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Protection of Tomato, *Lycopersicon esculentum* from Wilt Pathogen, *Fusarium oxysporum* f. sp. *lycopersici* by Arbuscular Mycorrhizal Fungi, *Glomus sp.*

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A B S T R A C T

Arbuscular Mycorrhizal Fungi benefits plants by improving the uptake of phosphate and other nutrients from soil and also increases the disease tolerance to the host plant. The fungal pathogens cause huge loss to vegetable crops when infected. In order to overcome this problem, the influence of AMF in the control of fungal plant pathogens is studied. AMF, *Glomus sp*. was isolated from the rhizosphere soil of tomato plants. The wilt pathogen, *Fusarium oxysporum* f.sp. *lycopersici* was cultured from the infected roots of tomato plants. The interaction of AMF and soil borne pathogen was carried out in tomato by *in vitro* and pot culture experiment using *Glomus* sp. and wilt causing pathogen, *Fusarium oxysporum* f.sp. *lycopersici*. *In vitro* interaction of AMF root and fungal pathogen resulted in the formation of clearing zone around the root indicating the production of antimicrobial compounds from the mycorrhizal root that arrested the mycelial growth of the fungal pathogen. The pot culture experiment revealed that the pre AMF – post pathogen inoculation to tomato reduced the disease incidence, and increased the plant growth, dry weight, N, P, K content, chlorophyll content and yield of the plant.

Introduction

Arbuscular Mycorrhizal Fungi is used as a potential factor in integrated plant protection. Mycelium of AMF functions as root hair and protects roots against soil borne pathogen (Azcón and Barea, 1997). It has been recognized that mycorrhizal symbiosis play a key role in nutrient cycling in the ecosystem and protects plant against environmental stress and plant diseases thereby improving the plant health (French,2017). The control of root rot diseases produced by fungi *viz*., *Pythium, Pytophthora, Fusarium, Verticillium* and *Rhizoctonia*is associated with AMF (Linderman, 1995). Also the increase in the absorption of nutrients, mainly phosphorus, supports the plant to withstand the attack of pathogenic microorganisms (Trotta *et al.*,1996). The interaction of the AM fungus *Glomus fasiculatum* with a wilt-causing soil borne pathogen *F.oxysporum* in cowpea

(*Vigna ugniculata*) reduced the severity of the disease (Sundaresan *et al.,* 1993). Hence mycorrhizae holds its potential use in control of soil borne pathogen and the presence of AMF in soil caused a 10-20 % reduction of wilt disease in cotton (Naraghi *et al.,* 2007). The interactions between *G.intraradices* and the root pathogen *Fusaium oxysporum* f.sp *chrysanthemi*ina compartmentalized *in vitro* systemelucidated a significant negative correlation between conidia production and *G.intraradices* hyphae or spore concentration (Arnaud *et al.,* 1995). The mode of action of AMF biocontrol activity is assumed to be the direct interactions between AMF and pathogens, but mycorrhiza-mediated triggering of plant defence reactions have also been proposed (Whipps, 2004). In addition, antagonism from bacteria inhabiting the mycorhizosphere has also been suggested as a possible mechanism (Budi *et al.,* 1999).The phenomenon of AMF protecting plants from root pathogens is known from studies involving root-infecting pathogens *viz.*, *Phytophthora parasitica*, *Fusarium sp*. and root-invading nematodes (Dodd, 2000) of tomato (*Lycopersicum esculentum* Mill.) and alfalfa (*Medicago sativa* L.) (Dehne and Schonbeck, 1979).*G. mosseae* induced local and systemic resistance to *P. parasitica* and was effective in reducing symptoms produced by this pathogen (Maria *et al.,* 2002).The capability of AMF in imparting disease tolerance in tomato (*L. esculantum*) due to *Fusarium oxysporum* f. sp. *lycopersici* is experimented.

