
T_0	61.0b	51.0b	19.6b	64.6a	12.3b	8.3	2.3	51.6b
T_1	70.6a	60.0a	22.3a	70.0a	14.0a	9.4	2.5	68.a
T_2	37.3c	18.6c	14.3c	36.3b	7.6c	5.4	1.4	0.0c
T_3	64.3ab	56.0a	19.6b	63.0a	12.6ab	9.6	2.0	67.3a
T_4	66.3ab	58.3a	21.0ab	68.6a	13.8ab	9.1	2.5	71.6a
LSD (0.05)	7.9	4.7	2.0	8.1	1.6	NS	NS	10.2
CV	7.3	5.3	5.8	7.3	7.5	26.3	24.0	10.8

T_0 = Control, T_1 = *G. mosseae* 10 g/kg soil, T_2 = *F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL, T_3 = *G. mosseae* 10 g/kg soil + *F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL, T_4= *F. oxysporum* f. sp. *melongenae* @ 1×10^7 spores/mL + 0.2% Knowin (carbendazim) 50 WP; column having similar letter(s) do not differ significantly

Table 4. Effect of co-inoculation of *G. mosseae* and *F. oxysporum* f. sp. *melongenae* on mycorrhizal spore production in soil and root mycorrhization of eggplant at different growth stage

Treatment	Number of spores/100 g soil			Root mycorrhization (%)		
	30 DAT	60DAT	90 DAT	30 DAT	60 DAT	90 DAT
G. mosseae @ 10g/kg soil	117 a	145 a	166 a	26.66 a	58.00 a	74.00 a
G. mosseae @ 10g/kg soil + *F. oxysporum* @ 1×10^7 spores/mL	110 b	132 b	149 b	20.00 b	48.33b	60.00 b
LSD (0.05)	4.89	5.85	5.47	4.03	4.03	4.53
CV	1.90	1.86	1.53	7.62	3.34	2.98

DAT=Days after transplanting, Mean having similar letter in a column are statistically similar at 5% level of significance

Table 5. Effects of inoculation of eggplant with *G. mosseae* on the incidence of *Fusarium* wilt

Treatment	Disease incidence (%)							
	20 DAT	30 DAT	40 DAT	50 DAT	60 DAT	70 DAT	80 DAT	90 DAT
F. oxysporum @ 1×10^7 spores/mL	17.38 a	40.55 a	46.65 a	58.15 a	65.44 a	70.66 a	79.03 a	89.34 a
G. mosseae 10 g/kg soil and *F. oxysporum* @ 1×10^7 spores/mL	7.54 c	10.35 c	10.68 c	13.66b	10.74b	8.23 b	6.92 b	3.69 b
F. oxysporum @ 1×10^{7}spores/mL and 0.2% Knowin 50 WP	16.64 b	15.73 b	13.55b	14.74 b	11.36 b	7.77 b	5.66 b	2.65 b
LSD (0.05)	0.166	2.593	1.835	1.807	1.842	1.381	1.610	1.237
CV	1.032	10.265	6.768	5.371	5.484	4.147	4.575	3.401

DAT = Days after transplanting, Mean having similar letter in a column are statistically similar at 5% level of significance

Discussions

Management of wilt in vegetable crops especially eggplant is largely depends on chemical pesticide application in Bangladesh (Faruq *et al.,* 2006). Indiscriminate use of chemical pesticides manifest resistance in pest population and residual effect of pesticides are the cause of many ailments in human being. As an alternative to chemical pesticides, mycorrhizal association of roots is a widespread strategy for facilitating mineral acquisition and induction of defense mechanisms against pathogens.

Fusarium oxysporum f. sp. *melongenae* is ubiquitous and persistent inhabitant in soil which makes it difficult to manage by conventional chemical pesticides. Although using resistant cultivar is one of the best options in some cases, however, the possibility of emerging of new races could breach the resistance of the host, led us to explore the efficacy of endotrophic myorrhizal fungi (*G. mosseae*) to enhance host tolerance, which has recently received much attention because of their influence in plant growth promotion, yield increment and disease control (Abbasi *et al.,* 2015).

