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Reducing nitrogen fertilization application in Cucumber by mycorrhiza colonization of the plant

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ABSTRACT- The effect of different nitrogen levels on plant growth, phenol content, antioxidant and Nitrate reductase (NR) activity of cucumber (*Cucumis sativus* cv. Super) inoculated with mycorrhiza was studied. A factorial experiment based on a completely randomized design (CRD) with 6 replicates was designed. Treatments were three levels of nitrogen (NO_3-50, NO_3-75 and NO_3-100) and two mycorrhiza inocula (1000 spore (AM1), 2000 spore (AM2) and no mycorrhiza treatment (AM0) as the control. Results showed that the root fresh weight increased by mycorrhiza inoculation at all nitrogen levels, while it was unaffected by nitrogen levels and mycorrhiza inoculation. FV/FM was higher in NO_3-75 mycorrhizal inoculated plants compared to that of AM0 ones. Antioxidant activity of plants increased due to mycorrhiza symbiosis in nitrogen deficiency treatments, so that AM1 and AM2 increased antioxidant activity in NO_3-50 and NO_3-75 treatments, respectively, as compared to the AM0 plants. The highest NR activity was observed in the NO_3-50 treatment. However, mycorrhiza inoculation decreased NR activity of the plants at all nitrogen levels. Therefore, it can be concluded that mycorrhiza inoculation, especially the 1000 spores treatment (AM1), and a moderate level of NO_3 (NO_3-75) can be used and these application levels can be very effective for greenhouse cucumber production.

INTRODUCTION

Nitrogen (N), as an essential nutrient element, which consists of about 3-4% of the dry matter of plants, often becomes a limiting factor for plant growth (Makhziah et al., 2013). Proteins increase vegetative and reproductive growth of plants. The production of proteins, in turn, depends on the supply of nitrogen (Lawlor, 2008). Nitrogen is also important for plant growth because it is one of the basic elements for the synthesis of chlorophyll and several enzymes and it is involved in cell division and growth (Liu et al., 2013).

Since N plays an important role in crop yield and quality (Chen et al., 2004), its application in agricultural practices is continuously increasing. Nitrate is the major source of N for higher plants. Uptake of nitrate by plants results in high nitrate accumulation in plants, especially in vegetable crops (Chen et al., 2004). On the other hand, application of nitrogen to produce more yields has become increasingly a potential pollutant (Zand-Parsa et al., 2006). Application of nitrogen fertilizers is more than what the plant needs and the nitrogen use efficiency is less than 10% (Dai et al., 2011); therefore, large amounts of unused N in soils lead to many environmental problems (Gollany et al., 2004).

Azcon et al. (1992) reported that mycorrhizal fungi are important in ecological agriculture as biofertilizers, biological protectors, and biological control agents. The positive effect of mycorrhiza on plant growth, especially under nutrient limited conditions, has been established by some researchers (Mortimer et al., 2009; Goss and de Varennes, 2002). Smith and Read (1997) demonstrated that *Arbuscular mycorrhiza* hyphae spread in the soil around the root and provided the surface area, and caused absorption of nutritional elements such as phosphorus, nitrogen, zinc, and copper for the host plant. Symbiosis relation between the mycorrhizal fungi and their host plants can also improve transferring large amounts of nitrogen in different plants (He et al., 2007; Mortimer et al., 2009; Li et al., 2006). Also, it has been reported that mycorrhiza fungi increased phosphorous content of plants and positively affected nitrogen uptake by plants (Tufenkci et al., 2005).

The two most important nitrogen sources for plant and mycorrhiza are nitrate and ammonium (Masoumeh et al., 2009). The colonization and growth of mycorrhiza is correlated with the nitrogen status in the soil and it is inhibited by high nitrogen levels (Azcon et al., 1992). The transfer and metabolism of nitrogen is dependent on the biosynthesis and flux of carbon by host plant to mycorrhizal fungi (Liu et al., 2013).

N deficiency and ecession is a nutritional stress on plant which activates defense plant system, such as antioxidant and non-antioxidant activities (Chen et al. 2013). On the other hand, it has been shown that mycorrhiza inoculation increased total phenol content leaves and flowers in artichoke plants (Ceccarelli et al.,

2010). Total phenol content of grape significantly increased by all strains of mycorrhiza inoculation compared to the control plants (Eftekhari et al., 2012).

