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The effect of mycorrhizal fungi on antioxidant activity of various cultivars of onion (*Allium cepa* L)

M. Mollavali[1*], S. Bolandnazar[1], H. Nazemieh[3], F. Zare[1], N, Aliasgharzad[2]

[1]*Department of Horticulture, Faculty of Agriculture, University of Tabriz, Tabriz, Iran*

[2]*Department of Soil Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran*

[3]*Research Center for Pharmaceutical Nanotechnology and Faculty of Pharmacy, Tabriz University Of Medical Sciences, Iran*

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Abstract

Onion (*Allium cepa* L.) is one of the most important vegetables in the diet of Iranian people. Considering the importance of the organic crops production with high nutritional value, an experiment was conducted to study of the effect of mycorrhizal fungi on the antioxidant capacity of Onion (*Allium cepa* L.). Antioxidant activity of five cultivars of onion (*Allium cepa* L.) affected by mycorrhizal fungi were determined using ABTS, DPPH and FRAP assays (P≤ 0.01). Onion growth, mycorrhizal colonization rate, mineral nutrient concentrations and total flavonoid content were also quantified. Results indicate that inoculated plant was contained higher antioxidant activity than non-inoculated ones. The highest antioxidant activity was found in inoculated plants with *Glomus versiforme*. Results revealed that red cultivars namely Azarshahr and Rosita had higher antioxidant activity as compared to pink, yellow and white cultivars. Azarshahr cultivar contained highest antioxidant activity at 57.516±0.076, 57.266±0.016 and 7.617±0.012 (µM Ascorbic acid/g FW) for DPPH, ABTS and FRAP assays, respectively. The antioxidant activity of different cultivars of onion affected by mycorrhizal fungi showed the following order: red Azarshahr> red Rosita> yellow Gholi Ghesse> pink Horand> white Kashan. A significant positive correlation was obtained between ABTS, DPPH and FRAP assays (P≤ 0.01).

* **Corresponding Author:** M. Mollavali ✉ mohannamollavali@yahoo.com

Introduction

The human body is exposed reactive oxygen species (ROS) due to numerous physiological and biochemical processes. A vast amount of evidence implicates that ROS are able to attack lipid membranes, proteins and DNA, and lead to some detrimental effects (Devasagayam *et al.*, 2004). Antioxidants are now known to play an important role in protecting against disorders caused by oxidant damage. Antioxidants can delay or inhibit the initiation or propagation of oxidative chain reactions and thus prevent or repair damage done to the body's cells by oxygen (Firuzi *et al.,* 2011). Although the synthetic antioxidants have been widely used by the food industry, because of their possible toxicities the development and use of more effective antioxidants of natural origin is highly desirable (Augustyniak *et al.,* 2010; Rodil *et al.,* 2012).

Onion (*Allium cepa* L.) has A, B1, B2, C vitamins, nicotinic acid, pantothenic acid and important substances such as calcium, phosphorus, potassium and traces of Fe, Al, Cu, Zn, Mn and I (Augusti, 1990).

Moreover, onions contain organosulfur compounds which can help to lowering blood pressure and cholesterol levels in the human body (Sampath Kumar *et al.*, 2010). The high antioxidant activity of *Allium* species was reported by numerous researchers (Velioglu *et al.*, 1998; Yin and Cheng, 1998). Onion contains a wide variety of free radical scavenging molecules, including phenolic compounds, nitrogen compounds, vitamins, terpenoids, and some other endogenous metabolites, which have protective effects against the development of cardiovascular and neurological diseases, cancer and other disorders that are caused by oxidative stress (Griffiths *et al.*, 2002). Organosulfur compounds in onion are known to lower blood pressure and cholesterol levels in the human body (Sampath Kumar *et al.*, 2010). Onion bulbs are rich sources of dietary flavonoids. The antioxidative and antiradical activities of onion were shown to be highly dependent on the content of phenolic compounds (Cao *et al.*, 2007; Jeong *et al.*, 2009).

