J. Crop Prot. **2021, 10 (4): 669-684**

Research Article

The morphological and physiological traits of *Cucumis sativus*-*Phelipanche aegyptiaca* association affected by arbuscular mycorrhizal fungi symbiosis

Nayerehalsadat Hosseini Faradonbeh[1], Ebrahim Izadi Darbandi[1*], Hassan Karimmojeni[2] and Ahmad Nezami[1]

1. Department of Agrotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan, Iran.

Abstract: The plant symbiotic fungi, Arbuscular mycorrhizae (AM), increases host competency and causes partial control of Egyptian broomrape *Phelipanche aegyptiaca* (Orobanchaceae). In this study, a greenhouse experiment was designed to investigate the AM efficacy on the morphological and physiological traits in the association of cucumber and *P. aegyptiaca*. Findings showed that the broomrape contamination increased the activity of ascorbate, peroxidase, and catalase in cucumber. In contrast, AM decreased ascorbate, peroxidase activity and increased total phenolic compounds. However, AM in *P. aegyptiaca*-infected genotypes had no significant effect on malondialdehyde and hydrogen peroxide content. In AM inoculated treatments, the height and number of cucumber leaves were unaffected by *P. aegyptiaca* infestation. Also, AM decreased the harmful effects of the *P. aegyptiaca* by reducing the total dry weight and number of attachments, increasing the leaf area, the shoot, and the dry root weight of cucumber genotypes. Despite the positive effect of AM, about 35 and 50% reduction in shoot and dry root weight of cucumber indicated high susceptibility of the host. Overall, It seems that the AM cannot be effective as a primary broomrape control strategy in cucumber.

Keywords: antioxidative upregulation, host susceptibility, phenolic, nonchemical management, boomrape

Introduction

Broomrape *Phelipanche aegyptiaca* is the holoparasite of Dicotyledoneae. Because of the specific biology of broomrape, the most damage to the host plant occurs before the emergence of the broomrape stem on soil surfaces. According to the reports, a severe broomrape infection can cause 50-100% yield loss of sensitive host plants (Goldwasser *et al.,* 2003, Samejima and Sugimoto, 2018). Broomrape also has important hosts in Cucurbitaceae like melons and cucumber (Joel *et al*., 2013).

Like other stresses and based on host susceptibility and resistance, the parasites change the activity of the antioxidant system of the infected host. All forms of reduced oxygen, including superoxide ($O_2^{\circ -}$) hydroxyl radical (°OH) and H_2O_2 are known as Reactive Oxygen Species (ROS). These species are made under stress conditions and initiate plant defense

Handling Editor: Eshagh Keshtkar

* Corresponding author: e-izadi@um.ac.ir
Received: 01 August 2020, Accepted: 10 November 2021
Published online: 11 December 2021

reactions. The accumulation of reactive oxygen species leads to oxidative stress that can damage cell components such as membranes, proteins, and DNA (Hsieh *et al*., 2002). The hydrogen peroxide molecule is more dangerous among the reactive oxygen species because it can pass through the membrane and reach intracellular organelles (Verma and Dubay, 2003). The increase of this substance can be measured as an indicator of oxidative stress in stress conditions. Hydrogen peroxide (H_2O_2) accumulation in response to infestation by Egyptian broomrape has also been reported (Pérez-de-Luque *et al*., 2006; Mabrouk *et al*., 2007). The balance between H_2O_2 production and elimination is essential for plant survival. On the other hand, the accumulation of ROS in the host also reflects the reaction of the plant to the presence of the parasitic plant, and faster accumulation of H_2O_2 can mean more rapid initiation of defense response and subsequent more resistance (Mor *et al*., 2008; Torres *et al*., 2010). To counteract oxidative stress, the plant subjected to stress adopts two different defense mechanisms: enzymatic, including catalase, peroxidase, superoxide dismutase, glutathione reductase, glutathione peroxidase (Hsieh *et al*., 2002) and non-enzymatic mechanisms such as flavonoids and anthocyanins, carotenoids, vitamin E, and alkaloids (Shahid, 2014) to neutralize free radicals and prevent them from damaging the cell. Among the effective enzymes, catalase serves as a high-efficiency catalyst with high energy use efficiency and is superior to other enzymes (Sharma, 2013). The literature emphasizes the importance of these enzymes in coping with stress conditions. However, severe stresses cause irreversible damage to these enzymes activity (Youssef and Azooz, 2013; Bocova *et al*., 2012). According to the findings of Gonzalez-verdejo *et al.* (2006), the exogenous application of catalase due to H_2O_2 degradation inhibits the parasite weed growth. The researchers found that catalase-increased activity inhibited elongation of the root of the parasite. Various literature has reported an increase in the phenolic acid content of the infected hosts. In *Vicia* sp. and *Petroselinum* sp., an increase in the phenolic compounds content has been observed in treatments infected with Egyptian broomrape (Goldwasser *et al*. 2000, 2002). Pérez-de-Luque *et al*. (2005a, b) also reported increased total soluble phenolic compound content in some chickpea genotypes. Peroxidase such as guaiacol peroxidase also had higher activity in resistant chickpea genotypes. Gonzalez-Verdejo *et al*. (2006)-noted that increased peroxidase activity in the root of the parasite causes faster host infestation and overcomes ROS accumulation at the infestation site. These researchers pointed out that peroxidases play an essential role in producing extracellular ROS to destroy the host cell wall, which accelerates the elongation of the parasite's root.

The germination in chemo-parasites such as broomrape is induced by chemical signals of the host root. Strigoles (SLs) are the most important chemical signals. This chemical compound group acts as the plant hormones, and chemical signals in the rhizosphere are stimulants of symbiotic microorganisms and parasitic plants (Boyer *et al.,* 2013). In addition to the induction of broomrape germination, SLs have other functions at low concentrations, like hyphal branching of AM, growth of plant pathogens, and asymmetric growth of root (Brewer, 2013; Boyer, 2013). The fact that strigolactones play an important role for parasites and AM allows for using the symbiotic potential as a management strategy. Umehara *et al.* (2008) showed that AM colonies could decrease infection in the maize-striga association. Also, AM-inoculated chickpea had less ability to stimulate broomrape seed germination by reducing the secretion or production of strigolactones in mycorrhizal plants (Steinkellner *et al.,* 2007). Reduced output of strigolactones in mycorrhizal plants has also been demonstrated in tomatoes. Plants can take advantage of other benefits of AM, such as increasing plant competence and tolerating biotic and abiotic stresses (Al-Karaki, 2006, Ortas *et al.*, 2001) According to the reports, AM inoculation has caused a yield increase and a decrease in chemical fertilizer (Ortas, 2003, 2010). Cagras *et al.* (2000) used *Glomus mosseae* and *G. fasciculata* spores to inoculate cucumber. They found that the inoculated plants have higher P, Zn, and Mn absorption. *G. caledonium, G. etunicatum, G. clarum,* and *G. mosseae* inoculation in cucumber significantly increased the seedling survival, the yield, and the concentration of zinc and phosphorus (Ortas, 2010).

In this research, the effect of AM on two cucumbers genotypes and their interaction with Egyptian broomrape was investigated to get a better understanding of the possible effect of AM as a broomrape nonchemical control method.