The objective of the present research was to study the effect of i) reduction of nitrogen rates and ii) beneficial effects of mycorrhiza inoculation to compensate the reduction of nitrogen rates on plant growth, antioxidant amount, and nitrate reductase activity of cucumber (*Cucumis sativus* cv. Super N3) under greenhouse condition.

MATERIALS AND METHODS

This experiment was conducted in a plastic greenhouse in the Department of Horticulture Science of Isfahan University of Technology, Isfahan, Iran . The temperature in greenhouse was 30-35 ℃ and relative humidity was 30-35% between July and October 2013. The study was carried out as a factorial experiment based on CRD with 6 replications. The inoculation dosages of mycorrhiza were 1000 spore (AM1; 50 g), 2000 spore (AM2; 100 g) and non-mycorrhizal inoculated plants (AM0) as controls. Nitrogen treatments were 52.5 (50% of Johnson nutrient solution= NO_3-50), 78.75 (75% of Johnson nutrient solution= NO_3-75) and 105 (100% of Johnson nutrient solution= NO_3-100) mg L^{-1} of Johnson nutrient solution (Jones, 1930). The components of Johnson nutrient solution are introduced in Table 1.

Seeds of cucumber (*Cucumis sativus*cv. Super N3) were germinated in peat at 25 ℃ for 30 days. The seedlings with uniform sizes were transplanted to 3-L pots containing sand which had received mycorrhiza inoculum of *Glomus mosseae* according to mycorrhizal treatments from Touran Biotech Company (Shahroud, Iran). The interaction of mycorhiz and root was observed with light microscopic through examination of all roots, stained with Chlorazol Black E at the root surface. The pots were irrigated daily with about 50 ml Johnson nutrient solution with defined treatments.

Chlorophyll content was measured using chlorophyll meter (SPAD-502, Minolta Corp., Ramsey, NJ, USA) and FV/FM was measured by chlorophyll florescence (OS- 30, USA) after 3 weeks. Nitrate reductase (NR) activity was measured according to Sagi et al. (1997) after one month.

In order to determine total phenol content in 1 g of the leaves, McDonald et al.'s (2001) procedure was followed. Briefly, each leave sample was mixed with 5 ml folin ciocaltea and 4 ml aqueous Na_2CO_3 separately. The phenol concentration was determined at 765 nm as gallic acid equivalents per gram (mg GAE g^{-1} DW) by a spectrophotometer.

Antioxidant activity of cucumber leaves was determined following Koleva et al.'s (2002) procedure. Briefly, 3 mg of samples were dissolved in 5 ml methanol stock and 1.4 ml of this solution was blended with 0.6 ml of DPPH solution. After 30 min, the absorbance of the solution was recorded at 515 nm by a spectrophotometer (V-530, JASCO, Japan) against methanol as a blank. The 0.2 mM of DPPH solution in methanol was used as a stock of DPPH for determination of the free radical scavenging activity of the samples.

The antiradical activity was calculated by the following equation:

$$\text{Antioxidant activity} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

$A_{control}$: absorbance of the DPPH solution

A_{sample}: absorbance of the DPPH solution after the addition of the sample

At the end of the experiment when plants started anthesis (30 days after transplanting), plants were harvested, fresh weights of roots and shoots were measured and then, all the samples were oven dried at 70°C for 3 days and the dry weights were measured by an analytical balance (to 0.001 decimal places).

Mycorrhizal dependency for shoot (MDS) and root (MDR), which shows the dependency of yield to mycorrhiza inoculation (M1 and M2) compared with the non-inoculated treatment, was also calculated using the following equation (Planchette et al., 1983), which shows the dependency of yield to mycorrhiza inoculation (M1 and M2) compared with the non-inoculation media:

$$MDS = \frac{FWS\ mycorrhiza - FWS\ non\text{-}mycorrhiza}{FWS\ mycorrhiza}$$

FWS= Fresh weight of shoot

$$MDR = \frac{FWR\ mycorrhiza - FWR\ non\text{-}mycorrhiza}{FWR\ mycorrhiza}$$

FWR= Fresh weight of Root

Nitrogen dependency for shoot (NDS) and root (NDR) was calculated by the following modified formula:

$$NDS = \frac{FWS\ full\ nitrogen - FWS\ nitrogen\ deficit}{FWS\ full\ nitrogen}$$

FWS= Fresh weight of shoot

$$NDR = \frac{FWR\ full\ nitrogen - FWR\ nitrogen\ deficit}{FWR\ full\ nitrogen}$$

FWR= Fresh weight of Root

Shoot nutrient efficiency (SNE) was calculated following Sepeher et al.'s (2012) procedure, using the following equation