There are many onion types, which differ in shapes, sizes, color and flavor, containing different concentrations of phenolic compounds and flavonoids (Crozier *et al.*, 1997; Yang *et al.*, 2004; Shon *et al.*, 2004). Generally, red cultivars are suggested to contain the highest phenolics and flavonoids and show highest antioxidant activities among cultivars (Lachman *et al.*, 2003; Gorinstein *et al.*, 2009; Jeong *et al.*, 2009; Kaur 2009). Plant phenolics show marked qualitative and quantitative variation not only in different genetic levels (between and within species and cultivars) but also between different physiological and developmental stages (Bunning *et al.,* 2010). They also vary in response to environmental factors, such as light intensity and nutrient availability (Bilyk *et al.*, 1984; Patil *et al.*, 1995; Sellappan and Akoh 2002; Yang *et al.*, 2004; Mogren *et al.*, 2007).

Soil organisms play a crucial role in the functioning of agricultural ecosystems. Mycorrhizal fungi are one of the most important soil microorganisms and major components of a sustainable soil-plant system. The association of mycorrhizal fungi with plant roots has mutual benefits for both the plant host and the fungus (Harley and Harley, 1987; Hodge, 2000). Previous studies have indicated that inoculation of onion roots with mycorrhizal fungi increased the uptake of P, N, K, Ca, Mg, Na by colonizing onion roots and forming a network of fungal hyphae in the soil (Abbott and Robson, 1982; Mosse, 1973; Tinker, 1978; Bolandnazar, 2009). Arbuscular mycorrhizal fungi also can function as biofertilizer, protecting against soil-borne pathogens and bioprotectant (Gianinazzi and Vosatka, 2004; Vosatka and Albrechtova, 2008). Onions have an inefficient root system and needs a high amount of fertilizer to obtain a good yield. Given the effect of mycorrhizal fungi in nutrient uptake, inoculating onion roots with mycorrhiza seems to be useful for growing onion. On the other hand, it has been shown that inoculation with mycorrhizal fungi increased the antioxidant activity in onion by increasing phenolic compounds as a consequence of defense mechanisms (Perner *et al.*, 2008).

Although a great deal of research has been carried out on the antioxidant properties of the *Allium* species, no data are available on the antioxidant properties of Iranian domestic cultivars. On the other hand, given the important role of mycorrhizal fungi in increasing antioxidant activity, it would be interesting to investigate and compare the effect of different species of mycorrhizal fungi on the antioxidant activity of *Allium* species. The main objectives of this study were: 1) to assay and compare the antioxidant activity of Iranian *Allium* cultivars; 2) to investigate the effect of mycorrhizal fungi on the antioxidant activity; and 3) to characterize the optimal mycorrhizal species which induce highest antioxidant activity.

Material and methods

Plant samples

Onion cultivars and mycorrhizal species

In the present study, we compared the antioxidant activity of five cultivars of onion, including four Iranian cultivar (red Azar-shahr, white Kashan, yellow Gholi Ghesse, pink Horand) and a commercial cultivar (red Rosita), affected by three species of mycorrhizal fungi (*Glomus versiforme, G. intraradices, G. mosseae*).

All onion cultivars were long-day and prepared from Agricultural Research Station of the University of Tabriz, Iran. Mycorrhizal species (isolated from Tabriz Plain) was obtained from Department of Soil Science, University of Tabriz (Aliasgharzad *et al.*, 2001).