Materials and Methods

The greenhouse experiment was conducted in a completely randomized design with two cucumber cultivars (Khassib and Argeto) and four replications at the Isfahan University of Technology, Iran (32°43′ E, 51°31′ N) from April to June 2017. Treatments included 1) the cultivation of each cultivar without any treatment as the control 2) the cultivation of cultivars infected with *P. aegyptiaca* 3) the cultivation of cultivars inoculated with AM, and 4) the cultivation of cultivars inoculated with AM and infected with *P. aegyptiaca*.

Cucumber cultivars Khassib is generally used in the greenhouse, but, Arego is an outdoor cultivar. Two-thirds of pots (30cm height and 25 cm diameter) were filled with farm soil, and then, according to El-Halmouch *et al.*(2006), 50 mg of *P. aegyptiaca* seeds were mixed into the soil. Before sowing the cucumber seed, the inoculation of AM was done by adding 15 g of the soil containing AM spores to appropriately 2 cm around the cucumber seed.

The *P. aegyptiaca* seeds were gathered from infected tomato fields. Commercial soil containing 50 to 100 spore.g soil^{-1} of *Glomus mossea* (27%), *G. intraradi* (27%), *G. hoi* (26%), *G.etanicatum* (3%), *G.clarideum* (3%), *G. versiform* (3%), *G. fasicultum* (3%), *G. caledonium* (3%), *G. acalospora longula* (3%) and *G.margarita* (2%) were used as AM treatments. The environmental conditions of the greenhouse were set at 25/15 °C as the day/night temperature, 65-75% relative humidity, and 300 µmol m^{-2} s^{-1} photosynthesis active rate. During growth season and after the emergence of one *P. aegyptiaca* spike in 90% of pots, a well-grown leaf was taken and kept at -80 °C for physiological traits measurements.

Traits measurement

Analysis of lipid peroxidation

Zhou and Leul (1998) method was used to determine malondialdehyde (MDA) content. The leaves were selected from upper two-thirds of the plant. Leaf samples (0.2 g) were homogenized and extracted in 10 ml solutions of 0.25% $S_2O_2N_4H_4C$ (thiobarbituric acid (TBA)) and 10% Cl_3CCOOH (trichloroacetic acid (TCA)). The extract was heated in a water bath at 95 °C for 30 minutes and immediately cooled down on the ice. After centrifugation at 5000 g for 10 min, the absorbance was measured at 532 and 600 nm (subtracted for correction of non-specific turbidity). The MDA content was expressed as µmol g^{-1} FW using an extinction coefficient of 155 mM.cm^{-1}.

H_2o_2 analysis

H_2O_2 was measured by the method of Velikova *et al.* (2000). Fresh samples (0.2 g) were extracted with 5 ml of 0.1% TCA (w/v), placed in an ice bath, and centrifuged at 12000 g for 15 min at 4 °C. Then 0.5 ml of 100 mM phosphate buffer (pH 7.0) and one ml of 1 M potassium iodide were added to 0.5 ml of the supernatant. The absorbance was read at 390 nm, and a standard curve was used to calculate the H_2O_2 content.

Enzyme activities

About 0.5 g of fresh samples were homogenized in 8 ml of 50 mM K_3PO_4 (potassium phosphate buffer) pH 7.8 in ice-cold mortars for enzyme analysis and centrifuged at 14000 g at 4 °C for 30 min. The obtained supernatant was used for further biochemical analysis (Nakano and Asada, 1981). Peroxidase (POX-EC 1.11.1.7 extinction coefficient = 26.61 mM^{-1} cm^{-1}) activity was determined according to the method of Zhou and Leul (1998). The reaction mixture (3 ml) was composed of 50 mM potassium phosphate buffer pH 7.0, 1% guaiacol, 0.4% H_2O_2 and 100 µl enzyme extract. Variation in absorbance because of oxidation of guaiacol was assayed spectrophotometrically (U-1800 UV/VIS, Hitachi, Japan) at 470 nm. POX activity was expressed as a unit per milligram of protein (Herzog and Fahimi, 1973). One unit of POX activity indicates the amount of enzyme that catalyzes the oxidation of 1.0 µM of guaiacol in 1 min.

Catalase activity (CAT-EC1.11.1.6, extinction coeffient. = .39.4 mM^{-1} cm^{-1}) was assayed by measuring the degradation of H_2O_2 for 1 min at 240 nm (Aebi, 1984). Reaction mixture (3 ml) contained 50 mM K_3PO_4 buffer (pH7.0), 2 mM EDTA-Na_2, 10 mM H_2O_2 and 100 µl enzyme extract. CAT activity was expressed as units per mg of protein (Chance and Maehly, 1955). The amount of CAT required to decompose 1.0 µM of H_2O_2 per min was defined as one unit of CAT activity.

Determination of ascorbate peroxidase (APX, EC1.11.1.11, extinction coefficient = 2.8 mM^{-1} cm^{-1}) activity was measured in a reaction mixture (3 ml) containing 100 mM phosphate buffer (pH 7.0), 0.1 mM EDTA-Na_2, 0.3 mM ascorbate, 0.06 mM H_2O_2 and 100 µl enzyme extract. The change in absorption was read at 290 nm for 1 min after the addition of H_2O_2 (Nakano and Asada, 1981). APX activity was expressed as a unit per mg of protein (Herzog and Fahimi, 1973). One unit of APX activity represents the amount of the enzyme that catalyzes the oxidation of 1.0 µM of ascorbate in one minute.

Total phenolic compounds

Total phenolic compounds were measured by the method of Kofalvi & Nassuth (1995). Fresh leaf (0.1 g) was homogenized by 5 ml ethanol 95% after putting at 25 °C for 24 h, 1 ml ethanol 95%, 4 ml distilled water, 1 ml $NaCO_3$, and 0.5 ml Folin's reagent were added to 1 ml of the sample, and then phenolic content was measured by p-Coumaric acid as a standard at 725 nm.

Morphological traits

In addition to the mentioned traits, shoots and roots dry weight of cucumber were measured at the end of the experiment and after plant ripening. For this purpose, the plants were separated from the crown, placed in appropriate pockets, and then dried in the oven at 70 °C and weighted after four days. Before drying the cucumber roots, the *P. aegyptiaca* necrotic nodes and attachments were isolated in infected treatments. Also, the total attachment number.$plant^{-1}$ (TAN), and attachment dry weight (g). $plant^{-1}$ (ADW) were measured as the *P. aegyptiaca* traits. For this purpose, the root washing method in a fine-mesh sieve was used. The total dry weight of *P. aegyptiaca* attachments was also measured after drying the entire parasite attachment by the technique used for the dry weight of cucumber genotypes.

Data analysis

For data analysis, generalized linear models employed in PROC GLIMMIX of SAS (version 9.4; SAS Institute, Gary, NC). The least squared means (LSMEANS) was used to compare means at the 5% level of significance according to Fisher's least significant difference (Fisher's LSD). According to the Shapiro-wilk test, no statistical transformation was necessary.

Results

Physiological traits

Malondialdehyde (MDA)

Analysis of variance of the relevant data showed that the main effect of the treatments, the effect of the cultivar, and the interaction of treatment and cultivar on the content of malondialdehyde were significant (Table 1).