$SNE=(SDW_{nitrogen\ déficit}/SDW_{full\ nitogen})\times 100$

SDW=shoot dry weight

$RNE=(RDW_{nitrogen\ déficit}/RDW_{full\ nitogen})\times 100$

RDW=Root dry weight

Stress tolerance indexes were calculated using the following modified equation (Farshadfar et al., 2013):

Stress Tolerance; $TOL=FWS_{full\ nitrogen}-FWS_{deficit\ nitrogen}$

FWS= Fresh weight of shoot

The present study was arranged in a factorial design with 6 replications. Data were analyzed using Statistix 8 (Tallahassee FL, USA). All data were subjected to two-way ANOVA and the means were compared for significance by the least significant difference (LSD) test at $P < 0.05$.

RESULTS AND DISCUSSION

Shoot and root fresh weights, shoot dry weight, chlorophyll content, and antioxidant activity increased with both AM1 and AM2 applications compared to non-mycorrical treatment (AM0). Root dry weight did not change significantly with AM application. FV/FM decreased with AM1 and AM2 applications compared to AM0. Total phenol content increased in AM1 and AM2 compared to AM0 and was the highest in AM2 treatment. Conversely, NR activity decreased in AM1 and AM2 compared to AM0 and was the lowest in AM2 treatment (Table 2).

By increasing NO_3-50 level to NO_3-75 and NO_3-100, shoot and root fresh weights, shoot dry weight, and chlorophyll content increased significantly. However, root dry weight and FV/FM did not change significantly. Antioxidant activity and NR activity increased in both NO_3-75 NO_3-100 treatments compared to NO_3-50 treatment and was the highest in NO_3-100 treatment. Total phenol content decreased by increasing NO_3 to NO_3-100 level compared to NO_3-50 to NO_3-75 levels (Table 2).

Mycorrhizal dependency of root was higher in AM1 than AM2 treatment, and the same results were observed for MDS. Nitrogen dependency for shoot and root was higher in NO_3-50 treatment than NO_3-75 treatment. Shoot nutrient efficiency was higher in NO_3-75 treatment than NO_3-50 treatment. Conversely, Stress Tolerance to N deficiency was lower in NO_3-75 treatment than NO_3-50 treatment. Root nutrient efficiency was not affected by NO_3 content (Table 4).

Table 1. The components of Johnson nutrient solution

Compound	M.W	Stock conc. M	Stock conc. gL^{-1}	ml stock per liter final sol.	Element	Final conc. ppm	50% ppm	75% ppm
KNO3	101.10	1.0	101.10	6.0	N	224	112	168
Ca(NO3)2.4H2O	236.16	1.0	236.16	4.0	K	235	117.5	176.25
NH4H2PO4	115.08	1.0	115.08	2.0	Ca	160	80	120
MgSO4.7H2O	246.49	1.0	246.49	1.0	P	62	62	62
					S	32	32	32
		mM			mg	24	24	24
KCl	74.55	50	3.72	1.0	Cl	1.77	1.77	1.77
H3BO3	61.84	25	1.54	1.0	B	0.27	0.27	0.27
MnSO4.H2O	169.01	2.0	0.33	1.0	Mn	0.11	0.11	0.11
ZnSO4.7H2O	287.55	2.0	0.57	1.0	Zn	0.13	0.13	0.13
CuSO4.5H2O	249.71	0.5	0.12	1.0	Cu	0.03	0.03	0.03
H2MoO4	161.97	0.5	0.081	1.0	Mo	0.05	0.05	0.05
Fe-EDTA	346.08	50	21.53	1.0	Fe	2.80	2.80	2.80

Changes in potassium and calcium elements were adjusted by chloride salts.

Table 2. Main effect of *Arbuscular mycorrhiza* (AM) on some characteristics of cucumber

Arbuscular mycorrhiza treatment	Shoot fresh weight/ plant (g)	Root fresh weight/ plant (g)	Shoot dry weight /plant (g)	Root dry weight /plant (g)	Chlorop-ıyll content (SPAD value)	FV/FM	Antioxida-nt activity (% inhibition)	Total phenol content (mg GAE g^{-1} FW)	NR activity (mg g^{-1} FW)
AM0	144.1 b	19.2 b	11.0 b	16.7 a	7.05 b	15.08 a	0.15 b	0.22 c	0.07 a
AM1	160.4 a	34.1 a	14.3 a	15.7 a	13.53 a	0.03 b	0.48 a	87.31 b	0.06 b
AM2	158.0 a	31.3a	14.0 a	15.9 a	12.04 a	0.04 b	0.44 a	89.13 a	0.05 c

Means with different letters in each column are significantly different at $P < 0.05$ according to the LSD test.