Onion cultivation

A pot experiment was performed from May to October 2012 to study the influence of mycorrhizal fungi on antioxidant activity in onion (*Allium cepa* L.) at the research station of Tabriz University, Tabriz, Iran. After disinfection of onion seeds with sodium hypochlorite (1%) for 10 minutes they were sown in a sandy loam soil that was autoclaved in 121°C for 2h. Physical and chemical characteristics of the soil were shown in Table 1. Fifty grams of mycorrhizal fungus inoculum (a mixture of spores, hyphae, AM root fragment and soil) were mixed into one kg of soil (Aliasgharzadeh *et al.*, 2001). The control pots received the same amount of sterilized inoculum. The temperatures during the experiments in the greenhouse were 26°C day/18°C night and the relative humidity was 50–70%. Onion plants were grown at 200 μmol m^{-2} s^{-1} light intensity. Three plants from each treatment were sampled randomly every week for 50 days (from emergence to transplanting) to determine the incidence of root colonization. The experiment was set as factorial based on a completely randomized block design with three replications. The first factor was five cultivars of onion (red Azar-shahr, white Kashan, yellow Gholi Ghesse, pink Horand and red Rosita) and three species of mycorrhizal fungi (*G. versiforme, G. intraradices, G. mosseae*) was considered as second factor (Table 1 near here).

Harvest

Plants were harvested 4 month after transplanting when 80% of the onion plants had fallen leaves. Bulbs fresh and dry weight (after 48 h in 72°C at oven) were recorded.

Mycorrhizal Colonization and Mineral Element Concentration

Bulb dry weight samples were ground and analyzed for total N (Baker and Thompson, 1992), P, K (Cottenie, 1980). 1cm root fragments were washed and cleared by 10% KOH at 90°C for 1 hour and then acidified with 1% HCl and stained with 0.05% (v/v) trypan blue in lactoglycerol at 90°C for 30 min (Phillips and Hayman, 1970). 30 root segments were put on slides and mycorrhizal colonization percentage was determined using grid line intersection method (Furlan and Fortin, 1973).

Total flavonoid content

Total flavonoid content of bulbs was determined following aluminum chloride colorimetric assay method described by Chang *et al.* (2002). 1 g of the fresh onion bulb samples ground in liquid nitrogen were extracted with 96% aqueous ethanol (1 g fresh weight/4 mL).

0.5 ml of extracts or standard solution of quercetin

(0, 20, 40, 60, 80 and 100 µg/ml) was added to 0.1 ml of 1 M potassium acetate and 0.1 ml 10% aluminium chloride was added. Then 1.5 ml methanol and 2.8 ml Distilled water were added and the solution was mixed well and absorbance was measured with spectrophotometer (Spekol 1500 Germany) at 415 nm after 30 min in room temprature.

Antioxidant activity

Extraction

The outer skin of the onions was removed and cut into small cubes and frozen at -70°C. 1 gram of each frozen sample was homogenized by 3 ml of 80% methanol. Extracts were centrifuged at 10,000 × *g* for 10 min at 4°C and used to test their antioxidant activity. The antioxidant potential of onion extracts was assessed by three common methods, including: 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP).

DPPH assay

The DPPH method is widely used to determine free radical scavenging activity of purified phenolic compounds as well as plant extracts. The antioxidant capacity of the onion was determined using a DPPH method according to Brand-Williams *et al.* (1995). Twenty-four mg of DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) was dissolved in 100 ml methanol for preparing 6×10^{-5} mol/l stock solution and then stored at -20° C until needed. Aliquots (0.1 ml) of methanol extract were added to 3.9 ml of DPPH solution and the decrease in absorbance was determined at 515 nm with spectrophotometer model Spekol 1500, Germany. Radical scavenging activity was expressed as the inhibition percentage and was calculated by the following formula:

% radical scavenging activity = (control OD - sample OD/control OD) × 100

ABTS

ABTS assay was done according to the method of Re *et al.* (1998). Stock solution was concluded of 7 mM 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2.45 mM potassium per sulfate solution. These reagents combined and made up to volume in a 10 ml volumetric flask and was covered with aluminum foil. The stock solution kept in the dark; at room temperature for 12–16 h (the solution is stable for 3 days). The $ABTS^{\bullet+}$ solution was diluted with PBS (Phosphate buffered saline) pH 7.4, and equilibrated at 30°C. The solution was diluted by mixing 1ml ABTS solution with 120 ml PBS to obtain an absorbance of 0.7 (±0.02). PBS (pH 7.4) was used as blank. Then 1ml of diluted ABTS solution was added to 10 µl of methanol extracts and the absorbance at 734 nm was measured immediately 1 min after mixing and reading continued every minute for 5 min. The percentage inhibition was calculated from the absorbance values at 5 min as follows:

