Saudi Journal of Biological Sciences 28 (2021) 5442–5450

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Mycorrhizal fungi induced activation of tomato defense system mitigates Fusarium wilt stress

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ARTICLE INFO

Article history:
Received 26 February 2021
Revised 1 July 2021
Accepted 5 July 2021
Available online 10 July 2021

Keywords:
Fusarium oxysporum f. sp. *lycopersici*
Oxidative stress
Osmolytes
Antioxidants
Phosphatases
Solanum lycopersicum
Soil borne fungi

ABSTRACT

The fungus *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is known to cause vascular wilt on tomato almost over the world. Inoculation of FOL reduced plant growth and increased wilt of tomato. The following study examined the possible role of arbuscular mycorrhizal fungi (AMF) consortium comprising of *Rhizophagus intraradices*, *Funneliformis mosseae* and *Claroideoglomus etunicatum* against FOL in tomato and explored in an inducing plant systemic defense. AMF inoculation reduced the wilt disease within vascular tissue and *in vivo* production of fusaric acid was observed which may be responsible in reduced wilting. FOL had an antagonistic effect on AMF colonization, reduced the number of spores, arbuscules and vesicles. AMF also inhibited the damage induced by Fusarium wilt through increasing chlorophyll contents along with the activity of phosphate metabolising enzymes (acid and alkaline phosphatases). Moreover, tomato plants with mycorrhizal inoculation showed an increase in the level of antioxidant enzymes including glutathione reductase, catalase, and etc. with an ultimate influence on the elimination of reactive oxygen species. Moreover, rise in phosphatase along with antioxidant enzymatic systems and enhanced photosynthetic performance contributed to induced resistance against FOL in tomato.
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1. Introduction

Tomato (*Solanum lycopersicum* L.) a member of the family *Solanaceae*, is an important food crop consumed worldwide. Tomato is rich in nutrients and anti-cancer and anti-oxidative compounds like lycopene and flavonoids (Gerszberg et al., 2015).

Cellular processes like photosynthesis, respiration, plasma membrane functions and water conductivity are affected by pathogenic fungi (Berger et al., 2007). This biotic stress leads towards the over accumulation of toxic reactive oxygen species (ROS) thus inducing oxidative stress in plant (Vellosillo et al., 2010). Excess accumulated ROS interacts with the cellular constituents including lipids, proteins and nucleic acids, thus hinders the normal working of the cell (El-Rahman et al., 2012; Egamberdieva et al., 2017; Hashem et al., 2017). One of the biotic stress induced by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is responsible for intense yield losses of tomato due to wilt disease (Nirmaladevi and Sirnivas 2012; Akhter et al., 2015).

To alleviate the adverse effect of wilt disease caused by FOL biologically, the biologists are looking for alternative means, arbuscular mycorrhizal fungi (AMF) one of the most effective biological strategy reported to control wilt diseases (Al-Hmoud and Al-Momany, 2015). AMF are ubiquitous and improves the plant growth and development via enhancing the nutrient uptake and the rhizospheric soil health (Nahiyan, and Matsubara 2012; Al-Hmoud and Al-Momany 2015). AMF induced resistance by enhancing the accumulation of defense related proteins, osmolytes and strengthening of the antioxidant system (Alqarawi et al., 2014; Abd_Allah et al., 2015; Akhter et al., 2015). The antioxidant system constituted of the reactive and non-reactive components which can mediate the elimination of ROS, hence protect the plants from the stress induced by oxidative burst (Nahiyan and Matsubara, 2012; Egamberdieva et al., 2017). Superoxide dismutase (SOD),

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.07.025
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catalase (CAT), ascorbate peroxidase (APX) and etc. (El-Rahman et al., 2012; Kurabachew and Wydra, 2014; Abd_Allah et al., 2015), exhibit a close coordination in neutralizing the ROS. AMF induced positive changes reflect as an improvement in growth of host plants and subsequently improve their potential to withstand the stress triggered deleterious changes (Al-Hmoud and Al-Momany 2015; Hashem et al., 2016). Amelioration of the stress induced by the AMF has also been observed by active involvement of key phytohormones like auxins, cytokines, jasmonates and etc. (Cao et al., 2011; Beneduzi et al., 2012; Denancé et al., 2013; Hashem et al., 2015). Petti et al. (2012) and Buhrow et al. (2016) have demonstrated that the up-regulated expression of genes encoding indole acetic acid, indole butyric acid, and nine-*cis*-epoxy carotenoid dioxygenase involved in the synthesis of abscissic acid improved tolerance to *Fusarium* head blight in barley. Therefore, symbiotic association between AMF and plants provides new avenues for developing alternative strategies against plant pathogenic fungi (Nahiyan and Matsubara, 2012; Lewandowski et al., 2013; Song et al., 2015).

