Growth, physiological, and biochemical responses of thyme (*Thymus vulgaris* L.) to the application of arbuscular mycorrhizal fungi under cadmium stress conditions

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Abstract

Thyme (*Thymus vulgaris* L.) is one of the most important medicinal plants used in various pharmaceutical, osmotic, health, and food industries. Arbuscular mycorrhizal fungi (AMF) symbiosis is viewed as one of the several methods to improve growth under heavy metals stress. To investigate the effects of cadmium (Cd) and AMF bio-fertilizers on the growth and morpho-physiological characteristics of thyme, a greenhouse experiment was performed in three replications. Experimental treatments included Cd at three levels 0, 75, and 150 mg/kg of soil and AMF at three levels without inoculation, inoculation with *Funneliformis etunicatum*, and *Funneliformis mosseae*. Cadmium stressed plant showed reduced plant height, number of leaves, stem fresh and dry weight, and root fresh and dry weight while AMF inoculation enhanced the increased means of these traits considerably. Inoculation with *F. mosseae* also ameliorated the Cd stress (150 mg/kg) induced reduction in plant height, number of leaves, and stem and root dry weight by 13.41%, 8.42%, 30.3%, and 22.2%, respectively. Cadmium stress reduced membrane stability index while AMF inoculation enhanced membrane stability index considerably. An increase in soluble carbohydrate and proline content was observed due to Cd stress and AMF inoculation caused a further increase in these two metabolite contents ensuring better growth under Cd stressed conditions. Results indicated that *F. mosseae* had a higher efficiency in increasing morphological traits and improving physiological characteristics than *F. etunicatum*. Overall, AMF inoculation, especially *F. mosseae* significant ameliorative potential for Cd toxicity in thyme plants.

Keywords: antioxidant activity; bio-fertilizers; heavy metal toxicity; number of leaves; proline content

Introduction

Thyme (*Thymus vulgaris* L.), an aromatic perennial subshrub belonging to the Lamiaceae family, is commonly known for its therapeutic properties since ancient times. The whole plant and its various extracts

are used in the medicine, cosmetic, and food industry. The genus *Thymus* is one of the important members of the Lamiaceae family and comprises more than 325 species worldwide (Kucukaydin *et al.*, 2020). Among the thyme species, 18 species have been identified from Iran, three of which are called Iranian thyme, Danai thyme, and Marandi thyme are exclusive of Iran (Kalvandi *et al.*, 2014; Pirbalouti *et al.*, 2015). Thyme contains about 0.8 to 2.6% essential oil of dry matter, mostly phenols, monoterpene hydrocarbons, and alcohols.

Cadmium (Cd) is included in the list of highly toxic environmental pollutants due to a considerable threat to all organisms, including plants and humans (Kabir *et al.*, 2018). Cadmium is believed to accumulate in both water and soil due to phosphate fertilizers and industrial activities (Kaya *et al.*, 2019). Song *et al.* (2016) reported that Cd contamination is one of the major environmental problems in the agricultural system due to its long stay in the soil for thousands of years. It has been reported that Cd affects animal and human health through the food chain by taking it up by plant roots (Ali *et al.*, 2013). Plants do not require cadmium, but being poisonous to plants it can disrupt various physiological events (Kaya *et al.*, 2019), such as restriction of respiration and photosynthesis (Song *et al.*, 2019), disruption of the water relations (Belimov *et al.*, 2015) and uptake of nutrient (Rasheed *et al.*, 2018), and change in enzyme activity (Kaya *et al.*, 2020). Besides, Cd toxicity can generate reactive oxygen species (ROS), causing oxidative stress by increasing lipid peroxidation and accumulating H_2O_2 (Vasiljeva *et al.*, 2018). The ROS molecules lead to oxidative damage of lipids, proteins, and nucleic acids resulting in cell death because of lipid peroxidation, membrane damage, and key enzymes' inhibition (Akram *et al.*, 2018). Plants possess protective machinery to counteract the deleterious effects of Cd stress and minimize the accumulation of ROS (Chmielowska-Bak *et al.*, 2018) by increasing the activities of key enzymatic antioxidants, such as peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) (Kaya *et al.*, 2019).