Table 3. Main effect of different NO_3 concentration on some characteristics of cucumber.

Nitrogen level	Shoot fresh weight/plant (g)	Root fresh weight/plant (g)	Shoot dry weight/plant (g)	Root dry weight/plant (g)	Chlorophyll content (SPAD value)	FV/FM	Antioxid-ant activity % inhibition)	Total phenol content (mg GAE g^{-1} FW)	NR activity (mg g^{-1} FW)
NO_3-50	136.2 b	6.1 b	10.8 b	12.25 a	7.72 b	4.83 a	0.26 c	61.51 b	0.06 c
NO_3-75	158.2 a	8.8 a	13.1 a	12.40 a	10.88 a	5.98 a	0.46 b	65.50 a	0.07 b
NO_3-100	158.1 a	8.2 a	12.9 a	12.18 a	9.02 ab	4.34 a	0.75 a	49.65 c	0.09 a

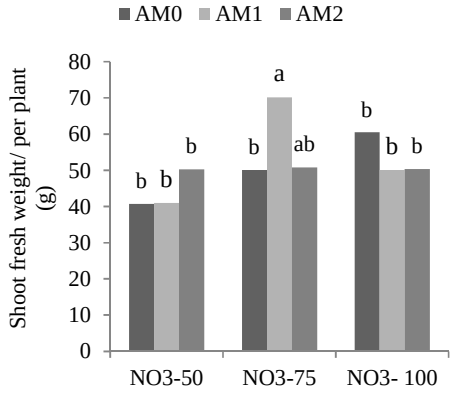
Means with different letters in each column are significantly different at $P < 0.05$ according to the LSD test.

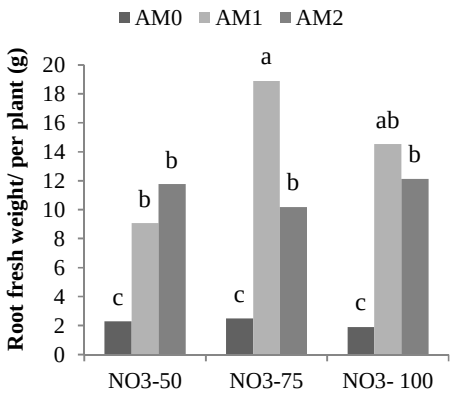
Table 4. The effect of mycorrhiza/NO_3 levels on MD, ND, NE and TOL of root and shoot

Arbuscular ycorrhiza treatment	MDR	MDS	Nitrogen level	NDS	NDR	SNE	RNE	TOL
AM1	0.14	0.10	NO3-50	0.13	0.25	83.72	100.57	21.9
AM2	0.06	0.08	NO3-75	-0.0006	-0.07	101.55	101.80	-0.1
T-test	0.01	0.41		0.027	0.02	0.02	0.27	0.002

Mycorrhizal dependency for shoot (MDS) and root (MDR); Nitrogen dependency for shoot (NDS) and root (NDR); Shoot nutrient efficiency (SNE); Root nutrient efficiency (RNE); Stress Tolerance (TOL)

Fresh weight of shoots increased significantly in NO_3-75×AM1 treatment but it did not show any significant difference between other treatments (Fig 1a). Root fresh weight increased significantly by AM1 and AM2 applications compared to non-mycorrical treatment (AM0) in all levels of NO_3 and the most root fresh weight was observed in NO_3-75×AM1 treatment (Fig 1b). Dry weight of shoots increased significantly in NO_3-75 and NO_3-100 treatments compared to NO_3-50 treatment in all mycorrhiza levels and the lowest dry weight of shoots was found in NO_3-50 treatment with all mycorrhiza levels (Fig 1c). Root dry weight increased by increasing NO_3 from NO_3-50 to NO_3-75 and NO_3-100 in all mycorrhiza levels. However, in NO_3-50 treatment, by applying mycorrhiza (AM1 and AM2), root dry weight significantly increased compared to the AM0 treatment (Fig 1d).