ΔA= (A0sample- A5 sample/A0sample) – (A0ABTS-A5ABTS/A0ABTS)×100

FRAP

Total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay (Benzie *et al.*, 1999). The reagents included 300 mM Acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6- tripyridyl-*s*-triazine) solution in 40 mM HCl and 20 mM $FeCl_3{\cdot}6H_2O$ solution. The fresh working solution was prepared by mixing these reagents in the volume ratio 10:1:1 (v:v:v), respectively. L-ascorbic acid was used to prepare a standard solution (100µM- 1000 µM). Then, 100µl methanol extract was mixed with 3 ml of working FRAP reagent and absorbance was measured at 0 minute at 593 nm. After that, samples incubated at 37°C in a water bath and absorption were taken again after 4 minutes. The total antioxidant capacity of FRAP was calculated by using the equation,

FRAP value (µM) = (Change in absorbance of the sample from 0 to 4 minute/change in absorbance of the blank from 0 to 4 minutes) × FRAP value of standard (1000 µM).

FRAP value of Ascorbic acid is 2.

ΔA sample= (A0sample-A5sample⁄A0sample) - (A0solvent-A5solvent/A0solvent).

Percent inhibition values were obtained by multiplying ΔA_{sample} values by 100.

Statistical analysis

Data were analyzed according to experimental design and means were compared by the Duncan's multiple range test. Multivariate analysis of variance (MANOVA) was applied to evaluate the effect of treatments on onion with a significance level of P< 0.01. All statistical analyses were carried out by using the "SPSS" software package (v. 18.0, SPSS, Inc).

Results

Mycorrhizal Colonization and plant growth

Mycorrhizal root colonization rates were different between onion cultivars and mycorrhizal fungi speices. Highest mycorrhizal root colonization was achived in red Azar-shahr with *G. versiforme* and white Kashan showed the lowest mycorrhizal root colonization (Table 3). Roots of non-inoculated plants remained free of AM colonization. As shown in table 1. The effect of cultivars and mycorrhization on fresh and dry weight of bulb was significant. Gholi Ghese cultivar and *G. versiforme* showed highest fresh and dry weight of bulb (Table 3 near here).

Table 1. Physical and chemical characteristics of the soil used at the experiment.

Clay (%)	Silt (%)	Sand (%)	K (mg/kg)	P (mg/kg)	Total N (%)	Organic carbon (%)	pH	E.C (dS/m)	Saturation (%)
15	18	76	313	3.1	0.12	1.6	7.8	3.22	37

Mineral nutrient concentrations

Mycorrhization, affected bulb N, P and K concentrations significantly (Table 2). Bulb N, P, and K concentrations were significantly increased by using *G. versiforme* (Table 3). Onion cultivars significantly affected on N and P concentrations whereas, bulb K concentration was not affected by cultivars (Table 2). Gholi Ghesse and red Azar-shahr cultivars showed highest N and P concentration, respectively. Moreover, white Kashan had lowest N and P concentration of bulb (Table 3).

Antioxidant Activity and total flavonoid content

The effect of cultivar, mycorrhizal inoculation and interaction between treatments on flavonoid content was significant (P≤0.01) (Table 2). red Azar-shahr followed by red Rosita showed highest total flavonoid content and white Kashan showed lowest flavonoid content. Moreover, mycorrhizal inoculatin caused to increasing in total flavonoid content of onion samples and highest total flavonoid content obtained by using *G. versiforme* (Table 4).

Table 2. Analysis of variance for root colonization, nitrogen (N), phosphorus (P) and potassium (K) of leaves, fresh (FW) and dry weight (DW), total flavonoid content and antioxidant activity of onion bulb affected by cultivar and mycorrhizal inoculation.