Therefore, the present study was aimed to assess the influence of *Fusarium oxysporum* f. sp. *lycopersici* on the development of tomato and FOL induced wilt severity and the impact of AMF (*Rhizophagus intraradices, Claroideoglomus etunicatum, Funneliformis mosseae,*) were observed in mitigating the disease severity via enhancing the antioxidant metabolism, phytohormones homeostasis and osmolytes accumulation.

2. Material and methods

2.1. Plant material

Certified tomato seeds (*Solanum lycopersicum* L., cv Red Rock) were treated with sodium hypochlorite (NaOCl, 5.0%, v/v) for 5 min to surface sterilize the seeds and washed afterwards with double distilled water. The seeds were sown in plastic plates (25x25x5 cm) containing autoclaved peat, perlite and sand (1:1:1, v/v/v) under controlled conditions (day/night temperature of 26/16 °C; Relative humidity, 56%) for two weeks after germination. The developed seedlings used for pathogenicity testing and pot experiments.

2.2. Fusarium oxysporum f. sp. Lycopersici isolation and inoculum preparation

Fusarium oxysporum f. sp. *lycopersici* (FOL) was isolated from tomato fields in Saleheyah Al Gadidah city (30.685725, 31.882915), Sharqia Governorate, Egypt (Fig. 1). Root tissue fragments of symptomatic tomato plants were surface-sterilized and plated on potato dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA) amended with 50 mg/L of antibiotic tetracycline. The inoculated PDA plates were incubated at 25 °C for 7–10 days under standard conditions according to Summerell et al. (2003), for the development mycelial growth of *Fusarium oxysporum* (Summerell et al., 2003) and sub-cultured onto PDA slants. The developed mycelia and conidia were characterized according to Booth (1977) and Nelson et al. (1983).

2.3. Disease incidence and severity assessment

Three weeks old healthy tomato seedlings were inoculated by standard root dip method as described by Nirmaladevi and Sirnivas (2012). The tomato seedlings were gently uprooted. The root tip (about 1 cm) was slightly trimmed and immersed for 30 min in the conidial suspension (10^6 CFU [colony forming units] ml^{-1} with sterile deionized water) of phytopathogen (FOL), carboxymethyl cellulose (CMC, 0.05%, w/v) used as adhering agent. Seedlings dipped in sterile water with CMC served as control. Afterwards, the seedlings were transplanted to plastic pots (25 cm diameter), containing autoclaved soil and sand (1:1). Five seedlings per pot were transplanted. Tomato plants were placed in a greenhouse where temperatures range varied between 25 and 30 °C. The plantlets were watered two times per week (50 mL/ pot) and fertilized once a week with NPK (15:15:15). Disease incidence was assessed after 6 weeks of inoculation. The disease index used throughout the experiments calculated as percentage according to the next equation:

$$\text{Disease incidence } (\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants}} 100$$

The brownish discoloration of the xylem vessel (percent invaded vessels) was confirmed and measured by slitting the stem (Johnson et al.,1982).

2.4. Arbuscular mycorrhizal fungi (AMF) and its application

The endophytic AMF (*Rhizophagus intraradices*, *Funneliformis mosseae, Claroideoglomus etunicatum*), were isolated previously from Talh trees (*Acacia gerrardii*) roots grown natively in Khuraim Meadow in Riyadh, Saudi Arabia (Hashem et al. 2016) according to the protocol as narrated by Daniels and Skipper (1982) and modified by Utobo et al. (2011). The trap culture protocol of Stutz and Morton (1996) was followed in this study. The inoculum of AMF was added to each pot at the application rate of 25 g of trap culture (counting approx.100 spores/g trap culture)/pot. Pots without mycorrhiza served as the control.

2.5. Experimental design, treatments and plant growing conditions

Completely randomized design experiment with ten replicates (one plant/each pot) was laid out to study the effect of AMF on FOL in tomato. The treatments were given as follows:

(1): Control (Without FOL and AMF inoculation); (2): FOL only; (3): FOL + AMF; (4): AMF only. The pots were placed in growth chamber. The disease incidence was assessed after 6 weeks of inoculation, subsequently the plant samples were collected for analyses.