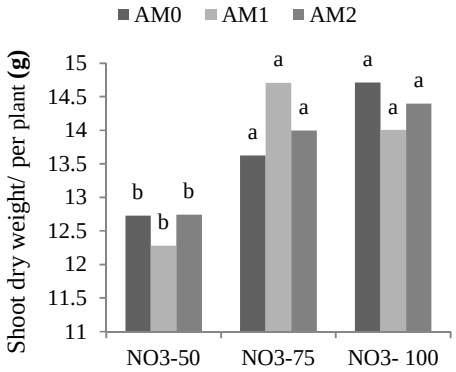


Fig. 1. Interactive effect of mycorrhiza inoculation and nitrogen concentration on shoot fresh weight (a), root fresh weight (b), shoot dry weight (c) and root dry weight (d) per plant. Bars with different letters across treatments are significantly different at $P < 0.05$ according to the LSD test.

Mycorrhiza application in NO_3-50 treatment increased SPAD values significantly when AM2 application was used. The highest SPAD value was observed in NO_3-100×AM1 treatment. However, no significant differences were found between NO_3-100×AM0 and NO_3-100×AM2 treatments and between NO_3-75 treatments in none of three levels of mycorrhiza applications (Fig 2).

No significant differences were observed in FV/FM between the different treatments. However, the highest and lowest levels of FV/FM were found in NO_3-100×AM0 treatment and NO_3-50×AM1 treatment, respectively (Fig 3).

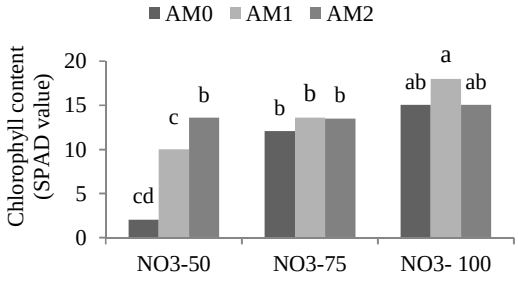


Fig. 2. Interactive effect of mycorrhiza inoculation and nitrogen concentration on SPAD value. Bars with different letters across treatments are significantly different at $P < 0.05$ according to the LSD test.

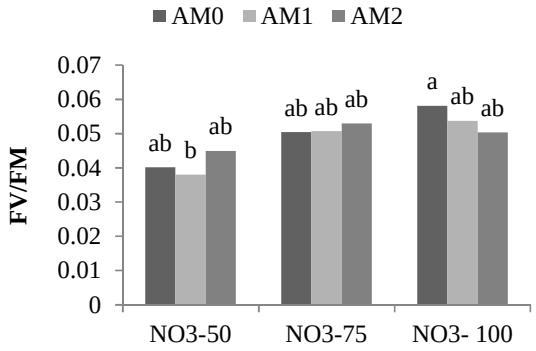


Fig. 3. Interactive effect of mycorrhiza inoculation and nitrogen concentration on FV/FM. Bars with different letters across treatments are significantly different at $P < 0.05$ according to the LSD test.

Phenol content of cucumber leaves increased by AM1 and AM2 applications compared to AM0 treatment in all levels of NO_3. The highest phenol content was observed in NO_3-75×AM1 treatment (Fig 4).

Antioxidant activity decreased in NO_3-75 and NO_3-100 treatments, especially with AM2 application and the highest antioxidant activity was found in NO_3-50 treatments with all three levels of AM applications (Fig 5).

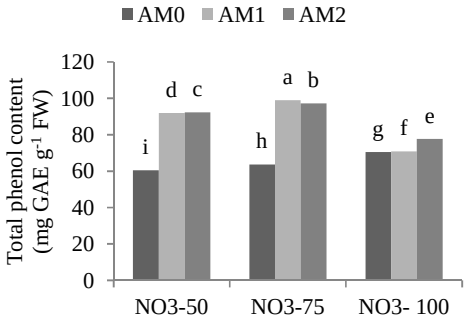


Fig. 4. Interactive effect of mycorrhiza inoculation and nitrogen concentration on total phenol content. Bars with different letters across treatments are significantly different at $P < 0.05$ according to the LSD test.

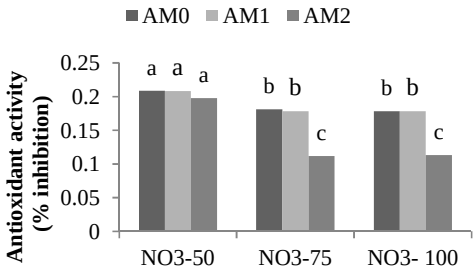


Fig. 5. Interactive effect of mycorrhiza inoculation and nitrogen concentration on antioxidant activity. Bars with different letters across treatments are significantly different at $P < 0.05$ according to the LSD test.