Materials and Methods

Arbuscular Mycorrhizal fungi

AMF culture, *Glomus* sp was isolated from the rhizosphere soil of tomato crop by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). The AMF spores isolated were identified as *Glomus sp*. according to the species description and pictures available in the INVAM website (International Culture Collection of Vesicular-Arbuscular Mycorrhizal Fungi). The AMF culture is maintained by soil trap culture method in which 1 to 3 maize seeds were placed in 10cm plastic cups containing sterilized soil. Three days after the germination of maize seeds, single spore of the AMF strain was placed on fine roots or root tip of the seedling. Ten spores per seedling was inoculated in this way. After 10 days of inoculation the seedlings were transferred to pots containing sterilized soil. The pots were maintained in a greenhouse for 3 months to develop the AMF inoculum. This AMF culture, *Glomus sp*. is deposited in the Department of Microbiology, AC&RI, Madurai and used for interaction studies with Fusarium wilt pathogen in tomato plants.

Fungal pathogen

The wilt pathogen, *Fusarium oxysporum*was isolated from tomato variety, PKM-1 showing typical wilt symptom. The isolate was purified in Potato Dextrose Agar (PDA) medium by single hyphal tip method (Rangaswamy, 1972) and maintained on (PDA) at 30°C.

Laboratory assay to study the interaction of AMF and root pathogen

The antagonistic effect of the mycorrhizal fungus, *Glomus* sp. was tested against *Fusarium oxysporum* f. sp. *lycopersici* by dual culture technique. The plain agar was prepared by adding 20g of agar into 1litre of distilled water and autoclaved at 15 lb pressure for 20 minutes. The mycorrhizal maize roots of 2cm length were washed in 0.05% Tween 20 solution, soaked in 2% chloramines T solution for 20 minutes and rinsed thrice in sterile distilled water. The root pieces were subsequently rinsed in 100mg/l

gentamycin antibiotic solution. Then the root pieces were washed thrice in sterile water. Thus surface sterilized root bit was placed at the center of one half of the petriplate containing 15ml of plain agar medium under aseptic conditions. A loopful of fungal pathogen was placed at the center of the other half of the petriplate. The petriplate was incubated in the inverted position at room temperature for the fungal growth. The following treatments was used for the lab assay of AMF-pathogen interaction.

T1 - AMF, *Glomus sp.* uncolonized root + *Fusarium oxysporum*
T2 - AMF, *Glomus sp*. colonized root + *Fusarium oxysporum*
The formation of clearing zone around the AMF, *Glomus sp*. colonized root was examined.

Pot culture experiment to study the interaction of AMF and root pathogen

The interaction of AMF, *Glomus sp*. with soil borne pathogen, *Fusarium oxysporum* f.sp. *lycopersici* in tomato (*L. esculantum*) was studied in pot culture experiment.

Seeds

Seeds of tomato (*L. esculantum*) var. PKM-1 were obtained from Department of Olericulture, Horticultural College and Research Institute, Periyakulam.

AMF Inoculant

The AMF culture, *Glomus* sp. was isolated from tomato rhizosphere soil and multipliedin maize roots. The culture was maintained in the Department of Microbiology, AC&RI, Madurai and the inoculum contained spore population of 200 spores / 50g of soil. The AMF inoculum of 50g was spread 2.5cm below the soil surface at the time of treatment.

Preparation of root pathogens

Fusarium oxysporum f. sp. *lycopersici* was isolated from diseased tomato roots and maintained on Potato Dextrose Agar (PDA). The isolate of *Fusarium oxysporum* f. sp. *lycopersici* was multiplied on sand maize medium containing sand and ground maize grains mixed in the ratio of 19:1, moistened and autoclaved in saline bottles at 15 lb/inch^2 pressure for two hours and incubated at 28±2^{o}C for 21 days. This sand maize medium containing the pathogen at five percent level was mixed with sterile soil and filled in earthen pots of 30cm height. The germination percentage on 7th day after sowing and the disease incidence on 45th day after sowing were assessed.

Seeds and Sowing

The seeds of tomato were surface sterilized with 0.1% $HgCl_2$ for three minutes and washed three times successively in sterile distilled water and 10 sterilized seeds were sown in pots.
After germination, only 3 plants were maintained in each pot.