2.6. Photosynthetic pigments

Tomato leaves (100 mg) were first extracted in acetone, then absorbance was measured at 622, 645, and 470 nm on spectrophotometer (Lichtenthaler and Wellburn, 1983). Chlorophyll and carotenoids contents were estimated by following formulae

$$Chla = 11.75A_{662} - -2.35A_{645}$$

$$Chlb = 18.61A_{645} - -3.96A_{662}$$

$$C_{x+c} = (1000A_{470} - -2.27C_a - -81.4C_b)/227$$

where: Chl a: chlorophyll *a* contents; Chl b: chlorophyll *b*, and C_{x+c}: carotenoids contents

2.7. Determination of leaf relative water content

Relative water contents (LRWC) of leaves were estimated by punching discs from the leaf of each treated plant. After calculating the fresh weight, the same leaf discs were kept on water for 4 h for the calculation of turgid weight. The leaf samples were dried in oven at 85 °C to obtain dry weight (Smart and Bihgham, 1974). Calculation of leaf water content was done by the following formula:

Fig. 1. Location epidemic area of tomato cultivation in Saleheyah Al Gadidah city (30.685725, 31.882915), Sharqia Governorate, Egypt.

$$\text{LRWC} = \frac{\text{fresh weight of leaf}}{\text{turgid weight of leaf}} \times 100$$

2.8. Determination of antioxidant enzyme activities

Frozen leaf tissue (0.4 g) samples were homogenized in pre-chilled mortar and pestle using 4 mL ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 4 % (w/v) polyvinyl pyrrolidone. The mixture was centrifuged at 14000 rpm at 4 °C for 30 min and the supernatant was used as enzyme source. Superoxide dismutase (SOD, EC1.15.1.1) activity was determined according to the Beauchamp and Fridovich (1971). Ascorbate peroxidase (APX, EC1.11.1.1) activity was assayed by observing the change in absorbance at 290 nm. While, APX activity was calculated by using molar extinction coefficient (ε) of 2.8 mM^{-1} cm^{-1} for AsA and activity expressed as U mg^{-1} protein (Nakano and Asada, 1981). For the measurement of dehydro ascorbate reductase (DHAR, EC: 1.8.5.1) activity, the method of Nakano and Asada (1981) was employed. Glutathione reductase (GR, EC1.6.4.2) activity was estimated by following the protocol of Smith et al. (1988).

2.9. Estimation of fusaric acid

Fusaric acid was estimated using thin-layer chromatography. Spots are developed on the chromatogram descending for 10–12 h in *sec*-butanol formic acid–water solvent system (75:15:10 v/v). The chromatograms were placed under hood (14–16 h) for drying and bromophenol blue was sprayed. Fusaric acid gives a yellow color (Stefan, 2005).

2.10. *Estimation* of *AMF colonization*

At the harvesting time, the AMF spores were isolated from the the soil substrate from every treatment by wet sieving and decanting method as described by Daniels and Skipper (1982) and modified by Utobo et al. (2011). The intensity of mycorrhizal colonization (mycelium, vesicles and arbuscules) was determined by the following formula:

$$\text{AMF root colonization } (\%) = \frac{\text{total no. of AMF postive segments}}{\text{total no. of segments studied}} \times 100$$

2.11. Statistical analysis

The experimental data were analyzed by employing two-way analysis of variance (ANOVA) with the help of Statistical Analysis System (SAS version 9.1) software. Significant differences between means were calculated by the least significant differences (LSD) test at P = 0.05. Additionally, the correlation coefficients were calculated for the studied parameters.

3. Results

3.1. Influence of FOL and AMF on tomato growth parameters

The morphological growth parameters were significantly higher in AMF inoculated treatment in comparison to control treatment (not inoculated with FOL). AMF exhibited significant improvement in the growth with an increase in shoot and root length (30.07% and 26.29%, respectively). However, upon inoculation with FOL

Table 1
Effect of *Fusarium oxysporum* triggered wilt disease on the length (cm / plant) and dry weight (gm/ plant) of shoot and root in *Solanum lycopersicum* with and without AMF inoculation. Data presented is mean of three replicates.

Treatments	Shoot height (cm)	Shoot dry wt (g)	Root depth (cm)	Root dry wt (g)	Shoot height/Root depth	Shoot / Root dry wt
Control	29.86b	0.5093b	14.3b	0.257b	2.100b	1.992b
Fusarium Only	8.93d	0.1963d	5.53d	0.1133d	1.621c	1.789c
Fusarium + AMF	21.63c	0.3593c	9.6c	0.1776c	2.253a	2.019a
AMFOnly	42.7a	0.706a	19.4a	0.3813a	2.201a	1.859b
LSD at 0.05:	4.78	0.066	1.642	0.036	0.42	0.58

Table 2
Effect of *Fusarium oxysporum* triggered wilt disease on chlorophyll pigments (mg/ g fresh wt) and net photosynthetic rate (mmol CO_2 M^{-2} S^{-1}) in *Solanum lycopersicum* with and without AMF inoculation. Data presented is mean of three replicates.