Crops containing Cd play an important role in bringing this element into the human diet. Because more than 70% of Cd that enters the human body is transmitted through crops containing Cd (Rahimzadeh *et al.*, 2017). Cd's natural concentration in plants is reported 0.05-2 mg/kg (Usman *et al.*, 2019). There are two different ways to reduce the entry of Cd into the food chain. The first solution is to use refining methods to remove Cd from the soil. The second way to stabilize this soil element and reduce its attractiveness is through plants (Usman *et al.*, 2019). Biological fertilizers use the second method to reduce this metal in plants and the human food chain (Wuana and Okieimen, 2011).

Arbuscular mycorrhizal fungi (AMF) can form intimate associations with the roots of about 85% of all terrestrial plants. They can significantly increase a plant's soil nutrients' uptake, especially phosphorus. Arbuscular mycorrhizal fungi are soil fungi, ubiquitous in all terrestrial ecosystems (Lou *et al.*, 2014). They establish a symbiotic relationship with plant roots and, consequently, increase their host resistance to diverse biotic and/or abiotic stresses (Yao *et al.*, 2014; Zou *et al.*, 2014). Mechanisms used by AMF to reduce the stress of the heavy metal for plants include chelating and non-dynamics of heavy metals in foreign hyphae, improving mineral nutrition, especially phosphorus, altering root acidity, regulating gene expression in metal carriers, and affecting adsorption and absorption, etc. (Gupta *et al.*, 2010; Luo *et al.*, 2014). Numerous studies have also shown that living organisms, such as AMF, can alter the fragmentation (various forms and chemical forms) of heavy metals in the soil (Ruiz *et al.*, 2011). Whitfield and Richards (2004) found that AMF increased the absorption of nutrients and improved the thyme plant's growth under heavy metal stress. Zhang *et al.* (2018) reported that AMF inoculation enhanced phosphorus absorption and plant growth and leaf CAT activity while decreasing heavy metal levels in the shoot of sunflower. Arbuscular mycorrhizal fungi mediate plant interaction by directly influencing plant biomass and/or indirectly influencing plant photosynthesis and macronutrient acquisition (Yang *et al.*, 2016). The present experiment aimed to assess the effects of two AMF isolates on the morpho-physiological characteristics of thyme plants grown in contaminated soils with three different Cd concentrations.

Materials and Methods

Plant materials and experimental design

To investigate the effect of different levels of AMF bio-fertilizers on morpho-physiological characteristics of thyme under Cd stress, a factorial experiment based on a completely randomized design in three replications including Cd at three levels: 0, 75, and 150 mg/kg soil and AMF in three levels without inoculation, inoculation with *F. etunicatum* and *F. mosseae*) was implemented in the greenhouse of Mohaghegh Ardabili University's, Ardabil City, Iran, in 2018.

The non-polluted soil sample with sandy loam texture was prepared from 0-30 cm depth of soil. After drying and passing through the 2 mm sieve, the samples were uniformly mixed and the physical, chemical, and biological characteristics were measured. Soil texture by hydrometer method (Gee and Bauder, 1986), organic matter content by dichromate wet-oxidation method (Ben-Dor and Banin, 1989), acidity by pH meter, electrical conductivity by EC meter, and calcium carbonate equivalent by Machado *et al.* (2014) method was measured and the results are presented in Table 1.

Cadmium at three levels of 0, 75, and 150 mg/kg soil was thoroughly mixed with each pot's soil by spraying cadmium chloride (CdCl2- Sigma-Aldrich). To achieve natural contamination, Cd-contaminated soils were exposed to wet and dry cycles for four months. Each seedling was inoculated with 10 ml of a spore suspension of either *F. etunicatum* or *F. mosseae* (collected from Soil Biology Laboratory of Tabriz University, Iran), containing approximately 1000 spores, which was poured on the seedling roots. The control plants received 10 ml of sterile water. Plants were uniformed in size, age and transplanted in 20 × 35 cm plastic pots (one plant/pot) filled with 8 kg sandy-loam soil. Plants were watered as needed but were not fertilized, and each pot was placed in an individual saucer to allow reabsorption of irrigation water and avoid TM leaching. Plants were grown for three months in a greenhouse with 16 hours of daylight (20-28 °C). After the end of the three-month planting period, the aerial and root sections were harvested separately in each pot. Samples of fresh roots were prepared to determine the percentage of root colonization. The dry weight of roots and aerial parts was measured at 72 °C for 72 h after washing and drying (Gerdeman and Nicolson, 1963).

