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Effect of amino acid and mycorrhiza inoculation on sweet pepper growth under greenhouse conditions

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ABSTRACT- The production of greenhouse sweet pepper is increasing because of the consumer demand for sweet pepper year around. In this study, physiological characteristics of sweet pepper were evaluated in a greenhouse under different levels of arbascular mycorrhizal fungus, *Glomus intraradices*, (AMF) and various levels of amino acid (AA). Treatments included no AMF as a control (AMF1), 1000 spores (AMF2) and 2000 spores (AMF3) of the AMF and four amino acid concentrations including control (C), 3 g (AA1), 4.5 g (AA2) and 6 g (AA3) of AA. Results indicated that mycorrhiza inoculation and mixture of amino acid increased shoot and root fresh weights. AMF application did not affect transpiration, chlorophyll content, and P and K concentration; nevertheless, photosynthesis improved with AMF. Fruit quality also improved under AMF2 and AMF3 treatments. Finally, it was concluded that with higher AA concentration and 1000 spores of mycorrhiza, more positive effects on sweet pepper growth could be observed.

INTRODUCTION

Green pepper (*Capsicum annuum* L.) is one of the most important vegetables widely cultivated in fields and greenhouses in both soil and soilless substrate around the world. On the other hand, demand for organic production is increasing and organic horticulture discouraged the use of chemical fertilizer (Perner et al., 2007). Mycorrhiza inoculation and amino acid application can be used instead of chemical fertilizer for vegetable production.

Arbascular mycorrhiza is an endomycorrhiz which cause some fungal structures in root cortex which are called vesicles and arbuscles (Quilambo, 2003). Soil microbial communities, which mycorrhizal fungi are an integral component, are central to soil fertility and can affect crop productivity (Rooney et al., 2009). Arbuscular mycorrhizal fungi (AMF) can increase plant growth and nutrient absorption and fruit yield (Al-Karaki et al., 2001; Hajiboland et al., 2010). Mycorrhizal application in pepper increased colonization rate and plant yield under stress conditions (Giri et al., 2007). Mycorrhiza can function in horticulture as a sustainable, biocontrol agent against pathogens, a bioprotectant against toxic stresses, and as a soil-improving anti-erosion agent (Vosátka and Albrecht ova, 2008). The improvement of vegetable nutritional quality can be the result of the existence of mycorrhizal symbiosis through activation of antioxidant, phenylpropanoid, or carotenoid pathways (Baslam et al., 2011).

Amino acid is an organic form of nitrogen (Cerdán et al., 2009) which improved plant growth and yield. Indifferent studies, increasing plant yield was observed in greenhouse tomato, Chinese cabbage and leafy radish by amino acid application (Koukounaras et al., 2013; Cao et al., 2010; Liu et al., 2008). Also. Amino acid application increased flower number, fruit set and fruit yield of tomato (Neeraja et al., 2005). It has also been reported that the foliar application of amino acid increased nitrogen uptake in plant (Liu et al., 2008;Junxi et al., 2010).

To date, numerous studies have been conducted on the use of amino acid and mycorrhiza symbiosis on the growth and yield of plants. Therefore, the present study was conducted to evaluate the effect of a) amino acid application b) mycorrhiza symbiosis and also c) the effect of the synchronic use of amino acid and mycorrhiza symbiosis on sweet pepper in terms of its growth, fruit yield, and photosynthesis and nutrients trait.

MATERIALS AND METHODS

This study was carried out as a factorial experiment based on CRD with 3 replications. Treatments were mycorrhiza inoculation including no mycorrhiza inoculation (AMF1) as control, mycorrhiza with 1000 spores per pot (AMF2) and mycorrhiza with 2000 spores per pot (AMF3) and amino acid concentration including control (C), 3 g (AA1), 4.5 g (AA2) and 6 g (AA3). Of AA.

Each pot was filled with the soil mixture up to 3 cm under its edge. Before transplanting the media, 100 g (1000–2000 spores per pot) AMF inocula, supplied by

Touran Biotech Company (Shahrood, Iran) with a known spore density (approximately 20–30 spores per g), were mixed with the growing media. In addition, the combined treatment was accomplished by using half inoculum amount of each species for a total of 100 g of inocula per pot (so that the total number of spores was similar). At the same time, in order to establish similar initial micro flora communities in all non-mycorrhizal treatments to mycorrhizal treatments, the filtrate of AMF inoculums (without AMF spores) was added into the non-mycorrhizal pots (Green et al., 1999).