		Mean square									
Source variation	of d.f	Root colonization	N	P	K	FW	DW	ABTS	DPPH	FRAP	Total Flavonoid
Cultivar (C)	4	2393.6**	23.08**	3.23*	397.7^{ns}	1049.7**	13.51*	1407.73**	1848.61**	28.54**	171.74**
Mycorrhization (M)	3	9552.9**	20.17**	8.91**	844.1*	862.7*	9.95*	934.58**	871.38**	17.53**	16.201**
C×M	12	282.3**	11.27*	1.65^{ns}	427.7^{ns}	529.8**	3.43^{ns}	512.83**	573.92**	9.014**	86.75**
Error	40	6.13	4.53	1.042	276.6	173.9	4.146	0.001	0.0171	0.000	0.002

*,** indicating significantly different at P≤0.05 and P≤0.01, respectively.

Antioxidant activity of five varieties of onions was determined by three different antioxidant assays, namely ABTS, DPPH and FRAP. The results of antioxidant activity obtained from onion samples are shown in Table 4(Table 4). Mycorrhizal inoculation and cultivar had significant effect on antioxidant activity of bulbs (P≤0.01). Antioxidant activity showed wide variation from 8.09±0.076 to 57.516±0.076, 9.581±0.016 to 57.266±0.016 and 1.019±0.012 to 7.617±0.012 in DPPH, ABTS and FRAP assays for white Kashan and red Azar-shahr, respectively. Total antioxidant activity of onion bulbs

is shown in Figures 1-3(Figs 1-3 near here). The main effect of cultivar on antioxidant activity showed the following order: red Rosita> red Azar-shahr> yellow Gholi Ghesse> pink Horand> white Kashan by ABTS and DPPH assays and red Azar-shahr> red Rosita> yellow Gholi Ghesse> pink Horand> white Kashan in FRAp assay (Table 4, Figs 1-3). Interaction between cultivars and mycorrhizal inoculation was significant (P≤0.01) and red Azarshahr onion samples inoculated with *G. versiforme* had highest antioxidant activity between treatments (Figs 1-3). ABTS, DPPH and FRAP assays of five cultivars showed a similar trend. Correlations among antioxidant activity based on ABTS, DPPH and FRAP assays were positively high and ranged between 0.709 and 0.892: the highest correlation was between ABTS and DPPH (0.892) and the lowest correlation was between DPPH and FRAP (0.709), the correlation between ABTS and FRAP was (0.819) (Table 5). The antioxidant activity of red Azarshahr and red Rosita was approximately 3-fold higher than white cultivars. For all assays red cultivars scored over the white, pink and yellow ones. *G. versiforme* and *G. intraradices* in all cultivars had the highest and lowest antioxidant activity, respectively (Table 4). All three methods of measurements resulted in significant interactions between cultivar and mycorrhizal fungus treatments (P≤0.01). The main effect of cultivar in ABTS and DPPH assays showed that the antioxidant activity of red Rosita was higher than those in red Azarshahr, whereas, after inoculation, red Azarshahr had the highest antioxidant activity in combination with *G. versiforme*, in all methods of measurements (Figs 1-3). Selected species showed varietal differences, accordingly, the highest antioxidant activity was found in inoculated plants with *G. versiforme* followed by *G. mosseae* and *G. intraradices* (Table 3). In all treatments, control showed the lowest antioxidant activity (Table 3 and Figs 1-3)(tables 4,5 near here).

Table 3. The effect of cultivar and mycorrhizal fungi on root colonization, nitrogen (N), phosphorus (P) and potassium (K) concentrations of leaves, fresh (FW) and dry weight (DW) of onion bulb

Treatments		Root colonization(%)	N(mg/g DW)	P(mg/g DW)	K(mg/g DW)	FW (gr)	DW(gr)
Cultivar	Gholi Ghesse	46.9b	15.81a	3.24ab	92.54ab	65.22a	7.07a
	White Kashan	15.97e	12.38b	2.6b	89.37ab	41.16b	4.87b
	Red Rosita	42.71c	13.28b	3.96a	91.3ab	46.06b	5.41ab
	Pink Horand	28.45d	12.65b	3.47ab	81.21b	44.98b	4.26b
	Red Azar-shahr	49.35a	14.15ab	3.71a	96.86a	48.27b	5.02b
Mycorrhiza	Without	0d	12.25b	2.84b	81.54b	44.61b	4.34b
	Glomus mosseae	40.46c	13.54ab	2.89b	92.74ab	56.55ab	5.22ab
	Glomus intraradices	50.9b	13.74ab	3.72ab	87.57ab	52.5ab	5.77ab
	Glomus versiform	55.34a	13.77a	4.32a	99.17a	62.89a	5.97a

Means followed by non-similar letters are significantly different at P≤ 0.01 according to Duncan's multiple range test.