Table 1. Some physical and chemical properties of soil

EC (dS/m)	pH	$CaCO_3$ (%)	Organic carbon (%)	N (%)	K (%)	P (%) (ppm)	Soil texture	Clay (%)	Silt (%)	Sand (%)
4	7.3	19.1	0.514	0.12	0.05	3.8	Sandy loam	14	36	50

Measurement of traits

The plant's morphological characteristics such as number of leaves, plant height, fresh and dry weight of stem and root organs were collected about two months after planting.

Total soluble carbohydrate

Samples of fresh leaves were weighed (0.2 g) and homogenized using 70% ethanol. Then they were filtered and pigments were removed by the use of benzene. An aliquot of 0.2 ml of leaf extract was added to 1.0 ml of 0.2% anthrone to react in a water bath for 10 min at 100 °C. The test tube 3 was soon cooled in an ice bath and then the absorbance was recorded at 620 nm, according to Yemm and Folkes (1953).

Proline assay

Proline was determined according to the method described by Bates *et al.* (1973). Approximately 0.5 g of fresh leaf material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid. Then, this aqueous solution was filtered through Whatman's paper No. 2. Finally, 2 ml of filtrated solution was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100

°C. The reaction mixture was extracted with 4 ml toluene, cooled to room temperature, and the absorbance was measured at 520 nm with a spectrometer.

Antioxidant enzymes assay

Samples were frozen in liquid nitrogen and stored at -30 °C. One g of frozen sample was homogenized in a mortar with 5 ml of 50 mM potassium phosphate buffer (pH 7.5) containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiotreitol and 2% polyvinyl pyrrolidone (PVP). The homogenate was centrifuged at 15,000 g for 25 min and the supernatant was used for antioxidant enzyme assay (Aghighi Shahverdi *et al.*, 2017).

Catalase assay

The CAT activity assay was performed using the Chance and Maehly (1995) method. Three ml reaction mixture containing 2.5 ml 0.05 mM sodium phosphate buffer (pH = 7), 30 μg protein solution was added to quettes. At the time of measurement, 30 μl 30% H2O2 was added to the reaction mixture and the absorbance at 240 nm, at 60 sec, and at 25 °C was recorded spectrophotometrically. The control contained 2.5 ml of sodium phosphate buffer and 30 μg protein. Catalase activity was reported based on absorption alternations per mg protein per min.

Peroxidase assay

Peroxidase activity was assayed adopting the method of Polle *et al.* (1994) and Aghighi Shahverdi *et al.* (2018). According to this method, POD activity was determined at 436 nm by its ability to convert guaiacol to tetraguaiacol ($\epsilon = 26.6$ mM^{-1}cm^{-1}). The reaction mixture contained 100 mM potassium phosphate buffer (pH = 7.0), 20.1 mM guaiacol, 10 mM H2O2 and enzyme extract. The increase in absorbance was recorded by the addition of H2O2 at 436 nm for 3 min.

Membrane stability index (MSI)

Membrane stability index was estimated by taking leaf samples in 10 ml of double-distilled water in two sets. One set was heated at 40 °C for 30 min in a water bath and measured for electrical conductivity (C1). The second set was boiled at 100 °C for 10 min before having its conductivity (C2) measured. MSI was calculated according to the formula (1) as described by Sairam (1994):

$$MSI = (1 - C1/C2) \tag{1}$$

Root colonization

To determine the percent of thyme root colonization by AFM treatment, Giovannetti and Mosse's modified method (1980) was used. The results showed that the root colonization average was above 40% in all treatments.