Malondialdehyde content was higher in *P. aegyptiaca* infected treatments than noninfected treatments (Fig. 1.a). However, the effect of mycorrhiza on malondialdehyde content in treatments was not significant. The highest and the lowest amounts of malondialdehyde was found in Argeto cultivar infected by *P. aegyptiaca*e without AM inoculation (7.52 $\mu mol.g^{-1}$) and Khassib cultivar inoculated by AM and no broomrape infection (2.98 $\mu mol.g^{1}$).

Table 1 The effect of arbuscular mycorrhizal fungi (AMF) and infection of *Phelipanche aegyptiaca* on cucumber physiological traits.

Infection of *P. aegyptiaca*	AMF-inoculation	Malondialdehyde (μmol.g^{-1})		H_2O_2 (μmol.g^{-1})		Ascorbate peroxidase (Unit mg^{-1} protein)		Catalase (Unit mg^{-1} protein)		Phenol (mg.g^{-1})	
		Khassib	Argeto	Khassib	Argeto	Khassib	Argeto	Khassib	Argeto	Khassib	Argeto
Yes	Yes	7.18	5.63	5.66	5.14	0.22	0.38	0.32	0.32	1.33	1.15
	No	5.50	7.52	7.19	4.42	1.58	0.13	0.47	0.15	0.85	1.12
No	Yes	2.98	3.74	2.28	1.83	0.09	0.02	0.19	0.14	1.09	0.86
	No	3.29	4.39	2.37	1.94	0.22	0.05	0.03	0.02	0.77	0.90
Treatment (T)		LSD (5%) = 0.72**		LSD (5%) = 1.28**		LSD (5%) = 0.07**		LSD (5%) = 0.05**		LSD (5%) = 0.01**	
Cultivar (C)		LSD (5%) = 0.51*		LSD (5%) = 0.91*		LSD (5%) = 0.05 **		LSD (5%) = 0.04*		N. S	
T × C		LSD (5%) = 1.02**		N.S		LSD (5%) = 0.10**		LSD (5%) = 0.07**		LSD (5%) = 0.03**	
CV (%)		13.91		32.38		20.88		22.96		1.70	

*,**: Indicate significant difference at the level 0.05 and 0.01, respectively.
NS: Non significantly different.

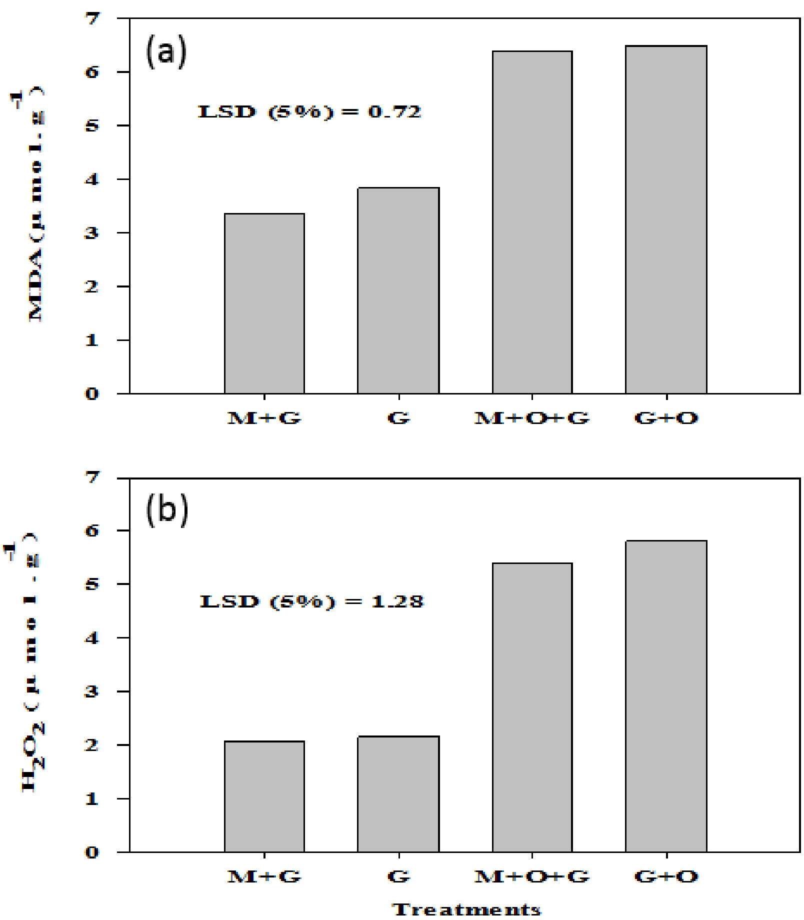


Figure 1 Effect of treatments on a) MDA b) H_2O_2 content of cucumber genotypes. M + G = Effect of AM inoculation on cucumber cultivars, G = cucumber cultivars, M + O + G = Effect of AM inoculation and *P. aegyptiaca* infection on cucumber cultivars, G + O = Effect of *P. aegyptiaca* infection on cucumber cultivars, MDA: Malondialdehyde. AM: *Arbuscular mycorrhiza*

Hydrogen peroxide (H_2O_2)
Like MDA, AM inoculation had no significant effect on hydrogen peroxide content either in *P. aegyptiaca* infection or in no broomrape infection treatment (Fig. 1.b). Accordingly, the highest amount of hydrogen peroxide (5.80 µmol.g^{-1}) was observed in broomrape-infected cultivars, with no significant difference between broomrape-infection and AM inoculation (5.40 µmol.g^{-1}) (Table 1).

Ascorbate peroxidase
There was a significant difference between the specific activity of the ascorbate peroxidase in the treatments with and without broomrape-infection (Table 1). Infection with the Orobanche increased the enzyme activity significantly (Fig. 2. a). The mean of enzyme activity was 0.86 and 0.13 (Unit mg^{-1}protein), in broomrape unninfected and infected treatments respectively,. Also, AM inoculation in the treatments decreased the specific activity of ascorbate peroxidase. The enzyme-specific activity in treatments was 0.13 and 0.05 (Unit mg^{-1}protein), respectively. Broomrape-infected Khassib cultivar with AM inoculation had the highest enzyme-specific activity (1.58 Unit mg^{-1}protein) and also the lowest enzyme activity belonged to noninfected Argeto cultivar with AM inoculation (0.02 Unit mg^{-1}protein).

Catalase
Contamination with *P. aegyptiaca* caused a significant increase in catalase-specific activity. However, this increase was not affected by AM inoculation (Table 1). The enzyme activity in *P. aegyptiaca* infected treatments was 0.32 µmol.g^{-1} (with AM inoculation) and 0.31 µmol.g^{-1}, (without AM inoculation) respectively (Fig. 2.b). Also, in treatments without *P. aegyptiaca* infection, AM inoculation increased catalase-specific activity. In general, the lowest and the highest catalase-specific activity was related to Argeto cultivar without AM inoculation and *P. aegyptiaca* infection (0.02 µmol.g^{-1}) and broomrape -infected Khassib cultivar without AM inoculation (0.47 µmol.g^{1}).

Total phenolic compounds
There was no significant difference between the content of total phenolic compounds in the two cultivars. The difference between treatments was significant. According to Table 1, the highest mean of total phenolic compounds was observed in AM inoculation and *P. aegyptiaca* infection in both cucumber cultivars (1.24 mg.g^{-1}). In no AM inoculation and broomrape infection treatment, the mean of total phenolic compounds was 0.84 mg.g^{-1} (Fig. 2.c).