NR activity of the leaves increased in NO_3-100 treatment in all levels of AM applications. However, there were not significant differences between these treatments and NO_3-50/AM2 and NO_3-75/AM2 treatments. NR activity was the lowest in NO_3-50/AM0, NO_3-50/AM1 and NO_3-75/AM0 treatments (Fig 6).

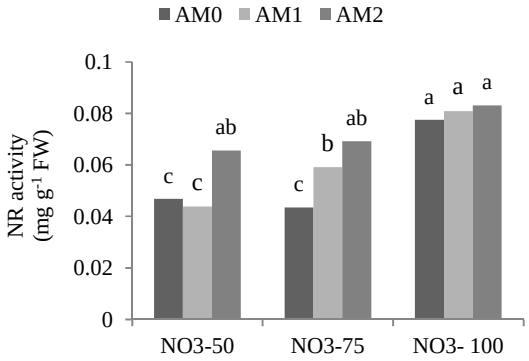


Fig. 6. Interactive effect of mycorrhiza inoculation and nitrogen concentration on NR activity. Bars with different letters across treatments are significantly different at $P < 0.05$ according to the LSD test.

Nitrogen fertilization at moderate concentration level increased the plant growth of some leafy vegetable crops, while high nitrogen level reduced plant growth by increasing N supply (Chen et al., 2004). In the present study, by increasing NO_3 level from NO_3-50 to NO_3-75 and NO_3-100, shoot and root fresh weights and shoot dry weight of cucumber significantly increased. Also, shoot fresh weight and root dry weight increased by mycorrhiza inoculation in NO_3-50 treatment. Similar results were reported by Vazquez et al. (2002) who found that shoot and root weights of alfalfa increased by mycorrhiza colonization under the lowest nitrogen concentration. However, Hays et al. (1982) reported that plant growth of blue grama was enhanced by increasing nutrient solution (N and P), no mycorrhizal infection occurred at high N and P levels and mycorrhiza inoculation caused a lower shoot and root weight at high N level (Hays et al., 1982). The amount and timing of N application can also alter plant morphology, nutrient availability, and net photosynthesis (Zapata & Zaharah, 2002). It seems that by increasing nutrient absorption, the photosynthesis traits improved and caused more assimilate production (Zapata & Zaharah, 2002). Nitrogen and phosphorous are critical determinants of plant growth and productivity, and plant growth and root morphology are important parameters for evaluating the effects of supplied nutrients (Zapata & Zaharah, 2002). N is required in the largest quantities, and its availability and internal concentration affect the partitioning of biomass between roots and shoots (Zhao et al., 2008). Although high N availability affects root and shoot biomass production (Barraclough et al., 1989), P plays an important role in lateral root morphology and

root branching (López-Bucio et al., 2003) and influences not only root development, but also the availability of nutrients (Jin et al., 2005).

In the present experiment, MDS did not show any significant effect on shoot fresh weight, which shows that shoot fresh weight did not depend on mycorrhiza application (Table 3), except in NO_3-75×AM1 treatment and AM2 application (Fig 1a). Also, in the medium N deficiency, mycorrhiza improved the root fresh weight effectively in NO_3-75×AM1 treatment (Fig 1b). These results supported the data of MDR which showed that generally AM1 application was more effective in increasing root fresh weigh (Table 3), especially in the NO_3-75 treatment. In agreement with the present study, the result of another study showed that plant height of chickpea and shoot fresh and dry weights of this plant significantly increased by increasing N concentration and mycorrhiza inoculation (Tufenkci et al., 2005). Johansen et al. (1994) showed that dry weights of shoots and roots increased by increasing N concentration, and mycorrhiza colonization (*G. intraradices*) increased cucumber cv. Aminex weight at all nitrogen levels (100, 200, and 400 mg N per kg soil). In the present study, it seems that in the medium and the optimum N levels of the nutrient solution, mycorrhiza had the same effect on root fresh weight and in the high rate of N application, the mycorrhiza application was more effective in increasing root dry weight than shoot dry weight of cucumber (Fig 1c and 1d).