Statistical analysis

All data were analysed with Statistical Analysis System software (SAS Institute, Cary, NC, USA, Version 9.2), and the mean are significant differences were determined by LSD test at $p < 0.05\%$. The Pearson correlation coefficient was used to measure relationships between morph-physiological trains by using SAS software vr.9.2.

Results

Plant height

The effects of Cd, AFM, and Cd × AFM were significant (*p*=0.01) on plant height (Table 2). The highest plant height was achieved in the *F. mosseae* inoculation under no application Cd with an average of 49.4 cm, which was a 44.3% increase compared to the control treatment. Non-inoculation under the high Cd stress level (150 mg/kg) showed the lowest means of this trait (Table 3).

Table 2. Variance analysis (ANOVA) of the effect of AMF on the morpho-physiology characteristics of thyme (*Thymus vulgaris* L.) under Cd stress conditions

S.O.V	df	Mean square (MS)										
		Plant height	Number of leaves	Stem fresh weight	Stem dry weight	Root fresh weight	Root dry weight	MSI	Proline content	Soluble carbohydrate	POD activity	CAT activity
Cadmium (Cd)	2	417.6**	4604.3**	2436.7**	353.59**	2436.7**	417.7**	6.8**	0.1**	0.014**	19.37**	6.87**
AFM	2	124.6**	1285.3**	360.1**	98.54*	360.11**	124.7**	12.9**	0.021**	0.017**	27.49**	12.97**
Cd × AFM	4	8.61 **	49.1 **	7.8 **	13.77 **	7.77 **	8.60 **	0.14 ns	0.0008 ns	0.00016 ns	27.42**	0.14 ns
Erorr	54	0.34	4.00	1.00	0.33	1.00	0.34	0.39	0.003	0.0012	3.25	0.203
CV (%)	-	5.67	4.38	11.54	6.52	8.33	5.00	13.6	11.19	20.4	4.14	11.2

ns: non-significant; **: significant at 1% probably level

AMF: Arbuscular mycorrhizal fungi; MSI: Membrane stability index; POD: Peroxidase; CAT: Catalase

Table 3. Interaction AMF × Cd on morphological traits and POD activity of thyme (*Thymus vulgaris* L.)

Cd concentration (mg/kg of soil)	AMF treatment	Plant height (cm)	Number of leaves per plant	Stem fresh weight (g/plant)	Stem dry weight (g/plant)	Root fresh weight (g/plant)	Root dry weight (g/plant)	POD activity (U/mg protein.min)
Control	non-inoculated	27.49 d	163.1 e	87.3 d	14.16 d	95 d	17.29 d	43.6 i
	Funneliformis etunicatum	36.16 b	185.4 b	93.2 b	22.86 b	102 b	25.96 b	55.0 f
	Funneliformis mosseae	49.40 a	196.2 a	101.0 a	27.19 a	110 a	30.29 a	60.3 c
75	non-inoculated	24.84 e	158.3 f	81.7 f	11.56 e	90 f	15.26 e	47.6 h
	Funneliformis etunicatum	26.66 d	173.1 d	86.6 d	13.36 d	96 d	16.46 d	56.3 e
	Funneliformis mosseae	28.76 c	180.7 c	89.4 c	15.46 c	98 c	18.56 c	64.4 b
150	non-inoculated	20.66 h	146.8 h	71.4 j	7.36 h	80 k	10.46 hi	53.6 g
	Funneliformis etunicatum	22.26 g	153.6 g	76.7 h	8.96 g	85 h	12.06 g	59.3 d
	Funneliformis mosseae	23.86 f	160.3 ef	74.3 ij	10.56 f	83 ij	13.46 f	72.5 a

Means in each column followed by a similar letter (s), are not significantly different at 5% probability level, using LSD Test

Number of leaves

Cadmium stress, AMF, and interaction Cd × AMF treatments, as shown in Table 2 affected the leaves' number of thyme plants (*p*=0.01). The inoculation by *F. mosseae* under free Cd conditions showed the highest number of leaves (196.2 per plant). Non-inoculated treatment under 150 mg/kg Cd stress decreased number of leaves (9.99%) compared to the control treatment (Table 3).