Cucumber morphological traits
The effect of AM and infection of *P. aegyptiaca* on morphological traits are summarized in Table 2. Cucumber height was significantly affected by the cultivars and the treatments (Fig. 3.a). Broomrape infection caused a significant decrease in the height of the Khassib and the Argeto cultivars. AM inoculation had no significant effect on broomrape-infected and noninfected treatments. Therefore, its application had no improving effect on reducing the broomrape effect on cucumber height.

Leaf number was also significantly affected. There were significant differences between Khassib genotype with an average of 15 and Argeto cultivar with 10.60 leaves per plant. The highest number of leaves was observed in treatments that tomato cultivars inoculated with AM and uninfected with *P. aegyptiaca* (M + G) in wich have no significant difference with uninaculated AM tratments (14) (GAM inoculation did not reduce *P. aegyptiaca* damage to leaf number, and the mean of both treatments with and without AM inoculation was 10.62 leaves per plant (Fig. 3.b).

However, the interaction between genotype and treatment on leaf area was not significant, this trait was significantly affected by treatments and cultivars. Application of mycorrhiza increased the leaf area so that the leaf area was the highest in AM inoculation and no broomrape infection treatment (13634.42 mm^2). The leaf area in infected treatments with *P. aegyptiaca* but inoculated with AM (M + O + G) was 6719.05 mm^2, which no significance different with infected *P. aegyptiaca* treatments but AM uninoculated plants (G + O) treatment (5796.9 mm^2). According to the results, the lowest leaf area was also observed in genotype with *P. aegyptiaca* infection and no AM inoculation (4159.95 mm^2) (Fig. 3.c).

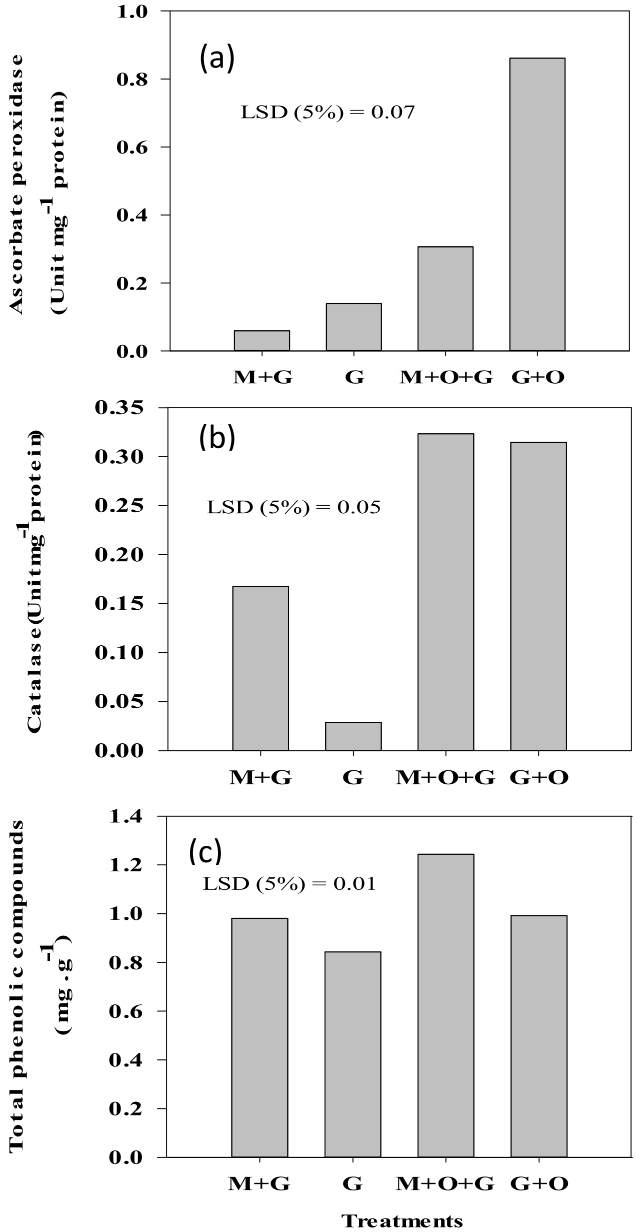


Figure 2 Effect of different treatments on a) Ascorbate proxidase b) Catalase specific activity and c) total phenolic compounds content of cucumber cultivars.

M + G = effect of AM inoculation on cucumber cultivars, G = cucumber cultivars, M + O + G = effect of AM inoculation and *P. aegyptiaca* infection on cucumber cultivars, G + O = effect of *P. aegyptiaca* infection on cucumber cultivars.

AM: *Arbuscular mycorrhizae*.

Table 2 Effect of arbuscular mycorrhizal fungi (AMF) and infection of *Phelipanche aegyptiaca* on cucumber morphological traits.

Infection of *P. aegyptiaca*	AMF inoculation	Height (cm)		No. of leaves		Leaf area (mm^2)		Root dry weight (g)		Shoot dry weight (g)	
		Khassib	Argeto	Khassib	Argeto	Khassib	Argeto	Khassib	Argeto	Khassib	Argeto
Yes	Yes	40.88	66.73	12.00	9.25	5956.29	5637.5	0.97	1.14	6.86	6.27
	No	21.64	63.73	13.25	8.00	3705.70	4614.2	0.52	0.35	5.22	4.65
No	Yes	86.58	95.60	19.25	12.75	12776.63	14492.2	3.28	2.87	12.35	12.94
	No	73.35	98.07	15.50	12.50	5844.62	7593.5	1.85	2.31	10.15	9.71
Treatment (T)		LSD (5%) = 13.40 **		LSD (5%) = 2.31 **		LSD (5%) = 1165.35 **		LSD (5%) = 0.53 **		LSD (5%) = 1.45 **	
Cultivar (C)		LSD (5%) = 9.46 **		LSD (5%) = 1.63 **		LSD (5%) = 824.02 *		N.S		N.S	
T × C		N.S		N.S		N.S		N.S		N.S	
CV(%)		18.99		17.52		14.90		31.12		16.50	

*,**: Indicate significant difference at the level 0.05 and 0.01, respectively.
NS: Non significantly different.