Treatments	Photosynthetic activity						
	Photosynthetic pigments (mg / g fresh wt)						Net photosynthetic rate
	Chl a	Chl b	Chl a + b	Chl a/b	Carotenoids	Total pigments	
Control	1.091b	0.6973b	1.788b	1.564d	0.3910d	2.179b	12.03b
Fusarium Only	0.5003d	0.1736d	0.674d	2.928a	0.8026a	1.476d	4.58d
Fusarium + AMF	0.7953c	0.4666c	1.262c	1.707c	0.5906b	1.852c	8.15c
AMF Only	1.640a	0.7990a	2.439a	2.053b	0.4113c	2.850a	14.63a
LSD at 0.05:	0.082	0.045	0.1073	0.492	0.032	0.12	1.24

reduction in root and shoot length was recorded (70.09% and 61.33%, respectively). In contrast an increase in shoot and root dry weights (27.86 % and 32.60 %, respectively) was observed upon inoculation with AMF. Fusarium infection reduced the dry shoot and root weight by 61.22% and 55.91%, respectively, however plants treated with both FOL and AMF together exhibited only 29.45% and 30.89% reduction (Table 6).

3.2. Influence of FOL and AMF on photosynthetic pigments

Tomato plants inoculated with AMF only exhibited a significant improvement in pigment content. Relative to the control increase in chlorophyll *a*, chlorophyll *b*, carotenoids and total pigments was 33.47%, 12.73 %, 4.93% and 23.54%, respectively (Table 7). Tomato infected with FOL exhibited a reduction of 54.14%, 75.10% and 32.26% in chlorophyll *a*, chlorophyll *b* and total pigments, respectively. However, AMF inoculation to FOL infected (FOL + AMF) plants significantly ameliorated the negative effects on pigment synthesis (Table 7). Net photosynthetic rate was maximum in AMF inoculated plants as compared to control as well as wilt infected plants. As compared to the control net photosynthetic rate was increased in AMF inoculated (17.77%) and decreased in FOL (61.93%) infected plants (Table 7), while co-inoculation of AMF and FOL (FOL + AMF) resulted a reduction of net photosynthetic activity as 32.25%.

3.3. Influence of FOL and AMF on leaf relative water contents

Mycorrhizae inoculated tomatoes showed an increase of 6.83% in the leaf relative water content (LRWC) as compared to uninoculatedplants, however FOL induced wilt resulted in 43.30% reduction in LRWC (Table 8). Theplants received AMF inoculum exhibited significant increase in flavonoid content under infectin free as well as diseased conditions. Relative to the control, an increase in total flavonoids was observed in AMF inoculated plants both without and with FOL (FOL + AMF) infected conditions (44.00% and 71.12%, respectively). However, FOL induced wilt resulted in 50.98% decline in LRWC (Table 8).

3.4. Influence of AMF on wilt development and accumulation of fusaric acid

FOL triggered wilt and its disease incidence potential in tomato was 85.22% and 90.51% as wilted plants and invaded vessels, respectively (Table 1). Moreover, the infection of tomato plants was accompanied with accumulation of fusaric acid (22.09 μg/ g root fresh weight) as wilt inducing agent. Inoculation tomato plant with the AMF, caused significant decrease in disease incidence (wilted plants and invaded vessels) and fusaric acid accumulation in roots (79.04% and 10.92%, respectively), compared to the plants without AMF (Table 1).

3.5. Determination of AMF root colonization and correlation with FOL development

Colonization and total spore number of AMF were lower significantly in FOL-inoculated plants (Table 2). FOL infection resulted in considerable reduction in the mycelia (39.43%), vesicles (55.85%) and arbuscules (52.14%) as well as total spore number of AM fungi (52.61%) compared to control mycorrhizal plants. The intensity of fungal infection (structural colonization) in tomato plants with M along The intensity of AMF as (M) and (A) were reduced in FOL plants significantly, however (P) had significantly higher intensity as compared non-diseased plants (Table 3). The intensity of AMF was always comparable to the infection of FOL. The Pearson's cor-

Table 3
Effect of Fusarium oxysporum triggered wilt disease on leaf relative water content (%) in Solanum lycopersicum with and without AMF inoculation. Data presented is mean of three replicates.