Table 4. The effect of cultivar and mycorrhizal fungi on antioxidant activity and total flavonoid of onion.

		Antioxidant activity			
Treatments		ABTS%	DPPH%	FRAP(µM AA/gr FW)	Total Flavonoid (mg/g FW)
Cultivar	Gholi Ghesse	34.86b	32.76b	4.48c	37.36c
	White Kashan	12.55d	11.74d	2.25e	31.26d
	Red Rosita	39.44a	42.23a	4.77b	37.78b
	Pink Horand	29.21c	26.71c	3.58d	31.30d
	Red Azar-shahr	37.34ab	40.89a	6.43a	39.11a
Mycorrhiza	Without	24.64c	25.63c	3.25d	33.97d
	Glomus	34.09b	31.32b	4.88b	35.36c
	Mosseae	23.77c	25.04c	3.56c	35.64b
	Glomus intraradices	40.22a	41.48a	5.53a	36.47a
	Glomus versiform				

Means followed by non-similar letters are significantly different at P≤ 0.01 according to Duncan's multiple range test.

Discussion

Mycorrhizal Colonization and plant growth

Highest colonization rate was obtained by using *G. versiforme* (Table 3). Aliasgharzad *et al.*, 2009 also reported the highest root colonization of onion plants in symbiosis with *G. versiforme*. Differences in mycorrhization rate among cultivars can be because of their different in root length. Low colonization rate of white Kashan cultivar may be due to thicker root system of this cultivar than others. onions have a spare and superficially root system without hair roots and such this roots have high mycorrhizal responsiveness (Plenchette *et al.*, 1983; De Melo *et al.*, 2003; Galván *et al.*, 2009). De Melo *et al.* (2003) reported that inoculation of *A. fistulosum* plants with *G. intraradices* resulted in increasing shoot dry biomass and root length up to 40-50%. Several studies demonstrated that response to arbuscular mycorrhizal colonization is different among cultivars of wheat (Azcon and Ocampo, 1981), barley (Baon *et al.*, 1993) and tomato (Bryla and Koide, 1990).

Table 5. Correlation coefficients (r) between parameters P≤ 0.01.

Variable	ABTS	DPPH	FRAP
ABTS	1		
DPPH	0.892**	1	
FRAP	0.819**	0.709**	1

** indicating significantly different at P≤0.01.

Mycorrhizal fungi can increase plant growth by increasing in nutrient uptake, forming a hyphal network and therefore improvement of water uptake. The positive effect of mycorrhizal fungi on growth of many plant species is well documented in earlier studies (Hayman and mosse, 1971; Plenchette *et al.*, 1983; Bolandnazar *et al.*, 2007, 2009; Wang *et al.*, 2011; Abdullahi and Sheriff, 2013). Inoculated onion samples showed higher fresh and dry weight than non-inoculated ones. This can be due to improvement of nutrient uptake by mycorrhization. The effect of mycorrhizal fungi on plant growth is different between plant species and even among cultivars (Plenchette *et al.*, 1983; Hetrick *et al.*, 1996). It seems that differences in tested cultivars, in response to mycorrhization caused to different fresh and dry weight in inoculated plants.