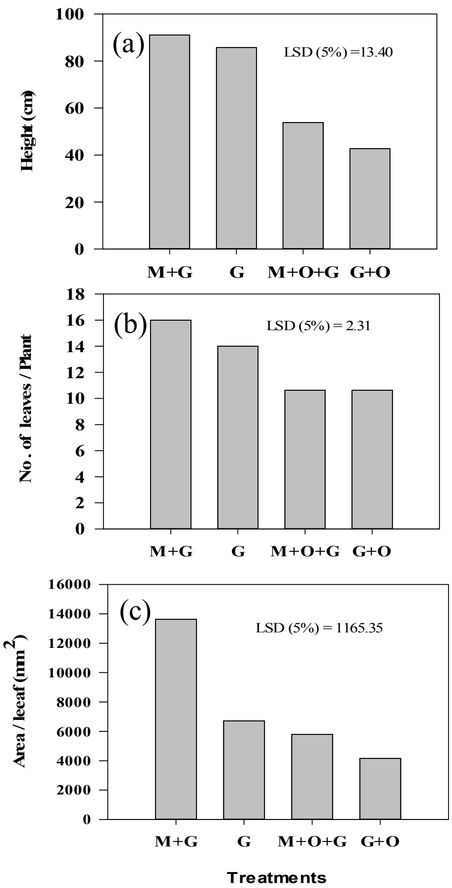


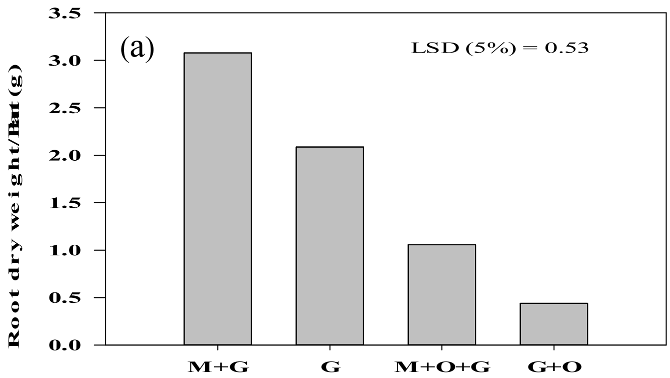
Figure 3 Effect of different treatments on a) Height b) Leaf No. c) Leaf area of cucumber genotypes.
M + G = effect of AM inoculation on cucumber cultivar, G = cucumber cultivars, M + O + G = effect of AM inoculation and *P. aegyptiaca* infection on cucumber cultivars, G + O = effect of *P. aegyptiaca* infection on cucumber cultivars.
AM: *Arbuscular mycorrhiza.*

There was a significant difference between the root dry weight of different treatments. The highest mean of root dry weight in both genotypes was 3.07 g in AM inoculation and no broomrape infection treatment. (Fig. 4.a) The lowest root dry weight was observed in broomrape infected genotype without AM inoculation in Argeto and Khasib cultivars (0.43 g). Shoot dry weight was also affected by different treatments (Table 2). Accordingly, the highest shoot dry weight was related to AM inoculation in each cultivar without *P. aegyptiaca* infection with an average of 12.65 g. Shoot dry weight mean in cucumber cultivars without AM inoculation, and broomrape infection was 9.93 g. Application of AM in both cultivars reduced the effect of broomrape infection on dry shoot weight (6.54 g). Shoot dry weight in the broomrape infected treatments without AM inoculation was 4.93 g, significantly different from other treatments (Fig. 4.b).

Broomrape traits

Arbuscular mycorrhizal fungi application effect was significant in both measured traits in broomrape (Table 3). AM inoculation reduced the total attachment number. Plant^{-1} of broomrape in both genotypes. Total attachment number. Plant^{-1} in the soil was 15.75 in no AM inoculation treatment vs. 10.25 in AM inoculation treatment.

The dry weight of broomrape was 0.62 g in AM inoculation and 0.81 g per plant in no AM inoculation, respectively.



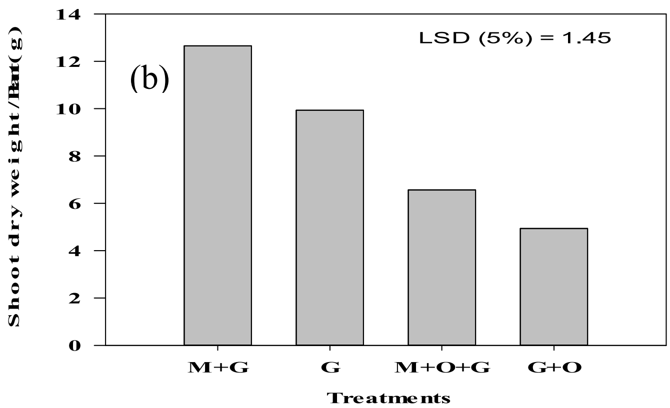


Figure 4 Effect of different treatments on a) Root dry weight b) Shoot dry weight of cucumber genotypes.
M + G = effect of AMF inoculation on cucumber cultivars, G = cucumber cultivars, M + O + G = effect of AMF inoculation and *P. aegyptiaca* infection on cucumber cultivars, G + O = effect of *P. aegyptiaca* infection on cucumber cultivars.
AM: *Arbuscular mycorrhizae*.

Table 3 Effect of arbuscular mycorrhizal fungi (AMF) on *Phelipanche aegyptiaca* traits.

Infection of *P. aegyptiaca*	AMF inoculation	No. of total attachment/plant			Total attachment dry weight./plant (g)		
		Kkhassib	Argeto	Mean[1]	Khassib	Argeto	Mean[1]
Yes	Yes	10.75	9.75	10.25[b]	0.60	0.64	0.62[b]
	No	17.75	13.75	15.75[a]	0.76	0.86	0.81[a]
No	Yes	----	----	----	----	----	----
	No	----	----	----	----	----	----
LSD (5%)		3.37			0.16		
CV (%)		23.81			20.85		

[1] For each trait, the same letters indicate no significant difference.

Discussion

According to the results, the infestation of *P. aegyptiaca* increased host defense responses. As an enzymatic antioxidant system, a significant increase in malondialdehyde and hydrogen peroxide are indicators of oxidative stress and increased specific catalase and ascorbate peroxidase activity. Other researchers have reported similar results (Goldwasser *et al*., 2000; Labrousse *et al.,* 2001, 2004; Gonzalez-Verdejo *et al.,* 2006; Pérez-de-Luque *et al.,* 2006; Hosseini *et al*., 2020). The catalase and peroxidase activity, phenolic, and protein content lead to some degrees of resistance (Demirbas and Okan 2017). Angeles Castillejo *et al*. (2004), reported that resistance in the tested chickpea genotype was related to the early stages of infection that are accompanied with necrosis of host and parasite tissues at the site of penetration and consequently the inhibition of contact with the host vascular system and the parasite development. Other studies have shown that parasite invasion can be inhibited in the cortex (Pérez-de-Luque *et al*., 2006), the endodermis (Pérez-de-Luque *et al*., 2005a), or pericycle. (Pérez-de-Luque *et al*., 2005b).

The role of peroxidases in this debate is also noteworthy (Mor *et al*., 2008). These enzymes are involved in the lignification and placement of phenols in the cell walls, developmental processes, defense mechanisms against pathogens, and other biotic and abiotic stresses. Peroxidases initiate cross-linkage proteins as the method of cell wall reinforcement in the presence of H_2O_2 soon after the pathogen attacks (Hammond-Kosack and Jones, 1996). The role of peroxidases in resistance and wall reinforcement has been confirmed in several pathosystems (Hammond-Kosack and Jones, 1996). Increased lignification and peroxidase activity in vetch Infected with Egyptian broomrape (Goldwasser, 2000). Also, increased expression of peroxidase-related genes in the process of resistance to Egyptian broomrape has been demonstrated (Vieira Dos Santos *et al*., 2003; Angeles Castillejo *et al*., 2004). Another study has shown that peroxidase activity in both susceptible and resistant sunflower genotypes has been greatly increased (Antonova and Ter Borg, 1996). In sunflower, production and secretion of phytotoxins in addition to the cell wall suberification was also observed. Phytoalexins are phenolic compounds and are considered as a protective response against *Orobanche cumuna*. (Serghini *et al*., 2001). In other studies, the induction of coumarin secretion the accumulation of phenolic compounds in pea (*Pisum* spp.) against *O. aegyptiaca and O. crenata* have also been shown. (Pérez-de-Luque *et al*., 2005a). The secretion of these substances inhibits more penetration to host tissue at the connection stage until the complete stop of the parasite and seedling death. In addition, in the resistant host, phenolic compounds are secreted into the apoplast during the penetration phase in the cells adjacent to the parasite attack site. At the same time, a toxic environment is created around the infestation site.