Treatments	LRWC %
Control	86.09b
Fusarium Only	48.82d
Fusarium + AMF	64.14c
AMF Only	92.40a
LSD at 0.05	2.57

Table 4
Wilting percentage (WP), percent invaded vessels (IV) and fusaric acid (µg/ 10 g root fresh weight) in tomato infected with Fusarium oxysporum f. sp. lycopersici. Data presented is mean of five replicates.

Treatments	WP (%)	Disease incidence	
		IV (%)	FA
Fusarium only	85.22a	90.51a	22.09a
Fusarium + AMF	15.19b	18.97b	9.16b
LSD at 0.05:	2.29	2.22	3.38

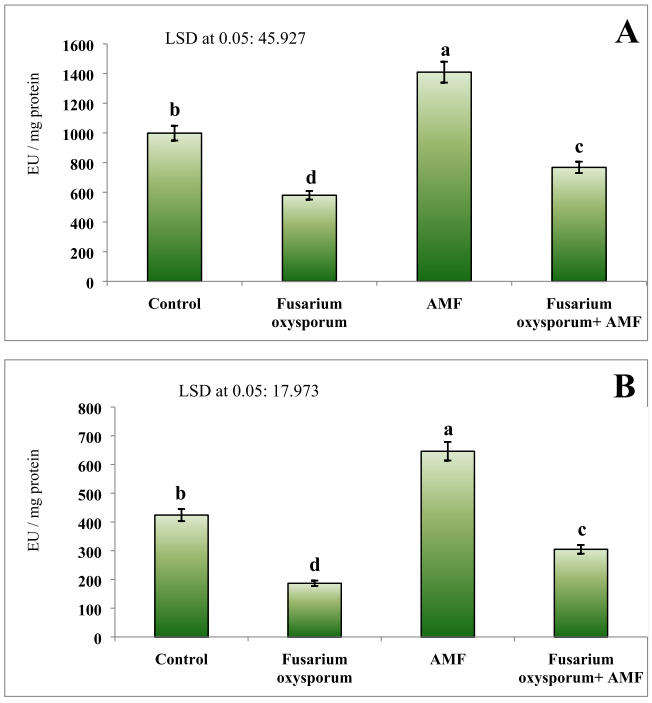

Fig. 2. A-B: Effect of *Fusarium oxysporum* triggered wilt disease on (**A**) acid and (**B**) alkaline acid phosphatase activity with and without AMF in *Solanum lycopersicum* L. Data presented are the means ± SE (n = 5).

relation coefficient between colonization of AMF and disease incidence of tomato caused by FOL was presented in the Table 4. The wilted plants have positive but non-significant effect on invaded vessels (IV), (M) and (V) as (0.937), (0.343) and (0.343) respectively, while non-significant and negative correlation were recorded for fusaric acid and (A) as (-0.597) and (-0.985), respectively. Invaded vessels (IV) showed negative correlation with fusaric acid, (M), (V) and (A). Fusaric acid showed positive and non-significant correlation with A (0.726) while negative correlation was recorded for both M and V. Mycelium showed highly significant and positive correlation for tomato vesicles, while vesicles showed negative correlation for (A) (Table 4).

3.6. Influence of AMF on phosphatase and antioxidant enzyme activity

Mycorrhizal inoculation on tomato plants resulted in significant increase activities of both phosphatase enzymes (acid & alkaline) as compared to un-inoculated control plants (Fig. 2A, B). In contrast, FOL inoculation of tomato plants caused drastic decline in the activity of acid and alkaline phosphatases by 41.91% and 55.97%, respectively, as compared with the un-inoculated control plants. However, the pre-inoculation of tomato plants with AMF resulted in strong induction of acid and alkaline phosphatases compared with treatment only inoculated with FOL. The Pearson's correlation coefficients between colonization and phosphatase enzymes is described in the Table 5. The mycelium showed non-significant but positive correlation against the vesicles (0.796) and arbuscules (0.752) while acid phosphatase (0.954) and alkaline phosphatase (0.965) recorded positive and significant correlation. On the other hand vesicles showed positive while non-significant correlation on arbuscules (0.932), ACP (0.786) and ALP (0.792). The arbuscules have positive and significant results on ACP (0.834) and ALP (0.824) respectively. ACP showed highly significant and positive correlation with Alkaline phosphatase activity (0.998). Tomato plants infected with FOL triggered an increase in SOD, APX, DHAR and GR activity. Tomato plants with FOL + AMF inoculation showed 51.75%, 12.75%, 18.83% and 26.38% increase in activity of SOD, APX, DHAR and GR, respectively, while AMF only treated plants resulted in 23.76%, 4.43%, 13.10% and 8.27% increase in activity of SOD, APX, DHAR and GR, respectively (Fig. 3 A-D).