Endotrophic mycorrhizae also establish an intimate relation with the host plants and enhance nutrient uptake and modulate a number of enzymatic activities that enhance tolerance against various biotic and abiotic factors. In the present study, significant effects of different treatments were recorded on vegetative and reproductive growth parameters of eggplant plants. Application of *G. mosseae* (T_1) into the soil exhibited the superior performances considering growth and yield parameters. Co-inoculation of *G. mosseae* with *F. oxysporum* f. sp. *melongenae* significantly improved the growth and yield parameters compared to both untreated control and negative control (T_2). Similar kinds of findings were reported by Elahi *et al.* (2010) where an application *G. mosseae* in eggplant seedling significantly influenced the shoot length, shoot diameter, number of leaves, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and fruit weight.

The present study also reflects that the abundance of spores of *G. mosseae* and percent mycorrhized root gradually increased with the time. The highest amount of spores and mycorrhized roots were found by the sole application of *G. mosseae* compared to untreated control treatment. But, spores and colonized roots were reduced substantially in case of *F. oxysporum* f. sp. *melongenae* inoculation which is similar to the findings of several researchers (Azcon-Aguilar and Bara, 1996; Hao *et al.,* 2005). Tahat *et al.* (2008) also reported that at 75 days of growth the highest number of AMF spores was found in corn as the host plant. However, Tahat *et al.* (2012) observed that the inoculum density from the three species used (100 spores/100 g soil) was sufficient to increase the nutrient absorption which resulted in strengthened root system through the positive effect on the host root colonization.

In this study, incidence of wilt disease was observed lowest when eggplants were co-inoculated with *G. mosseae* and pathogen meaning that the mycorrhizal fungi may colonize in the root system of host plant that enables protection against *F. oxysporum* f. sp. *melongenae*. This finding is in the similar line of Azcon-Aguilar *et al*. (2002) where protection against root pathogens was enhanced by mycorrhizal root colonization. Khaosaad *et al.* (2007) also found that pre-colonization of plant roots by endotrophic mycorrhizae suppress the activities of soil-borne fungal pathogens, and this was due to the release of volatile substances by endotrophic mycorrhizae. It can also be hypothesized that cell wall lignification and increased antioxidant activity by soil application of *G. mosseae* can be vital factors for reducing wilt disease of eggplant. Previously, Yanan *et al*. (2015) reported that arbuscular mycorrhizal fungi also enhanced protective enzyme activities to scavenge oxidative stresses resulting from the invasion of pathogens and prevented the plant cells from damaging severely. Thus, it is clear that mycorrhizal fungi (*G. mosseae*) inoculation can aid eggplants to enhance tolerance against wilt disease caused by *F. oxysporum* f. sp. *melongenae*.

Conclusion

It is obvious from the present study that eggplant root inoculation with *G. mosseae* enhances both vegetative growth and reproductive parameters like plant height, shoot and root length, their fresh and dry weight and fruit weight. It is also apparent that the effect of *G. mosseae* inoculation can be found at 30 DAT. But, the effectiveness of *G. mosseae* inoculation increases gradually to reduce wilt incidence of eggplant caused by *F. oxysporum* f. sp. *melongenae*. Co-inoculation study indicates that mycorrhizal spore can withstand at the presence of pathogen in soil which explains the sustainability of *G. mosseae* in eggplant field. Therefore, mycorrhizal fungus like *G. mosseae* can be adopted in the existing integrated disease management system for eggplant cultivation. Moreover, more intensive study needs to be undertaken to develop formulation of massive production of *G. mosseae* and their interaction with other bio-agents for more effective management of the *Fusarium* wilt disease of eggplant as well as other solanaceous crops in.

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Conflict of Interests

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

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