The effect of AM on the mentioned traits was not the same. However, there were no significant differences in malondialdehyde and hydrogen peroxide content due to AM inoculation. The ascorbate-specific activity was lower in AM inoculation treatments than treatments without AM inoculation. In treatments without *P. aegyptiaca* infection, AM inoculation increased specific catalase activity. However, *P. aegyptiaca* contamination neutralized the AM effect, and there was no significant difference between treatments. Mycorrhizal arbuscular fungi are abundant symbiotic microorganisms that coexist with plants in many plant families (Ortas, 2010). They play a crucial role in plant nutrition, stress resistance, and the expression of various oxidative enzymes. AM is capable of altering root enzymes, including peroxidase activity (Charron *et al.,* 2001). The results of different experiments indicate the difference in the results of AM application in the antioxidant system. Inoculation of tomatoes with AM increased peroxidase activity compared to the control treatment (ZhongQun *et al.,* 2010). It can be concluded that a decrease in the amount of strigolactones secretion induced by symbiosis with AM is a conservation phenomenon in plants. Since this beneficial association exists in most plant species globally, it can be used as a biocontrol strategy for economically important crops damaged by broomrape.

Similarly, peroxidase activity in the thin roots of *Pinus sylvestris* was decreased at the beginning of the experiment and gradually increased (Tarvainen *et al.,* 2004). The decrease in peroxidases was associated with an increase in the number of ECM morphotypes and root biomass. These results and Albrecht *et al.* (1994) show that AM increases peroxidase activity at the early stages of coexistence. The plant later controls this response and is thus avoided by the adult mycorrhiza (Munzenberger *et al.*, 1997). However, conflicting reports have also been published. For instance, peroxidases increased in the root of alfalfa colonized by adult mycorrhiza (*G. mosseae*) (Criquet *et al.,* 2000).

The height of Khassib and Argeto cultivars was not affected by mycorrhiza, while *P. aegyptiaca* infection caused a decrease in height in treatments with and without AM. Also, in the number of leaves, the mycorrhiza application did not reduce the damage of *P. aegyptiaca*. However AM inoculation increased leaf area in Khassib and Argeto cultivars in both infected and uninfected treatments with *P. aegyptiaac*.

AM application's shoot and dry root weight was significantly improved in the *P. aegyptiaca* infected and noninfected treatments. Despite the positive effect of AM on increasing host competence, *P. aegyptiaca* still decreased shoot and dry root weight by about 35 and 50%, respectively, indicating a high host sensitivity. The effect of AM on the traits related to *P. aegyptiaca* was significant. The decrease in TAN and ADW was observed in the AM application.

According to other research, mycorrhizal fungi as a *P. aegyptiaca* biocontrol agent has two main advantages. Initially, they are not pathogenic and have different benefits, such as improved water and nutrient availability for the plant (Baum *et al.,* 2015). These have led to their increasing use and breeding strategies and other biocontrol methods (Kohlen *et al.,* 2012). According to existing reports, the inoculation of sorghum (Lendzemo *et al*., 2001 and 2007) and maize (Sun *et al*., 2008) cultivars with AM has reduced the infection rate and biomass of *S. hermonthica*. The production and secretion of SL were significantly reduced by the coexistence of Mycorrhiza fungi in tomatoes. As a result, germination induction of *P. ramosa* in mycorrhizal tomato was markedly less than non-colonized tomato (López-Ráeza *et al.,* 2011).

Conclusion

Despite the effect of AM on the significant reduction of *P. aegyptiaca* and increased antioxidant activity of the phenylpropanoid pathway, and also increased host competency through improved morphological indices, due to the high susceptibility of the cucumber host to the broomrape, AM could not be used as a primary broomrape control strategy in this host.

However, its use in the integrated management of sustainable agroecosystems.

References

Aebi, H. 1984. Catalase in vitro. Methods in Enzymology, 105: 121-126.

Albrecht, C., Burgess, T., Dell, B. and Lapeyrie, F. 1994. Chitinase and peroxidase activities are induced in eucalyptus roots according to aggressiveness of Australian ectomycorrhizal strains of *Pisolithus* sp. New Phytologist, 127: 217-222.

Al-karaki, G. N. 2006. Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Scientia Horticulture, 109: 1-7.

Antonova, T. S. and Ter Borg, S. J. 1996. The role of peroxidase in the resistance of sunflower against *Orobanche cumana* in Russia. Weed Research, 36: 113-121.

Angeles Castillejo, M., Amiour, N., Dumas-Gaudot, E., Rubiales, D. and Jorrın, V. 2004. A proteomic approach to studying plant response to crenate broomrape (*Orobanche crenata*) in pea (*Pisum sativum*). Phytochemistry, 65(12): 1817-1828.

Baum, C., El-Tohamy, W. and Gruda, B. 2015. Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: A review. Scientia Horticulturae, 187 : 131-141

Bocova, B., Huttova, J., Liptakova, L., Mistrık, I., Olle, M. and Tamas, L. 2012. Impact of short term cadmium treatment on catalase and ascorbate peroxidase activities in barley root tips. Plant Biology, 56(4): 724-728.

Boyer, F., Saint germain, A. Pouvreau, J. and Clave, G. 2013. New Strigolactone Analogs as Plant Hormones with Low Activities in the Rhizosphere. Molecular plant. 8: 1-17.

Brewer, P. B., Koltai, H. and Beveridge, C. A. 2013. Diverse roles of strigolactones in plant development. Molecular plant. 6: 18-28.

Cagras, S., Sari, N. and Ortas, I. 2000. The effects of vesicular-arbuscular mycorrhizae on the plant growth and nutrient uptake of cucumber. Turkish Journal of Agriculture and Forestry, 24: 571-578.

Chance, B. and Maehly, A. C. 1955. Assay of Catalase and Peroxidase. Methods in Enzymology, 2: 764-775.

Charron, G., Furlan, V., Bernier, M. and Doyon, G. 2001. Response of onion plants to arbuscular mycorrhizae. 2. Effects of inoculum method and phosphorus fertilization on biomass and bulp firmness. Mycorrhiza, 11: 187-197.

Criquet, S., Joner, E., Leglize, P. and Leyval, C. 2000. Anthracene and mycorrhiza affect the activity of oxidoreductase in the roots and rhizosphere of lucerne (*Medicago sativa* L.). Biotechnology Letters, 22: 1733-1737.

Demirbas, S. and Okan, A. 2017. Physiological and biochemical defence reactions of *Arabidopsis thalana* to *Phelipanche ramosa* infection and salt stress. Fresenius Environmentah Bulletine, 26(3): 2275-2268.