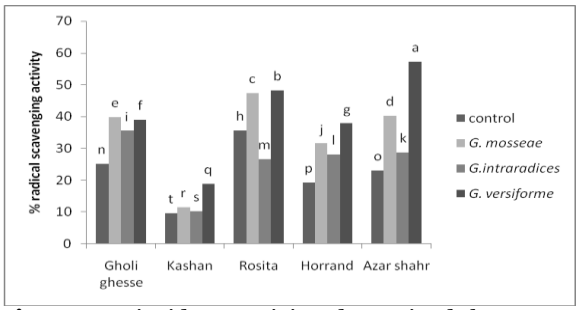

Fig. 1. Antioxidant activity determined by ABTS method in onion cultivars affected by mycorrhizal fungi.

Mineral nutrient concentrations

Mycorrhization of onion plants resulted in increasing of bulb N, P and K concentration. Mycorrhizal fungi is demonstrated to enhance uptake of mineral nutrient such as P, N, K, S, Zn and Cu (Sharma, 2004; Singh *et al.*, 2004). Onion samples inoculated with *G.versiforme* showed higher N, P and K concentration of bulb compared to plants inoculated with *G. intraradices* and *G. mosseae*. This result is in agreement with Charron *et al.*, 2001 which they reported that phosphorus concentration of inoculated onion plants with *G. versiforme* was higher than plants inoculated with *G. intraradices*. Changes in P uptake and growth of plants with arbuscular mycorrhizal colonization differ among plant species and cultivars (Plenchette *et al.*, 1983). Our results are supported by Bolandnazar *et al.*, 2007; Aliasgharzad *et al.*, 2009; Lenin *et al.*, 2010; Sridevi and Ramakrishnan, 2010).

Antioxidant Activity and total flavonoid content

In the present study it was shown that colonization with mycorrhizal fungi influenced total flavonoid content. Morandi and Bailey (1984) reported that mycorrhizal inoculation affects production of flavonoid compounds in soybean plants roots. These researchers also found that the effect of inoculation

with mycorrhizal fungi was significantly vary between the flavonoid compounds accumulated in plant roots. Mycorrhizal colonization increases production of new phenolic compounds during symbiosis and also can alter profile of flavonoids by changing in the expression of genes involved flavonoid and isoflavonoid biosynthesis (Ling-Lee *et al.*, 1977; Harrison and Dixon, 1993 and 1994; Devi and Reddy, 2002).

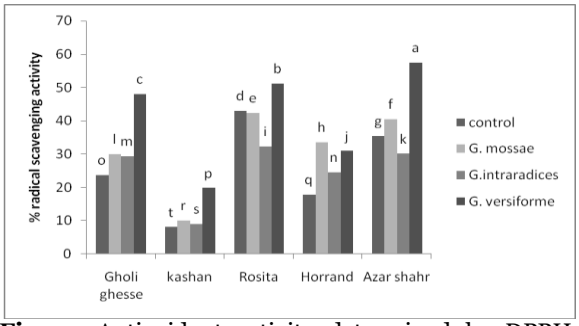

Fig. 2. Antioxidant activity determined by DPPH method in onion cultivars affected by mycorrhizal fungi.

All three methods of determination showed that the antioxidant activity was significantly enhanced by the application of mycorrhizal fungi (Figures 1-3). Plants are exposed to many unfavorable conditions such as biotic (viruses, bacteria, fungi, nematodes and other pests attacking plants) and abiotic heat, cold, drought, salinity, solar radiation, and nutrient deficiency) stresses. Their defense against stress by synthesizing phenolic compounds or induce or activate the antioxidant defense system (Winkel-Shirley, 2002). Mycorrhizal fungi promote antioxidant activity by utilizing various mechanisms such as: (a) enhancing nutrient uptake (b) increasing the efficiency of the host plants by increasing their growth (c) producing phytochemicals such as flavonoids. Some researchers in the recent years showed that mycorrhizal inoculation can increase in antioxidant activity and phenolic compounds (Huang *et al.*, 2011; Banuelos *et al.*, 2014). perner *et al.* (2008) revealed that inoculation of onion plants with arbuscular mycorrhizal fungi increased the antioxidant activity by increasing phenolic compounds as a result of plant defense mechanism. In this experiment, it seems that mycorrhizal application acts as biotic stress and enhanced antioxidant activity. Wu *et al.* (2006) reported that the levels of enzymatic and non-enzymatic antioxidant productions increased under drought stress. Inoculation of sugarcane plants with *G. mosseae*, reduced the production rate of O_2^- and improved antioxidant enzyme content during water stress in the leaves, consequently reducing the peroxidation of membrane lipids and enhanced drought tolerance (Wang *et al.*, 1995; Dudhane *et al.*, 2011). Our results are in agreement with the earlier report by Hernandez-Ortega *et al.* (2012) who found *Melilotus albus* roots of mycorrhized-plants had significantly higher antioxidant activity.