Table 5
Effect of *Fusarium oxysporum* triggered wilt disease on the AMF colonization characteristics; total spore number, percent colonization in terms of mycelium (M), vesicles (V) and arbuscules (A) in *Solanum lycopersicum*. Data presented is mean of thirty replicates.

Treatments	*Total spore number	Total colonization percent		
		Mycelium	Vesicles	Arbuscules
Fusarium + AMF	664.6b	57.3b	20.0b	34.6b
AMF Only	1402.3a	94.6a	45.3a	72.3a
LSD at 0.05:	352.118c	37.87	15.34	26.69

*Total spore number: spore per 250 g soil.

Table 6
Effect of *Fusarium oxysporum* triggered wilt disease on the Intensity of AMF structural colonisation is shown as poor (P), moderate (M) and abundant (A). Data presented is mean of thirty replicates.

Treatments	Intensity of Structural Colonization (%)								
	Mycelium (M)			Vesicles (V)			Arbuscules (A)		
	P	M	A	P	M	A	P	M	A
Fusarium + AMF	71.0a	24.0b	5.01c	83.3a	16.6c	1.7c	83.3a	10.0b	6.66b
AMFOnly	45.0b	27.3a	27.6a	63.0b	24.6b	12.3a	61.3b	26.3a	12.33a
LSD at 0.05:	18.37	2.03	10.82	12.47	50.3	7.21	11.04	8.92	4.38