The SPAD value indirectly quantifies chlorophyll content and plant nitrogen status (Wu et al., 2007). In the present study, higher SPAD value was observed in the mycorrhizal inoculated plants compared to the non-mycorrhizal inoculated ones at all nitrogen levels. Huang et al. (2011) stated that high chlorophyll content in the leaves of the mycorrhizal inoculated plants confirms that symbiosis plays a key role in modifying photosynthesis and metabolic activity. The SPAD values were higher in the mycorrhizal inoculated plants than in the non-mycorrhizal inoculated ones in the study of Busquet et al. (2010). Similar results were observed by Campanelli et al. (2013) who found that SPAD value of alfalfa increased by mycorrhiza inoculation under non-stress conditions, but was not influenced under salinity stress. Chlorophyll and carotenoid synthesis are dependent upon mineral nutritions. N is the most important elemental factor in chlorophyll biosynthesis. It might be due to the optimum availability of N, which plays a vital role in cell division and the formation of active photosynthetic pigments including chlorophyll. Green pigments in leaves also depend on P concentration (Daughtry et al., 2000).

Zhu et al. (2011) reported that Fv/Fm was stimulated by mycorrhiza inoculation under non-stress conditions and temperature stress condition. In the present study, mycorrhiza inoculation increased Fv/Fm in NO_3-75 treatment, while mycorrhiza symbiosis reduced Fv/Fm in NO_3-100.

In the present study, nitrogen at a moderate level (NO_3-75) increased total phenol content of cucumber. Giorgi et al. (2009) showed that by reducing N supplementation, total phenol content of yarrow increased. In our study, mycorrhiza inoculation with 2000 spore (AM2) significantly increased total phenol content of cucumber leaves. In agreement with the present study, the increase in total phenol content by mycorrhiza inoculation was also observed by Kapoor (2008), Al-Askar and Rashad (2010), and Mathur and Vyas (1996). Chen et al. (2013) reported that polyphenol content of cucumber increased by mycorrhiza inoculation under non-stress and low temperature stress conditions. Similar results were observed in the present experiment as both mycorrhiza inoculations increased total phenol content of cucumber under N deficit and normal N supply. The highest total phenol content was observed in the N deficit (NO_3-75) treatment by mycorrhiza inoculation with 1000 spores.

The highest deleterious effect of stress on cucumber was found in the NO_3-50 treatment, and Mycorrhiza application did not significantly improve it, because it caused the highest antioxidant activity to moderate the stress condition, but in NO3-75 and NO3-100 treatments, mycorrhiza application effectively decreased stresses on plants, due to not stimulating antioxidant activities. Giorgi et al. (2009) showed that nitrogen deficit reduced plant growth, total nitrogen, chlorophyll, and carotenoids contents. Nitrogen significantly increased total phenol compound and antioxidant contents of root and leaves of yarrow (Achillea collina Becker ex Rchb.) compared to the normal nitrogen supply condition (Giorgi et al., 2009). In agreement with the present study, increasing N supply significantly reduced antioxidant activity of chrysanthemum flower (Liu et al., 2010). Liu et al. (2010) reported that an excess N supply negatively affected the antioxidant activity and, thereby, reduced the quality of chrysanthemum. In agreement with the present study, Ordookhani and Zare (2011) reported that antioxidant activity of tomato increased by mycorrhiza inoculation as compared to non-mycorrhiza inoculated plants. Similar results were observed by Akhtar Maya and Matsubara (2013) who reported that mycorrhiza inoculation increased antioxidant activity of leaf, root, and tuber of cyclamen.

Nitrate reductase (NR) activity of cabbage, spinach and rape significantly increased with high N supplementation (Chen et al., 2004). Similarly, an increase in NR activity by mycorrhiza inoculation was observed in onion under water stress by Azcon and Tobar (1998) and in rice under low temperature stress by Liu et al. (2013). Caravaca et al. (2005) showed that drought stress caused a reduction in NR activity and mycorrhiza inoculation improved NR activity of the plants under drought stress. In the present study, it seems that by increasing N level of nutrient solution and NO_3 availability for plants, the NR activity increased to convert N to an usable form for improving plant metabolism. The application of mycorrhiza in high level (AM2) along with low NO_3 levels (NO_3-50 and NO_3-75) effectively increased the absorption of NO_3 which resulted in the same NR activity as was observed in the high level of NO_3 application; i.e., NO_3-100 (Fig 6). Evidence is on the rise that the AM symbiosis with host plants changes the nutrient uptakes of the plants (Li et al., 2006). Whether and how the plant uptake pathway for N is affected by the colonization with AM fungi is

currently unknown, but some pieces of evidence were presented that showed some plant N transporters were downregulated in colonized roots (Kobae et al., 2010). It was also shown that fungal N uptake system have a high affinity for the uptake of N from the soil (Pérez-Tienda et al., 2012), but how the expression of these transporters is regulated is largely unknown. The transcript levels of some of these transporters are substrate inducible, and regulated by the NH4+ supply and/or fungal NH4+ status (Lopez-Pedrosa et al., 2006). Other researches showed different results on the efficiency of mycorrhiza on N absorption in different plants (Mensah et al., 2015). Evidence was presented that there is a high intraspecific diversity in the efficiency with which fungi are able to improve the N nutrition of their host (Mensah et al., 2015).