Design and Treatment

Six treatments with three replications were arranged in completely randomized block design.
T1 Control – Uninoculated.
T2 Pathogen- *Fusarium oxysporum* f. sp. *lycopersici* inoculation at the time of sowing
T3 Arbuscular Mycorrhizal Fungi, *Glomus sp.*inoculation at the time of sowing.
T4 Simultaneous inoculation of AMF and Pathogen at the time of sowing
T5 Pre AMF inoculation at the time of sowing; Post Pathogen inoculation on7th day after inoculation of AMF
T6 Pre Pathogen inoculation at the time of sowing; Post AMF Inoculation on 7th day after inoculation of Pathogen.

Experimental observation

The plant growth biometric observation *viz*., dry weight / plant, fruit yield/plant, disease incidence, germination percentage and AMF spore count were recorded. The total chlorophyll content of the leaf sample was estimated following the method described by Mahadevan and Sridhar (1986). The plant analysis was done in the plant samples collected at flowering stage, dried at 60ºC for 3 days in a hot air oven and ground in a Wiley mill to pass through a 20-mesh sieve. The nitrogen content of the plant samples was analyzed by microkjeldhal method (Humphries, 1956), phosphorus content by Vandomolybdate yellow colour method (Jackson, 1973) and potassium content by flame photometry (Jackson, 1973).

Results and Discussion

Isolation of AMF strains

The spore shape and colour of the isolated AMF, *Glomus sp.* was found to be globose to sub globose and pale yellow colour (Plate.1). The *Glomus* spores have a spore wall and all layers originating from the wall of the subtending hyphae, with a variable number of layers (1-4). No flexible inner walls are formed. The spore wall is not continuous, with a pore at the subtending hyphae which may or may not be occluded. The AMF *Glomussp.* was cultured by soil trap culture method in pot culture with maize as host plant. The AMF *Glomus* sp. spore count was 385 spores / 100g of soil and root infection percentage was 80% in soil trap culture (Plate 2 and 3).

Isolation of soil borne pathogen from tomato

The pathogenic fungi, *Fusarium oxysporum* f. sp. *lycopersici* was isolated from wilt affected tomato. The fungal characteristics were the presence of micro and macro conidia and septate hyphae (Plate 4).

In vitro interaction of AMF and soil borne pathogen

In vitro interaction of AMF *Glomus* sp. and fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* in tomato resulted in the formation of clearing zone of 1.5cm around the mycorrhizal root. The zone was observed after twelve days of inoculation. In case of non- mycorrhizal root and fungal pathogen interaction, clearing zone was not observed around the root (Plate.5&6).

Interaction of AMF and soil borne pathogen *Fusarium oxysporum* f. sp. *lycopersici* in tomato under pot culture condition

The germination percentage was 93.8% in AMF alone inoculated seeds followed by pre AMF– post pathogen inoculated plants. AMF spore count was 165.4 spores per 100g of soil in AMF inoculated tomato. Also the AMF inoculated plants showed no disease incidence. Disease incidence percentage was 17.7% in pre AMF – post pathogen inoculated plants and 24.6 % in simultaneous inoculation of AMF and pathogen. The tomato plants inoculated with AMF alone recorded a dry weight of 29.6g at flowering stage. Pre AMF - post pathogen inoculation recorded 27.1g as against 15.3g in pathogen alone inoculated. Pre-pathogen and post AMF inoculation recorded a plant dry weight of 17.3g which was on par with pathogen alone inoculated plant of 15.3g. The maximum chlorophyll content of 2.7mg/g was recorded in AMF alone treated plants and 1.2 mg/g was found in pathogen alone treated plants followed by pre pathogen – post AMF treated plants.

The total nitrogen content was 1.89% due to AMF inoculation. The total phosphorus content was maximum in AMF alone

inoculated plant followed by pre AMF – post pathogen inoculated plants. The total phosphorus content was minimum in pathogen alone inoculated plant followed by pre-pathogen post AMF inoculated plants. The plants that received the mycorrhizal inoculum showed highest potassium content of 2.25%.