Stem fresh and dry weight

Stem fresh and dry weight significantly affected by Cd, AFM, and Cd × AMF (Table 2). As shown in Table 3, a drastic decline in stem fresh and dry weight was observed due to Cd stress. On the other hand, the inoculation of AMF enhanced these parameters and ameliorated Cd stress-induced decline. Due to the inoculation of AMF (*F. mosseae*) under 150 mg/kg Cd, stem fresh and dry weight

were enhanced by 3.9 and 30.3%, respectively, compared to non-inoculated and 150 mg/kg Cd. The lowest of these traits related to the non-inoculated AMF under the high level of Cd stress (Table 3).

Root fresh and dry weight

Root fresh and dry weight was significantly affected by Cd, AMF, and Cd × AMF treatments (Table 2). Root fresh and dry weight decreased drastically due to Cd stress and however, AMF inoculation increased their traits and ameliorated the Cd-induced reduction. Cadmium stress (150 mg/kg + non-inoculated) reduced root fresh and dry weight by 15.7 and 39.5%, while in AMF (*F. mosseae*) inoculated Cd stressed (150 mg/kg + AMF) plants reduction was only 12.6 and 22.1% (Table 3).

Membrane stability index

As shown in Table 2, the effects of Cd and AMF were significant on MSI (p=0.01). Membrane stability index decreased in Cd stressed (150 mg/kg) plants. This reduction was 19.8% compared to the control treatment. Inoculated plants with AFM showed enhanced MSI compared to the non-inoculated treatment, this increase was 25.1% under *F. mosseae* application (Table 4).

Table 4. The effects of Cd and AMF treatments on some physiological traits of thyme (*Thymus vulgaris* L.)

Cd concentrations (mg/kg of soil)	MSI (%)	Proline content (μmol/g FW)	Soluble carbohydrate content (μg/g FW)	CAT activity (U/mg protein.min)
Control	5.03 a	0.47 c	0.147 b	3.50 c
75	4.65 b	0.51 b	0.177 a	4.12 b
150	4.03 c	0.59 a	0.192 a	4.50 a
AMF treatments				
Non-inoculated	3.77 c	0.49 c	0.145 b	3.24 c
Funneliformis etunicatum	4.89 b	0.52 b	0.177 a	4.36 b
Funneliformis mosseae	5.04 a	0.53 a	0.194 a	4.51 a

Means in each column followed by a similar letter (s), are not significantly different at 5% probability level, using LSD Test

Free proline content

Leaves free proline content affected by Cd stress and AFM inoculation (Table 2). Cadmium stress and AMF application increased proline content. In comparison to control, we achieved 20.3 and 7.54% increases in 150 mg/kg Cd stress and *F. mosseae* inoculated treatments (Table 4).

Soluble carbohydrate content

As shown in Table 2, the effects of Cd stress and AMF inoculated treatments were significant on the soluble carbohydrate content. The highest soluble carbohydrate content was related to the 75 and 150 mg/kg Cd levels (0.177 and 0.192 μg/g FW) and inoculated with *F. etunicatum* and *F. mosseae* (0.177 and 0.194 μg/g FW). Control treatment (free Cd stress and non-inoculated treatments) showed the lowest means of the trait (Table 4).

Peroxidase activity

As shown in Table 2, POD activity is affected by Cd, AMF, and Cd × AMF treatments (p=0.01). Results indicated that the Cd stress and AFM inoculation treatments increased POD activity. According to the interaction effect of Cd × AMF, the highest and lowest POD activity (72.5 and 43.6 U/mg protein.min) was related to the inoculation of *F. mosseae* under 150 mg/kg Cd levels and non-inoculated under free Cd stress conditions, respectively (Table 3).

Catalase activity

The effects of Cd stress and AFM treatments were significant on CAT activity (Table 2). As shown in Table 4, Cd stress and AFM inoculated enhanced CAT activity. This enzyme's highest activity was observed in 150 mg/kg Cd levels and inoculation with *F. mosseae* which, compared to the control treatment, showed 22.2 and 28.1% increases, respectively. Control treatments had the lowest activity (Table 4).