CONCLUSIONS

In general, high nitrogen stress which resulted from low N supply and was observed in the NO_3-50 treatment had a deleterious effect on plant growth (decreasing root fresh and dry weights compared to high N supply treatments). Both mycorrhiza inoculation levels enhanced root fresh and dry weights while these parameters were unaffected by mycorrhiza inoculation in nitrogen stress conditions. The lowest total phenol content of tested plants was found in the NO_3-50 treatment. However, both mycorrhiza inoculation levels stimulated total phenol content of tested plants. Mycorrhiza inoculation with 2000 spores (AM2) was more effective than AM1 treatment. The highest NR activity was observed in the NO_3-100 treatment in all mycorrhiza inoculation levels while the lowest NR activity was achieved in the NO_3-50 and NO_3-75 treatments. Mycorrhiza inoculation increased NR activity. Based on the results of this study, it can be concluded that the low nitrogen supply caused high stress on cucumber production during the vegetative growth stage. Mycorrhiza inoculation can compensate for some parameters like root fresh and dry weights, phenol content and NR activity under high nitrogen deficit stress condition (NO_3-50), especially under high mycorrhiza inoculation condition (AM2). It seems that mycorrhiza inoculation could compensate for moderate N deficiency (NO_3-75) in most parameters and had the same results as NO_3-100 provided. Therefore, it can be concluded that by mycorrhiza inoculation, especially the 1000 spores treatment (AM1), a moderate level of NO_3 (NO_3-75) can be used and these application levels can be very effective for greenhouse cucumber production.

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واژه‌های کلیدی:
فعالیت آنتی اکسیدانی
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چکیده- این مطالعه به‌منظور بررسی تأثیر کاربردهای مختلف نیتروژن بر رشد، میزان فنل، آنتی‌اکسیدان و فعالیت آنزیم نیترات رداکتاز همراه با مایه‌زنی میکوریزا در رقم Super¬N3 خیار به‌صورت فاکتوریل در قالب یک طرح کاملاً تصادفی با ۶ تکرار با ۳ سطح نیتروژن NO3-50، NO3- 75 وNO3-100 و ۳ سطح مایه‌زنی میکوریزا شامل مایه‌زنی ۱۰۰۰ اسپور (AM1)، مایه‌زنی ۲۰۰۰ اسپور (AM2) و بدون مایه‌زنی میکوریزا (AM0) موردبررسی قرار گرفت. نتایج حاصل از ترکیبات نیتروژن با مایه‌زنی میکوریزا نشان داد که وزن‌تر ریشه با مایه‌زنی میکوریزا در تمام سطوح نیتروژن افزایش یافت، درحالی‌که وزن‌تر ساقه با سطوح نیتروژن و مایه‌زنی میکوریزا تحت تأثیر قرار نگرفت. FV / FMدر گیاهان با سطح NO3-75 و مایه‌زنی میکوریزا در مقایسه با تیمار بدون مایه‌زنی میکوریزا بالاتر بود. همزیستی میکوریزا فعالیت آنتی‌اکسیدانی گیاه را در تیمار بدون نیتروژن افزایش داد، به‌طوری‌که AM1 و AM2 فعالیت آنتی‌اکسیدانی را به ترتیب در NO3-50 و NO3-75 نسبت به گیاهان بدون مایه‌زنی میکوریزا افزایش دادند. بالاترین فعالیت آنزیم نیترات رداکتاز در NO3- 50و مایه‌زنی میکوریزا مشاهده شد و فعالیت آنزیم نیترات رداکتاز گیاهان در تمام سطوح نیتروژن کاهش یافت. در کل، می توان نتیجه گرفت که با مایه زنی میکوریزا، به ویژه تیمار ۱۰۰۰ اسپور (AM1)، و سطح متوسط NO3 (NO3-75) می تواند مورد استفاده قرار گیرد و این میزان مصرف می تواند برای تولید خیار گلخانه ای بسیار موثر باشد.