El-Halmouch, Y., Benharrat, H. and Thalouarn, P. 2006. Effect of root exudates from different tomato genotypes on broomrape (*O. aegyptiaca*) seed germination and tubercle development. Crop Protection, 25: 501-507.

Goldwasser, Y. H., Eizenberg, H., Golan, S. and Kleinfeld Y. 2003. Control of *Orobanche crenata* and *Orobanche aegyptiaca* in parsley. Crop Protection, 22: 295-305.

Goldwasser, Y. and Kleifeld, Y. 2002. Tolerance of parsley varieties to *Orobanche*. Crop Protection, 21: 1101-1107 https://doi.org/10.1016/S0261-2194(02)00066-2

Goldwasser, Y., Plakhine, D., Kleifeld, Y., Zamski, E. and Rubin, B. 2000. The differential susceptibility of vetch (*Vicia* spp.) to *Orobanche aegyptiaca*: anatomical studies. Annals of Botany, 85: 257-262.

Gonzalez-Verdejo, C. I., Barandiaran, X., Moreno, M. T., Cubero, J. I. and Di Pietro A. 2006. A peroxidase gene expressed during early developmental stages of the parasitic plant *Orobanche ramosa*. Journal of Experiment Botany, 57: 185-192.

Hammond-Kosack, K. E. and Jones, J. D. G. 1996. Resistance gene dependent plant defense responses. The Plant Cell, 8: 1773-1791.

Hosseini Faradonbeh, N., Izadi Darbandi, E., Karimmojeni, H. and Nezami, A. 2020. Physiological and growth responses of cucumber (Cucumis sativus L.) genotypes to Egyptian broomrape (Phelipanche aegyptiaca (Pers.) Pomel) parasitism. Acta Physiologiae Plantarum, 42(140) https://doi.org/10.1007/s11738-020-03127-8.

Hsieh, T. H., Lee, J. T., Charng, Y. Y. and Chan, M. T. 2002. How to define resistance to water deficit stress? Plant Physiology, 130: 618-626.

Herzog, V. and Fahimi, H. 1973. Determination of activity of peroxidase. Annals of Biochemistry 55: 554-562.

Joel, D. M., Lytton, J. G. and Musselman, J. 2013. Parasitic *Orobanchaceae*, Parasitic Mechanisms and Control Strategies. Springer. p: 325.

Kofalvi, S. A. and Nassuth, A. 1995. Influence of wheat streak mosaic virus infection on phenylpropanoid metabolism and the accumulation of phenolics and lignin in wheat. hysiological and Molecular Plant Pathology, 47(6): 365-377.

Kohlen, W., Charnikhova, T., Lammers, M., Pollina, T., Toth, P., Haider, I., Pozo, M. J., de Maagd, R. A., Ruyter-Spira, C., Bouwmeester, H. J. and Lopez-Raez J. A. 2012. The tomato Carotenoid Cleavage Dioxygenase8 (SlCCD8) regulates rhizosphere signaling, plant architecture and affects reproductive development through strigolactone biosynthesis. New Phytologist journal, 196: 535-547.

Labrousse, P., Arnaud, M. C., Seryes, H., Berville, A., and Thalouarn, P. 2001. Several mechanisms are involved in resistance of *Helianthus* to *Orobanche cumana* Wallr. Annals of Botany, 88: 859-868.

Labrousse, P., Arnaud, M. C., Griveau, Y., Fer, A., and Thalouarn, P. 2004. Analysis of resistance criteria of sunflower recombined in bred lines against *Orobanche cumana* Wallr. Crop Protection, 23: 407-413.

Lendzemo, V. and Kuyper, T. 2001. Effects of arbuscular mycorrhizal fungi on damage by Striga hermonthica on two contrasting cultivars of sorghum, sorghum bicolor. Agriculture, Ecosystem and Environment journal, 87: 29-35.

Lendzemo, V., Kuyper, T., Matusova, R., Bouwmeester, H. J., and Van Ast, A. 2007. Colonization by arbuscular mycorrhizal fungi of sorghum leads to reduced germination and subsequent attachement and emergence of Striga hermonthica. Plant Signaling and Behavior journal, 2: 58-62.

López-Ráeza, J. A., Charnikhovab, T., Fernándeza, I., Bouwmeester, H., and Pozoa, M. J. 2011. Arbuscular mycorrhizal symbiosis decreases strigolactone production in tomato. Journal of Plant Physiology, 168: 294-297.

Mabrouk, Y., Simier, P., Delavault, P., Delgrange, S., Sifi, B., Zourgui, L. and Belhad, O. 2007. Molecular and biochemical mechanisms of defense induced in pea by *Rhizobium leguminosarum* against *Orobanche crenata*. Weed Research, 47: 452-460.

Mor, A., Mayer, A. M., and Levine, A. 2008. Possible peroxidase functions in the interaction between the parasitic plant, *Orobanche aegyptiaca*, and its host, *Arabidopsis thaliana.* Weed Biology Management, 8: 1-10.

Munzenberger, B., Otter, T., Wustrich, D. and Polle, A. 1997. Peroxidase and laccase activities in mycorrhizal and non-mycorrhizal fine roots of Norway spruce (*Picea abies*) and larch (*Larix decidua*). Canadian Journal of Botany, 75: 932-938.

Nakano, Y. and Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxides in spinach chloroplasts. Plant Cell Physiology, 22: 867-880.

Ortas, I., Kaya, Z. and Çakmak, I. 2001. Influence of VA-mycorrhiza inoculation on growth of maize and green pepper plants in phosphorus and zinc deficient soils. In: Horst, W. J., Schenk, M. K., Burkert, A., Claassen, N., Flessa, H., rommer, W. B., Goldbach, H. E., Olfs, H. W., Romheld, V., Sattelmacher, B., chmidhalter, U., Schubert, S., von Wiren, N. and Wittenmayer, L. (Eds.), Plant Nutrition-Food Security and Sustainability of Agroecosystems. Dordrecht: Kluwer Academic Publication, Dordrecht. pp. 632-633.

Ortas, I. 2003. Effect of selected mycorrhizal inoculation on phosphorus sustainability in sterile and no-sterile soilsin the Harran Plain in south Anatolia. Journal of Plant Nutrition, 26(1): 1-17.

Ortas, I. 2010. Effect of mycorrhiza application on plant growth and nutrient uptake in cucumber production under field conditions.Spanish Journal of Agricalture Research, 8(S1): S116-S122.

Pérez-de-Luque A, Jorrín J., Cubero J. I. and Rubiales D. 2005a. Resistance and avoidance against *Orobanche crenata* in pea (*Pisum* spp.) operate at different developmental stages of the parasite. Weed Research, 45: 379-387.

Pérez-de-Luque, A., Lozano, M. D., Madrid E. and Rubiales D. 2005b. Histochemistry of the resistance to *Orobanche crenata* in *Medicago truncatula* and *Pisum sativum*. In: Ellis N, ed. Grain legumes annual meeting. Norwich, UK: John Innes Centre, 9.

Pérez-de-Luque, A., Gonzalez-Verdejo, C. I., Lozano, M. D., Dita, M. A., Cubero, J. I., González-Melendi, P., Risueno, M. C. and Rubiales, D. 2006. Protein cross-linking, peroxidase and b-1,3-endoglucanase involved in resistance of pea against *Orobanche crenata*. Journal of Experimental Botany, 57: 1461-1469.