Correlation analysis

The results of the simple correlation (Pearson) presented in Table 5. Based on this table's results, morphological traits such as plant height, number of leaves, stem fresh and dry weight, and root fresh and dry weight were positively and significantly correlated. Among the physiological parameters, soluble carbohydrates had a positive and significant correlation with stem fresh and dry weight, root fresh weight, MSI, proline content, and activity of CAT and POD enzymes.

Table 5. Correlation coefficients among morph-physiological traits of thyme (*Thymus vulgaris* L.) under AMF and Cd treatments

	PH	NL	SFW	SDW	RFW	RDW	MSI	PC	SC	POD	CAT
NL	0.90**	1									
SFW	0.90**	0.94**	1								
SDW	0.97**	0.95**	0.94**	1							
RFW	0.91**	0.95**	0.96**	0.95**	1						
RDW	0.97**	0.94**	0.95**	0.95**	0.95**	1					
MSI	0.11ns	0.20ns	0.15ns	0.13ns	0.05ns	0.16ns	1				
PC	-0.19ns	-0.10ns	-0.24ns	-0.15ns	-0.11ns	-0.23ns	0.33*	1			
SC	0.12ns	0.15ns	0.36*	0.33*	0.27*	0.23ns	0.29*	0.54**	1		
POD	0.07ns	0.20ns	-0.11ns	0.04ns	-0.08ns	0.01ns	0.15ns	0.46*	0.16ns	1	
CAT	0.04ns	-0.12ns	-0.12ns	-0.22ns	-0.17ns	-0.22ns	0.31*	0.39*	0.19ns	0.58**	1

-1	-0.9	-0.8	-0.7	-0.6	-0.5	-0.4	-0.3	-0.2	-0.1	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1

High negative correlation   Non-correlation   High positive correlation

ns: non-significant; * and ** significant at 5 and 1% probably levels, respectively
AMF: arbuscular mycorrhizal fungi; Cd: cadmium; PH: plant height; NL: number of leaves; SFW: stem fresh weight; SDW: stem dry weight; RFW: root fresh weight; RDW: root dry weight; MSI: membrane stability index; PC: proline content; SC: soluble carbohydrate; POD: peroxidase activity; CAT: catalase activity

Discussion

Cadmium stress has been shown to induce many morpho-physiological modifications in plants, which is dependent upon plant species, organ/tissue, the concentration of metal, and exposure period (Abdelhameed and Metwally, 2019). Cd stress drastically reduced growth parameters such as plant height, number of leaves, stem fresh and dry weight, and root fresh and dry weight in the current study. Heavy metals such as Cd appear to inhibit plant growth in a variety of ways. On the one hand, heavy metals, by reducing cell turgor, reduce cell division and inhibit cell growth, and on the other hand, by accumulating in the cell wall and entering the cytoplasm and disrupting the cell's natural metabolism, they lead to reduced growth (Abdelhameed and Metwally, 2019). An examination of the cabbage plant showed that by increasing Cd's concentration in the plant environment, the weight of the plant was reduced (Ali *et al.*, 2015).

In the current study, Cd stress decreased MSI. The first sign of Cd oxidative stress is the proliferation of lipid cell membranes, which is followed by an increase in lipid peroxidation, destroyed cell membranes, and ion leakage, and then a decrease in MSI and an increase MDA, which are two indicators for measuring damage to biological membranes (Siddhu and Ali, 2012).