The yield per plant inoculated with mycorrhiza alone was 42.7 fruits/ plant and minimum yield of 20.7 fruits / plant was found in plants inoculated with pathogen alone. Among the interaction between AMF and pathogen, pre AMF-post pathogen registered more yield of 36.2 fruits/ plant (Table 1).

The beneficial effects of AM fungus on the growth of various crop plants have been well documented. AM associations, in general, can reduce or even suppress damage caused by soil borne plant pathogen (Jalaluddin *et al.*, 2008). Since AM fungus are established in the roots of host plants, it can primarily reduce the diseases caused by soil-borne pathogens (Dehne, 1982) and mycorrhizae have been suggested as biocontrol agents (Ryan and Graham, 2002). The isolation and maintenance of AMF *Glomus* sp. MDU2 isolate was done by culturing in soil trap culture method using maize seedlings. After infection of the maize roots by these methods, the seedlings were transferred to pots containing sterilized soil and the AMF spores were allowed to multiply. Manfred *et al.*, (2006) used *Glomus mosseae* and *Glomus intraradices* to infect the roots of maize and Sharif *et al.*, (2006) studied the infection percentage of AMF in wheat and maize.

Fusarium oxysporum is a threatening fungal pathogen causing wilt disease in many crops. Wilt causing pathogen in tomato, *Fusarium oxysporum* f.sp. *lycopersici* was isolated from diseased plants. Resi *et al.,* (2005) found seven isolates of *Fusarium oxysporum* f.sp. *lycopercisi* from tomato crops distributed all over Brazil.*Trichoderma harzianum* and Arbuscular mycorrhizal fungi (AMF) were able to control the wilt pathogen, *Fusarium oxysporum* f. sp. *lycopersici* in tomato seedlings (Mwangi *et al.,* 2011; Sandani and Weerahewa, 2018).

In vitro interaction of AMF and soil borne pathogen

In vitro interaction between arbuscular mycorrhizal fungal root and fungal pathogen resulted in the reduction of mycelial growth ofthe fungal pathogen, *Fusarium oxysporum* f.sp. *lycopersici* of tomato. This elucidated the mode of biocontrol activity by AMF to be the direct interactions between AMF and pathogens. Mycorrhiza-mediated triggering of plant defence reactions have also been proposed (Manila and Nelson, 2014; Nasrin *et al.*, 2018).

Interaction of AMF, *Glomus sp.* and soil borne pathogen *Fusarium oxysporum* f.sp. *lycopersici* in tomato

AMF, when inoculated either after pathogen inoculation or simultaneously with pathogen, the degree of growth increment was less compared to plants inoculated with AMF alone. The presence of AMF in soil caused a 10-20 % reduction of wilt diseases in cotton (Naraghi *et al.,* 2007). The culture filtrate of *Rhizobium* spp. and arbuscular mycorrhizal fungus act as potential biological control agents against root rot fungal diseases of *Albizzialebbeck* (Kaushik and Kaushik, 1995).

AMF and pathogen interaction in nursery stage of tomato

AMF inoculation to soil before sowing induced seed germination at a faster rate and increased the germination percentage and also produced tallest seedlings. The AMF

inoculated plant produced more number of roots and higher dry weight of plant in the nursery. Thomson *et al.,* (1996) observed that the mycorrhizal tomato seedlings exhibited significantly higher dry matter than non-mycorrhizal plants. The arbuscular mycorrhizal inoculation in pepper seedlings increased the dry matter of the plant (Turkmen *et al.,* 2005).

AMF and root pathogen interaction on the root colonization percentage and Disease incidence percentage of tomato

AMF have been shown to increase resistance to root-infecting pathogenic fungi e.g. *Phytophthora parasitica* or *Fusarium* spp. and root invading nematodes. Cordier *et al.,* (1996) however, provided evidence for the benefits of pre-inoculation of plants with an AM fungus and showed bioprotection against *P.nicotinae* var. *parasitica* via localized and induced systemic resistance in mycorrhizal plants. An attempt to drive a relationship between AMF colonization and pathogen disease incidence percentage revealed the existence of a negative correlation between the two components. This indicated that AMF acted antagonistically to counteract the presence of pathogen resulting in the suppression of disease incidence percentage. The exact mechanism of suppression of pathogen by mycorrhizal fungus is not known.