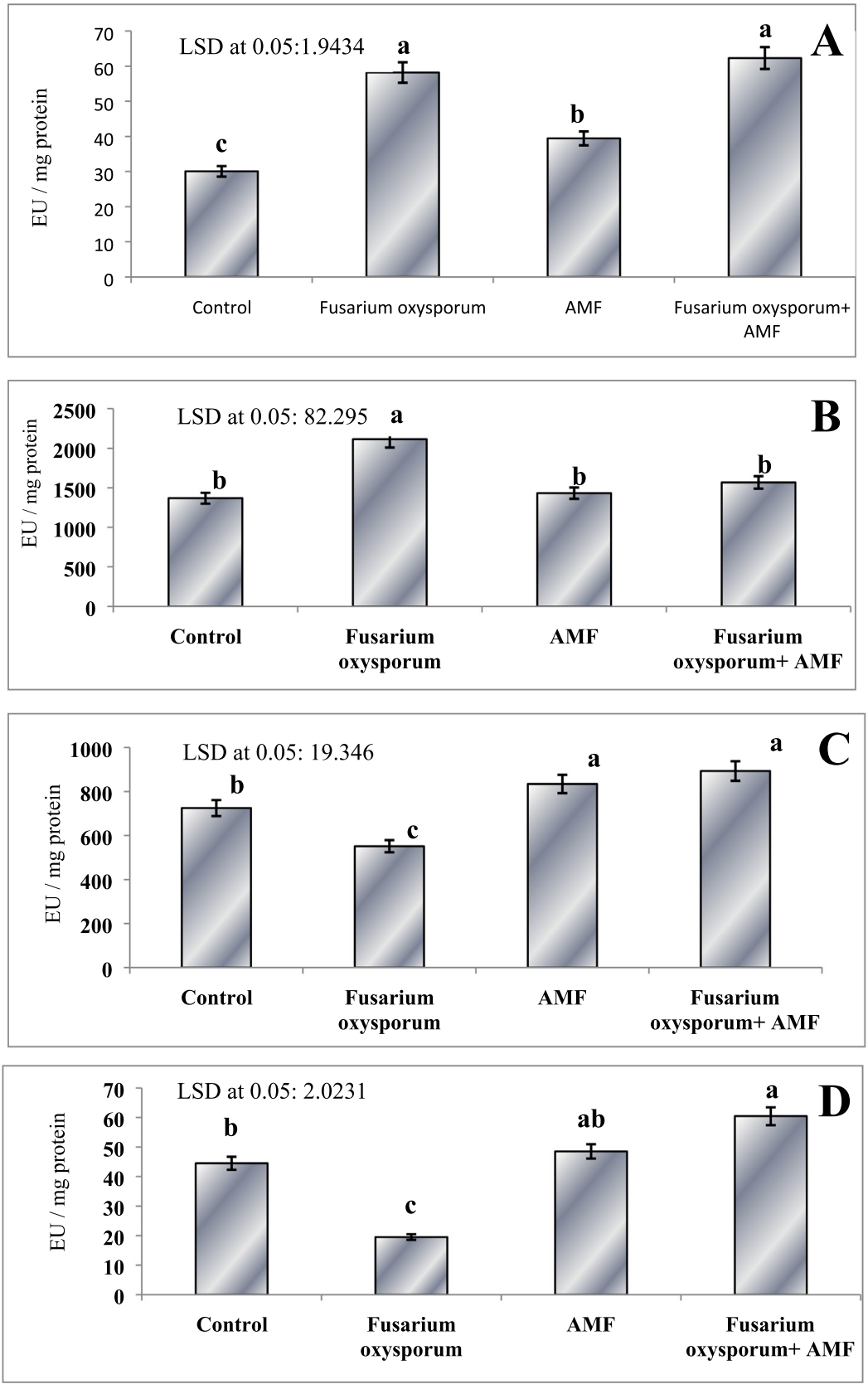

Fig. 3. A-D: Effect of *Fusarium oxysporum* triggered wilt disease on activity of (**A**) superoxide dismutase, (**B**) ascorbate peroxidase (**C**) dehydroascorbate reductase, (**D**) glutathione reductase with and without AMF in *Solanum lycopersicum* L. Data presented are the means ± SE (n = 5).

Table 7
Pearson Correlation Coefficients between Colonization and Disease incidence.

	WP	IV	FA	M	V	A
WP	1.00000	0.93731 0.2266	−0.59780 0.5921	0.34370 0.7766	0.34370 0.7766	−0.98512 0.1100
IV		1.00000	−0.28095 0.8187	−0.00511 0.9967	−0.00511 0.9967	−0.86346 0.3366
FA			1.00000	−0.95827 0.1846	−0.95827 0.1846	0.72669 0.4821
M				1.00000	1.00000 <0.0001	−0.50000 0.6667
V					1.00000	−0.823700.5634
A						1.00000

WP: Wilt plants; IV: invaded vessels; FA: Fusaric acid; M: Mycelium; V: Vesicles; A: Arbuscules.

Table 8
Pearson Correlation Coefficients between Colonization and Phosphatases enzymes.

	M	V	A	ACP	ALP
M	1.00000	0.79676 0.0578	0.75227 0.0845	0.95467 0.0030	0.96594 0.0017
V		1.00000	0.93295 0.0066	0.78697 0.0632	0.79285 0.0599
A			1.00000	0.83415 0.0390	0.82407 0.0437
ACP				1.00000	0.99884 <0.0001
ALP					1.00000

M: Mycelium; V: Vesicles; A: Arbuscules; ACP: Acid phosphatase; ALP: Alkaline phosphatase.