In our results, as Cd concentrations in the soil increased, leaf proline levels increased significantly, corresponding to the results of Dinakar *et al.* (2008) in peanuts, Zhao (2011) in wheat and corn. Plants exposed to adverse environmental conditions show enhanced synthesis and accumulation of osmolytes which have an important role in maintaining growth under stressed conditions (Alqarawi *et al.*, 2014). Proline is among the important osmolytes involved in the maintenance of tissue water content. In the current study, an increase in proline accumulation due to Cd stress was obvious and a further increase caused by AMF inoculation confirms the role of AMF in strengthening the stress tolerance mechanisms in plants. Under stress conditions, proline synthesizing enzymes' activity is upregulated while its catabolism is lowered (Hashem *et al.*, 2014). Proline and other osmolyte accumulation such as soluble carbohydrate help plants to maintain cellular water potential well below that of the soil solution. Our results of proline accumulation due to Cd stress are in agreement with the results of Irfan *et al.* (2014), Abd_Allah *et al.* (2015), and Hashem *et al.* (2016) in *Brassica juncea*, *Helianthus annus* L. and *Cassia italica* Mill, respectively. Enhancement in proline in our study supports the role of proline in maintaining growth under stress conditions. An increase in proline due to AMF inoculation is in corroboration with the results of Shekoofeh *et al.* (2012) and Hashem *et al.* (2016). Proline has a protective role for protecting membranes and other cellular molecules like enzymes and neutralizes toxic ROS, therefore contributing to better growth under stress conditions (Irfan *et al.*, 2014). By reducing water transfer to the leaves and slowing down the transpiration rate, Cd changes key enzymes' behaviour in the metabolism of sugars and increases the amount of soluble carbohydrates in the cell.

The coexistence of AMF with roots by absorbing water and nutrients increases photosynthesis, leading to more crop production and improved growth (Begum *et al.*, 2019). On the other hand, AMF by increases soil health parameters (soil moisture, fertility levels, and soil quality), nutrients uptake and regulation of Aquaporin gene (AQP), ABA-responsive gene, phytohormone biosynthesis pathways, and Transcription factors were improved stress tolerance (Begum *et al.*, 2019). These results are consistent with other researchers' findings on the positive effect of AMF in increasing plant dry matter (Wu and Xia, 2006). In another report, inoculation of plant roots of dill and cumin with two types of AMF significantly increased their aerial parts' dry weight compared to non-mycorrhizal plants (Kapoor *et al.*, 2004).

In general, AMF prevents carbohydrate intake by increasing the soluble sugars, and by maintaining the osmotic pressure and water content of the leaves, they lead to cell growth and increase plant growth (Yooyongwech *et al.*, 2013). Improved plant growth and production in AMF-associated plants under stress have been linked to the optimization of biochemical changes (Miransari, 2010). Yooyongwech *et al.* (2013) stated the AMF regulates growth under stress conditions by modifying proline and sugar contents. Cordero *et al.* (2004) showed that the levels of fructose, α-glucose, β-glucose, sucrose, as well as the total sugar content in peppers coexisting with AFM, were significantly higher than control plants (non-AFM). Another reason for these fungi' effect on increasing soluble carbohydrate content is to increase the levels of plant hormones such as cytokinin and gibberellin in inoculated plants. Increased levels of these hormones, especially cytokinin, can increase photosynthesis rate and ultimately increase the carbohydrate content of plants by transferring ions that are effective in opening the pores and regulating chlorophyll levels (Chanclud and Moral, 2016).

In the current study, the colonization of AFM to thyme plant in the Cd stress conditions was significantly higher than that in the free Cd stress conditions. Zhang *et al.* (2018) reported that the root with AMF inoculation often enhances phosphorous absorption and plant growth by tapping a larger soil volume than that without inoculation for the relatively immobile phosphorous solubilizing normally insoluble phosphorous sources through the excretion of various organic acids. Meanwhile, the higher phosphorous absorption by plants upon AMF inoculations also seemed to be due to the elevation of soil phosphatase activity, which may involve AMF directly and indirectly: AMF propagules can synthesize such enzymes, and

mycorrhizal roots may release more root exudates containing enzymes due to the improved nutrition and/or larger root system (Hu *et al.*, 2014). As a result, the observed large increases in the phosphorous acquisition and thus plant biomass of sunflower following both inoculation treatments were mostly due to the enhanced root mycorrhizal colonization rate and soil phosphatase activity (Zhang *et al.*, 2018).