Kapoor (2008) suggested that *Glomus macrocarpum* and *Glomus fasciculatum* inoculation increased growth and phenol concentration that were capable of imparting disease tolerance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato.

Influence of AMF on the growth of tomato

AMF alone inoculated plant recorded the maximum dry weight of 29.6 g per plant. Among the interaction between the AMF and pathogen, in the pre AMF inoculated plant, dry weight was maximum followed by simultaneous inoculation of AMF and pathogen.

Bayozen and Yildiz (2009) determined the mycorrhizal interaction with pathogen *Rhizoctonia solani* and observed that the pathogenicity was reduced in AM fungus inoculated plants.

Influence of AMF on the nutrition of tomato

AM fungal inoculation increased the total chlorophyll content of the plant significantly. Druva *et al.,* (2008) also observed that the mycorrhizalinoculated marsh plant improved its photosynthetic performance and also the inoculation with *Glomus epigaeum* increased the chlorophyll content in black gram and also increased the N, P and K content (Umadevi and Sitaramaiah, 1998).

The N, P and K contents were very high in AMF alone inoculated plant. Among the interaction between the AMF and pathogen, the pre AMF inoculated plant showed maximum N, P and K content followed by simultaneous inoculation of AMF and pathogen. Pre pathogen treated plants recorded minimum N, P and K content. The inoculation of AM fungus to pepper seedlings increased the total nitrogen content in the plants (Turkmen *et al.,* 2005).

Albizzia plants treated with *Glomus mosseae* recorded higher nitrogen concentration (Kaushik and Kaushik, 1995). Arbuscular mycorrhizal fungi, *Glomus intraradiaces* improved the phosphorus efficiency of plants (Seoud,2008). *Dalbergia sissoo* inoculated with *G.fasciculatum* increased the K uptake (Manoharachary *et al.,* 2008).

Table.1 Interaction effect of AMF and soil borne pathogen *Fusarium oxysporum f.sp. lycopersici* in tomato seedlings under pot culture condition

S. No	Treatments	Germination percentage on 7th day after sowing	AMF Spores/ 100g of soil	Disease incidence on 45th day after sowing (%)	Dry weight (g/plant)	Chlorophyll content (mg/g)	Total Nitrogen (%)	Total phosphorus (%)	Total potassium (%)	Yield (no.of fruits/plant)
1.	Uninoculated - control	66.6	0.0	12.0	21.3	1.7	1.49	0.38	1.67	26.6
2.	Pathogen *Fusarium oxysporum* f.sp. *lycopersici* alone	53.3	0.0	72.4	15.3	1.2	1.34	0.21	1.21	20.7
3.	AMF inoculated alone	93.8	165.4	0.0	29.6	2.7	1.89	0.50	2.25	42.7
4.	Pre AMF – Post Pathogen (15 days before inoculation)	86.6	130.9	17.7	27.1	2.2	1.67	0.46	1.85	36.2
5.	Pre pathogen – Post AMF (7 days before inoculation)	60.0	50.7	51.2	17.3	1.3	1.43	0.24	1.38	22.7
6.	Simultaneous inoculation of AMF and Pathogen	76.6	80.6	24.6	23.9	1.9	1.63	0.41	1.79	30.0
	SEd	3.0	3.8	2.0	0.7	0.0	0.04	0.01	0.09	1.2
	CD (p = 0.05)	6.6	8.3	4.0	1.4	0.1	0.08	0.02	0.18	2.7

Plate.1&2 Spore of *Glomus sp.* from rhizosphere soil of tomato & Multiplication of *Glomus* sp. by soil trap method in maize roots