4. Discussion

Tomato showed drastically reduced growth in plants inoculated with FOL. The phytotoxic potential of FOL is related to production of fusaric acid as wilt inducing agent which played a major role in a significant decrease of plant growth via photosynthesis inhibition (Landa et al., 2002; Wu et al. 2008). Our study revealed that there is a substantial accumulation of fusaric acid in Fusarium infected plants compared with control. In another context, water deficit stress developed by FOL which caused blocking of vascular system in tomato roots hence, significantly enhanced restricting plant growth rate when only limited resource was available during stress (Lima et al., 2019). Increased growth and biomass production with AMF inoculation was observed in both FOL inoculated and uninoculated plants. Several reports including Al-Askar and Rashad (2010) Nahiyan, and Matsubara (2012) and Al-Hmoud and Al-Momany (2015) has reported enhanced growth of AMF inoculated plants in both healthy and diseased conditions on different crops. The induction of defense associated proteins including pathogenesis-related proteins (PRP) and cell wall degrading chitinase and β-1,3-glucanaseby AMF are known to induce systemic resistance against Fusarium oxysporum (Pozo et al., 2010). Disease resistance in tomatoes could be due to improved growth conditions. however the mechanisms actually inovolved was not established. Production of PRP is considered an indicator of induced defense response whileaccumulation of chitinases and β-1,3-glucanase also linked with inducing resistance against *Alternaria solani* in tomato and AMF-colonization induced increase in growth of the host is mainly due to the increased nutrient acquisition particularly the phosphorous (Evelin et al., 2009; Beltrano et al., 2013, Huang et al., 2014; Hashem et al., 2015). The beneficial impact of associations between plant roots and AMF enhance uptake & mobility of nutrients like inorganic phosphate to host plants in exchange for fixed carbon source, food for AMF (Garcia et al., 2016; Bukovská et al., 2018). Phosphatases play their role in increasing the availability of phosphorous to plants (Liao et al., 2003). In our investigation, acid and alkaline phosphatases decreased with FOL infection, whereas AMF ameliorated the effect considerably. In accordance to our findings, Zhang et al. (2014) has also reported the reduced uptake of phosphorous in pea due to the reduction in phosphatase activity. Valliyodan et al. (2017) has demonstrated considerable enhancement in the activity of phosphate assimilating enzymes in soybean due to AMF. In our study root phosphatase activity was higher in mycorrhizae inoculated plants. Probably, the higher resistance of the existing acid phosphatase to the degradation by stress-induced enzyme as well as production of acid phosphatases were among the prime reasons for improved acid phosphatase activity (Jakobek and Lindgren 2002; Liao et al., 2003). Beltrano et al. (2013) and Zhang et al. (2014) advocates that improved root phosphatase activity regulates phosphorous transport and assimilation. Tomato plants infected with *Fusarium* wilt had shown reduced AMF colonization which may be due to the release of fusaric acid by FOL. Fusaric acid inhibit the growth of microflora either natural or beneficial to plants (Landa et al., 2002). Earlier Al-Askar and Rashad (2010) has demonstrated significant reduction in the AMF root colonization in beans due to *Fusarium* root rot disease. Similarly, Nahiyan and Matsubara (2012), Lewandowski et al. (2013) and Al-Hmoud and Al-Momany (2015) had also reported decline in the AMF root colonization in different crop plants infected with root pathogens. Moreover, fusaric acid produced by the *F. oxysporum* has the potential to inhibit photosynthesis and reduced chlorophyll synthesis (Wu et al., 2008), and it could have promoted the activity of chlorophyll degrading enzymes chlorophyllase concomitant with the decline in the Rubisco activase activity leading to reduced photosynthetic rate (Akhter et al. 2015). We also found significantly lower photosynthetic pigments in FOL-treated tomato plants. Our results are in line with the previous reports of other *F. oxysporum* attacks on watermelon (Wu et al., 2008); onion (Abdelrahman et al., 2016) and banana (Thakker et al., 2013). Moreover, the stom-

atal opening and closure as well as inhibition of chloroplast due to organelles damage caused by FOL and fusaric acid (Dehgahi et al., 2015) reduced stomatal conductance and activity of photosynthesis (Mcelrone et al., 2002; Wu et al., 2008). However, the AMF-inoculated plants maintained a higher photosynthetic activity as compared to the FOL infected plants. Sheng et al. (2008) suggested that improved mineral uptake particularly magnesium by AMF might be the reason and possible mechanism to induce an increase in chlorophyll contents. AMF protects photosynthetic apparatus via increasing electron transport (ETRI and ETRII) and decreasing the quantum yield of non-photochemical quenching Y (NPQ) as reported by Rehman et al. (2010). The antioxidant enzymes activity was elivayed in tomato plants by FOL induced stress and such activities were further up regulated by co-inoculation with FOL and AMF. The enhancement of antioxidant enzymes activity is mediated to decrease the oxidative stress and detoxify ROS triggered by the biotic stress of FOL (Vellosillo et al., 2010). Previously, Huang et al. (2014) also reported that in AMF-plant-pathogen interaction enhanced SOD gene expression resulted in lower accumulation of ROS (Huang et al., 2014). AMF may lead to the quick elimination of wilt-generated ROS, hence protecting the host plant against the deleterious effects of pathogen induced oxidative damage (Nahiyan, and Matsubara 2012; Song et al., 2015). The fungal colonization of xylem vessels induce symptoms like water stress (Yadeta and Thomma 2013; Akhter et al. 2015). The SOD expression was greater in lettuce inoculated with AMF under osmotic stress (Ruiz-Lozano, 2003). Additionally, AMF increased the tolerance of plants against water stress by increased antioxidant enzymes activity for the protection of lipids in membranes (Qun et al., 2007; Tang et al. 2009; Rasool et al., 2013; Hashem et al., 2015). Wilt disease caused by FOL induced significant reduction in tomato plant growth by limiting the root colonization of mycorrhizal fungi, plant growth, chlorophyll contents and induce oxidative damage. AMF inoculation restored the wilt-triggered growth reduction by enhancing the chlorophyll synthesis, level of antioxidant, phosphorous metabolism and by reduced oxidative stress. AMF also enhanced the accumulation of osmolytes providing extra osmotic strength to plants against FOL stress. Therefore, AMF has the prospective to improve the tomato growth and increase the plant's tolerance to *Fusarium* wilt.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP-2021/356), King Saud University, Riyadh, Saudi Arabia.

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