Samejima, H. and Sugimoto, Y. 2018. Recent research progress in combatting root parasitic weeds. Biotechnology and Biotechnology Equipment, 32(2): 221-240.

Serghini, K., Perez-De-Luque, A., Castejon-Munoz, M., Garcıa-Torres, L. and Jorrın, J. V. 2001. Sunflower (*Helianthus annuus* L.) response to broomrape (*Orobanche cernua* Loefl.) parasitism: induced synthesis and excretion of 7-hydroxylated simple coumarins. Journal of Experimental Botany, 52: 2227-2234.

Shahid, M., Pourrut, B., Dumat, C., Nadeem, M., Aslam, M. and Pinelli, E. 2014. Heavy-metal induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. Reviews of Environmental Contamination and Toxicology, 232: 1-44.

Sharma, I. 2013. Arsenic induced oxidative stress and antioxidant defense system of *Pisum sativum* and *Pennisetum typhoides*: A comparative study. Research Journal of Biotechnology, 8: 48-56.

Steinkellner, S., Lendzemo, V., Langer, I., Schweiger, P., Khaosaad, T., Toussaint, J. P. andVierheilig, H. 2007. Flavonoids and Strigolactones in Root Exudates as Signals in Symbiotic and Pathogenic Plant-Fungus Interactions. Molecules, 12: 1290-1306.

Sun, Z., Has, J. and Walter, M. H. 2008. Cloning and characterization of a maize carotenoid cleavage dioxygenase (ZmCCD1) and its involvement in the biosynthesis of apocarotenoids with various roles in mutualistic and parasitic interactions. Planta Journal, 228: 789-801.

Tarvainen, O., Markkola, A.M., Ahonen-Jonnarth, U., Jumpponen, A. and Strommer, R. 2004. Changes in ectomycorrhizal colonization and root peroxidase activity in *Pinus sylvestris* nursery seedlings planted in forest humus. Scandinavian Journal of Forest Research, 19: 400-408.

Torres, A. M., Avila, C. M., Gutierrez, N., Palomino, C., Moreno, M. T. and Cubero, J. I. 2010. Marker-assisted selection in faba bean (*Vicia faba* L.). Field Crops Research, 115: 243-252.

Umehara, M., A. Hanada, S. Yoshida, K. Akiyama, T. Arite, N. Takeda-Kamiya, H. Magome, Y. and Kamiya, K. Shirasu. 2008. Inhibition of Shoot Branching by New Terpenoid Plant Hormones. Nature, 455 (7210): 195-200.

Velikova, V., Yordanov, I. and Edreva, A. 2000. Oxidative Stress and Some Antioxidant Systems in Acid RainTreated Bean Plants: Protective Role of Exogenous Polyamines. Plant Science, 151: 59-66.

Verma, S. and Dubay, R. S. 2003. Lead toxicity induces lipid peroxidation and alters the activities of ntioxidant enzymes in growing rice plants. Plant Science, 164: 645-655.

Vieira Dos Santos, C., Delavault, P., Letousey, P. and Thalouarn, P. 2003. Identification by suppression subtractive hybridization and expression analysis of Arabidopsis thaliana putative defence genes during *Orobanche ramosa* infestation. Physiological and Molecular Plant Pathology, 62: 297-303.

Youssef, M. M. and Azooz, M. M. 2013. Biochemical studies on the effects of zinc and lead on oxidative stress, antioxidant enzymes and lipid peroxidation in Okra (*Hibiscus esculentus* cv. Hassawi). Science International, 1: 12-16.

Zhou, W. J. and Leul, M. 1998. Uniconazole-induced alleviation of freezing injury in relation to changes in hormonal balance, enzyme activities and lipid peroxidation in winter rape. Plant Growth Regulation, 26: 41-47.

ZhongQun, H., HaoRu, T., HuanXiu, L., ChaoXing, H., ZhiBin, Z. and HuaiSong, W. 2010. Arbuscular mycorrhizal alleviated ion toxicity, oxidative damage and enhanced osmotic adjustment in tomato subjected to NaCl stress. Am. Eurasian Journal of Agricalture and Environmental Science, 7: 676-683.

اثر هم‌زیستی قارچ‌های مایکوریزا آربسکولار بر خصوصیات مورفولوژیک و فیزیولوژیک در رابطه خیار *Cucumis sativus* - گل جالیز *Phelipanche aegyptiaca*

نیره‌السادات حسینی فرادنبه[۱]، ابراهیم ایزدی دربندی[۱*]، حسن کریم مجنی[۲] و احمد نظامی[۱]

۱- گروه اگروتکنولوژِی، دانشکده کشاورزی، دانشگاه فردوسی مشهد، مشهد، ایران.
۲- گروه زراعت و اصلاح نباتات، دانشگاه صنعتی اصفهان، اصفهان، ایران.
پست الکترونیکی نویسنده مسئول مکاتبه: e-izadi@um.ac.ir
دریافت: ۲۱ مرداد ۱۳۹۹؛ پذیرش: ۱۹ آبان ۱۴۰۰

چکیده: قارچ‌های مایکوریزا آربسکولار در کنترل نسبی گل‌جالیز مصری و افزایش شایستگی میزبان مؤثر هستند. در این پژوهش، اثر قارچ‌های مایکوریزا آربسکولار روی رابطه گل‌جالیز مصری و خیار و برخی خصوصیات مورفولوژیکی و فیزیولوژیکی میزبان و گل‌جالیز بررسی شد. نتایج نشان داد که آلودگی به گل‌جالیز به‌طور معنی‌داری باعث افزایش فعالیت آسکوربات پراکسیداز و کاتالاز در تمام ژنوتیپ‌های خیار گردید. کاربرد قارچ‌های مایکوریزا آربسکولار باعث کاهش فعالیت آسکوربات پراکسیداز و افزایش محتوای فنل کل در میزبان شد. کاربرد مایکوریزا در تیمارهای آلوده به گل‌جالیز اثر معنی‌داری بر محتوای مالون دی‌آلدئید و پراکسید هیدروژن نداشت با این‌حال، ارتفاع بوته‌های خیار و تعداد برگ نیز در تیمارهای کاربرد قارچ مایکوریزا آربسکولار تحت تأثیر آلودگی گل جالیز قرار نگرفت. کاربرد قارچ‌های مایکوریزا آربسکولار باعث کاهش اثر آلودگی گل‌جالیز بر روی سطح برگ، وزن خشک اندام هوایی و ریشه خیار گردید و موجب افزایش مقادیر این صفات نسبت به شاهد شد. هم‌چنین وزن خشک کل گل‌جالیز و تعداد اتصال آن نیز با کاربرد قارچ مایکوریزا کاهش یافت. علی‌رغم اثر مثبت کاربرد قارچ‌های مایکوریزا، کاهش ۳۵ تا ۵۰ درصدی در وزن خشک ریشه و اندام هوایی کلیه ژنوتیپ‌های خیار مورد آزمایش نشان از حساسیت بالای میزبان به انگلی شدن توسط گل‌جالیز داشت.

واژگان کلیدی: تنظیمات آنتی‌اکسیدانی، حساسیت میزبان، ترکیبات فنلی، مدیریت غیرشیمیایی، گل‌جالیز