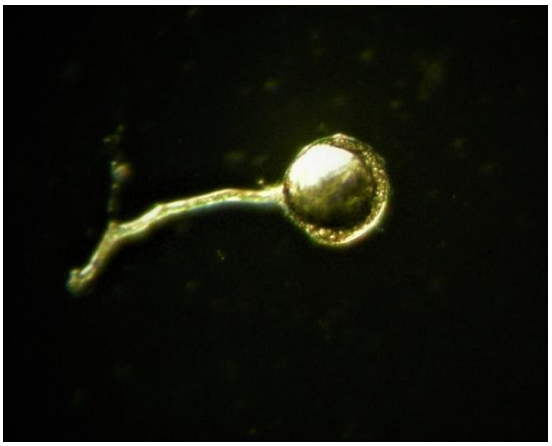


Plate.3&4 AMF, *Glomus* sp. infection in maize roots for multiplication as inoculum & Fungal colony of the wilt pathogen, *Fusarium oxysporumf.sp.lycopersici*

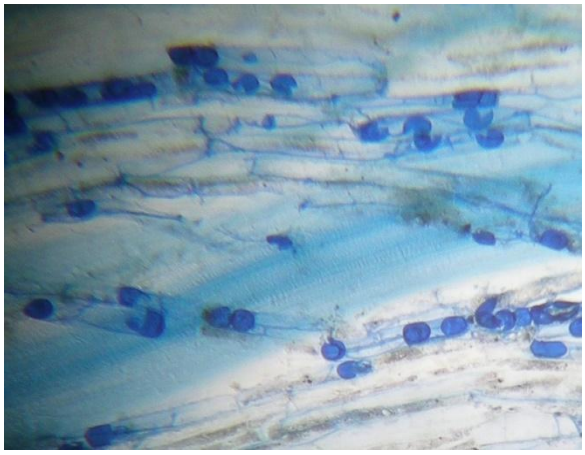
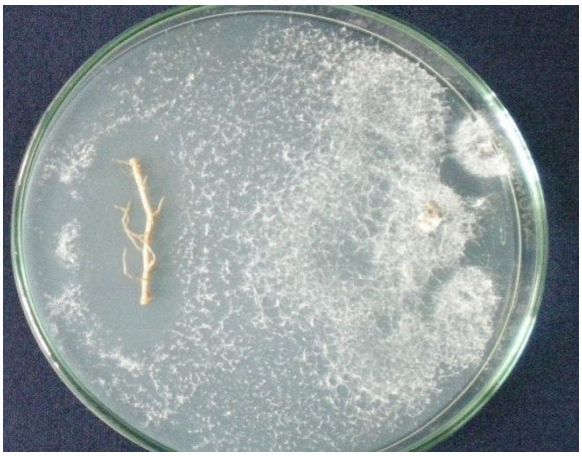


Plate.5&6 In vitro interaction between AMF un-colonized maize root and Fusarium wilt pathogen - no clearing zone formed around the root & In vitro interaction between AMF colonized maize root and Fusarium wilt pathogen in vitroby dual culture - clearing zone formed around the root



Influence of AMF on the yield of tomato

The highest yield was recorded in the AMF inoculated plants and those inoculated with pathogen (either pre or post AMF inoculation) registered a significantly lower yield. *Glomus fasiculatum* inoculation in soilless grown tomato plants increased the growth, yield, fruit properties and nutrient uptake (Dasgan *et al.*,2008) and similar observation is also made in many other crops (Haque and Matsubara,2018). The pot culture experiment revealed the interaction between AMF and soil borne pathogen *Fusarium oxysporum* f. sp. *lycopersici* of tomato. AMF inoculation was found to reduce the disease incidence percentage. Pre inoculation of AMF followed by pathogen reduced the disease infection better than simultaneous application of pathogen. The mycorrhizal inoculation showed vigorous growth of tomato seedlings and increased the crop nutrition and yield.AM fungi also interact with most crop plants including cereals, vegetables, and fruit trees, therefore, they receive increasing attention for their potential use in sustainable agriculture (Chen *et al.,* 2018).

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