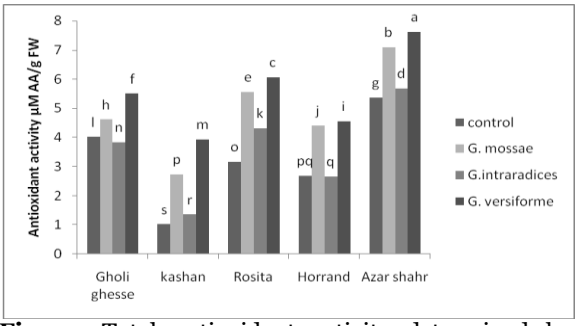

Fig. 3. Total antioxidant activity determined by FRAP method in onion cultivars affected by mycorrhizal fungi.

Results indicate that the effect of cultivar on antioxidant activity and total flavonoid content was significant ($P \leq 0.01$) (Table2). There is considerable variation in composition, concentration, and beneficial activities of antioxidant compounds between different varieties (Yang *et al.*, 2004). Racharla (2011) reported that distribution of flavonoids in varieties of onions is significantly different and the most differences was between red and white cultivars. Higher antioxidant capacity is associate with phenol content, the thiosulphinates and S-alk(en)yl-L-cysteine sulphoxides, which are responsible for pungency of onion. Onion cultivars are different in flavonoid content, flavor, pungency and have a different fraction of these compounds (Rice-Evans *et al.*, 1996; Xiao and Parkin, 2002; Shon *et al.*, 2004; Santas *et al.*, 2008; Beesk *et al.*, 2010). As shown in table 4 red Azar-shahr and red Rosita extracts significantly had higher antioxidant activity and total flavonoid content than three other cultivars. Our results suggested that the red onions had higher

antioxidant activities than yellow, pink and white onions. These results are in agreement with Gregorio *et al.* (2010), Gökçea *et al.* (2010) and Cheng *et al.* (2013) who found red cultivars have a higher flavonoid content and antioxidant capacity.

Conclusion

The results of our experiment may be useful to guide consumers to purchase varieties with high quality and health benefits. Also, regarding the effect of mycorrhizal effect on antioxidant activity may affect future efforts on manufacturers to produce high quality onion by using mycorrhizal fungi as biofertilizer. The results of the present study suggest that , inoculation with arbuscular mycorrhizal fungi can significantly increase antioxidant activity in onion plants by increasing in nutrient uptake and promote the flavonoid production. Moreover, *G. versiforme* is the most effective mycorrhizal fungus in increasing of antioxidant activity in onion plants. Our results provides clear evidence that the red onion cultivars possess higher antioxidant activities as compared to pink, yellow and white cultivars. The findings of the current study have shown a positive relationship between the results of ABTS assay, DPPH radical scavenging activity assay and FRAP assay. Mycorrhizal colonization developed at the different rate and level of intensity in five cultivars. The intensity of mycorrhizal infection was higher at red Azarshahr than commercial cultivar. The results suggested that red Azar-shahr variety of onion could be a promising source of natural antioxidants. Regarding to the antioxidant capacity of onion to decrease the risk of degenerative diseases, we recommend that using dietary onion rich in flavonoids, especially red cultivars, could have beneficial effects on subjects with cataracts. These findings should be followed by more studies to understand formation of phytochemicals such as flavonoids in onion plants response to mycorrhizal fungi symbiosis.

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