Abdelhameed and Metwally (2019) reported plants had developed a series of mechanisms to cope with Cd pollution, among which AMF is considered an effective strategy to alleviate Cd phytotoxicity. Cadmium can be immobilized in the fungal hyphae of internal and external origin that can fix Cd in the cell wall and store them in the vacuole or may chelate with some other substances in the cytoplasm and hence reduce Cd toxicity in the plants (Punamiya *et al.*, 2010; Begum *et al.*, 2019). Li *et al.* (2016) indicated that AMF was very effective in lowering Cd levels, which brought about Cd detoxification in rice plants.

The strong effects of AMF on plant development and growth under heavy metals stressful conditions are most often due to the ability of these fungi in increasing morphological and physiological processes that increase plant biomass and consequently uptake of important immovable nutrients (notably P) and thus reduced metal toxicity in the host plants (Kanwal *et al.*, 2015; Miransari, 2017). On the one hand, AM fungi can make a considerable contribution to nutrient (notably phosphorus) uptake to promote plant growth. Besides, AMF can alleviate heavy metals toxicity on plants in polluted soil by the reduction of heavy metal acquisitions, the biological dilution of heavy metals, and the decrease of oxidative stress (Neagoe *et al.*, 2014; Zhang *et al.*, 2018).

Antioxidant enzymes play an important role in scavenging ROS and averting the oxidative stress that prompted numerous sensitive molecules' damaging effects. In the present study, results showed an increase in CAT and POD activities of thyme with Cd application. These results are in good agreement with those of Chaturvedi *et al.* (2018) and Abdelhameed and Metwally (2019) that can be attributed to ROS's overproduction overexpression of genes coding for antioxidant enzymes. This result, which increases with increasing stress on the activity of antioxidant enzymes, indicates the effectiveness of the antioxidant system in plant protection and is compatible with the results obtained by Nas and Ali (2018) as a study the of effects of lead and Cd the growth and activity of some spinach enzymes. The highest activity of CAT enzyme was obtained in soil inoculation with *F. mosseae* mycorrhizal fungus, which was significantly higher than other treatments, and the lowest activity was observed in the soilless treatment of fungi (control) which is consistent with the results of Tan *et al.* (2015) on two species of *Solanum hoteinocarpum*.

Our result indicated that the application of AFM enhanced CAT and POD activities. In this regard, in a study conducted by Dehghani *et al.* (2017), AFM-corn had significantly higher CAT activity than non-AFM. The present study results are consistent with the results of Nareshkomar *et al.* (2015) on peanut plants.

The results indicate that these AMF strains mediate different tolerance strategies to alleviate Cd toxicity in their host plants and that inoculation with both AMFs can be used for Cd phytoextraction. In contrast, this *F. mosseae* strain can be useful for Cd of contaminated soil. The present study results showed that in the comparison between the two fungi, *F. mosseae* had a higher efficiency in increasing morphological traits and improving physiological traits compared to *F. etunicatum*.

Conclusions

In conclusion, Cd stress affected growth and morpho-physiological characteristics in the thyme plant. This study's overall results showed that the presence of Cd in the culture medium affected the growth and morphological characteristics of thyme and reduced growth and disruption of various physiological processes in plant structure. The increased activity of antioxidant enzymes in this study could reduce the production of reactive oxygen species (ROS) that can inhibit growth by creating oxidative stress. In fact, by increasing the CAT and POD enzyme effect, especially at a concentration of 150 mg/kg Cd and the loss of H_2O_2 in the plant, the lipid peroxidation index decreases. Also, thyme inoculation with AMF in terms of vegetative and

morphological traits such as plant height, number of leaves, stem fresh and dry weight, and root fresh and dry weight has reduced Cd stress. AMF inoculation (especially *F. mosseae*) mitigated Cd stress's damaging impact by reducing the enhancing the antioxidant activity, proline, and soluble carbohydrate contents and lipid peroxidation. The present study strongly supports employing AMF (especially *F. mosseae*) as the biological fertilizer for enhancing the Cd stress tolerance of thyme plants.

Authors' Contributions

RR collected and analyzed the data used in manuscript, and drafted the manuscript. SAH and BE supervised the project. AE and GS Edited final approval of the version to be submitted. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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