Pepper seeds (*Capsicum annum* cv. Gold flame) were germinated and transplanted into 3L pot in a plastic greenhouse in the Department of Horticulture Science at Isfahan University of Technology, Isfahan, Iran, with an average temperature of 30-35°C. All pots were irrigated by Johnson nutrient solution every day about 100 ml. Mycorrhiza inoculations were applied before transplanting according to treatment and amino acid was applied to media together with irrigation after seedling establishment.

One month after transplanting, leaf chlorophyll index was measured by using a nondestructive dual-wavelength chlorophyll meter (SPAD-502, Minolta Corp, USA) and photosynthesis parameters were determined from the youngest fully expanded leaf for 3 replications per treatment by portable area meter (Li-Cor Li-3000, USA). Photosynthetic water use efficiency (PWUE)= photosynthesis rate/stomata conductance ($\mu molCO_2/mol\ H_2O_2$)

After 124 days from transplanting seedlings, inoculated and un-inoculated plants (3 plants replication) were carefully uprooted. The roots were dipped in water to remove substrate particle and were washed. Shoots were excised from the roots using a steel blade; root volume was measured using the method of changes in the water volume (Haghighi et al., 2015). 124 days after transplanting, fruits were harvested and washed by using tap water. Fruits diameter and Total soluble solids (TSS) were measured with a digital caliper (Mitutoyo Corp, Japan) and refracto meter (PAL-1 Brix, Japan), respectively at the end of the experiment. Then, fruits were oven dried at 70°C to a constant weight. Fresh weights and dry weights of fruit were measured by an analytical balance. Then, fresh weights of the roots and the shoots were measured. All 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).

Total nitrogen (N) was quantified in samples of 0.1 g dry weight using the indophenols blue method (Novozamsky et al. 1974).P concentration was determined spectrophotometrically by the ammonium–vanadate–molybdate method (Gericke and Kurmies 1952). Potassium (K) concentration was measured using a flame (Haghighi et al., 2015).

Internal fungal structures (hype, arbuscules, vesicles) were examined under a stereomicroscope at × 100 magnification and the percentage of root length colonized was calculated using the gridline intersect method (Giovanetti and Mosse, 1980).

All data were subjected to two-way ANOVA by using Statistics 8 software (Tallahassee FL, USA) and the means were compared for significance by the least significant difference (LSD) test at $P < 0.05$.

RESULTS AND DISCUSSION

Shoot fresh and dry weight increased in AA3. Root fresh weight increased in AA2 and AA3 but root dry weight increased with amino acid application at all levels. Root volume increased in AA3 and statistically was the same as AA2. However, TSS and IWUE did not change with amino acid application. Fruit fresh and dry weight and fruit diameter increased with AA at all levels. Day to harvest decreased with AA (Table 1).

Chlorophyll content concentration and PWUE increased in AA3. Photosynthesis rate increased in AA2 and AA3 significantly. Compared with control, transpiration, N and K concentration and stomata conductance increased with AA statistically. Mesophyll conductance did not change with AA (Table 2).

Shoot fresh and dry weight, root fresh weight, root volume and TSS did not change significantly. Root dry weight fruit fresh and dry weight IWUE increased with AMF3. Fruit diameter increased with AMF2 and AMF3.Day to harvest decreased with AMF application (Table 3).Chlorophyll content, transpiration, P and K concentration were not affected by AMF. Photosynthesis rate increased with AMF application. Mesophyll conductance, PWUE and N concentration increased in AMF3 (Table 4).

Table 1. Effect of different amino acid concentrations on plant growth and fruit properties of pepper

	Shoot fresh weight (per plant g)	Shoot dry weight (per plant g)	Root fresh weight (per plant g)	Root dry weight (per plant g)	Root volume (ml)	TSS (%)	Fruit fresh weight(g)	Fruit dry weight(g)	IWUE	Fruit diameter (cm)	Day to harvest (Day)
C	38.22 b	3.05b	23.99bc	3.08b	28.88b	7.34a	78.08 b	6.79b	9.28a	5.63b	98.77a
AA1	34.67b	5.15b	18.76c	4.02a	31.11b	7.66a	103.28a	8.76a	10.73a	6.35a	96.11ab
AA2	31.67b	1.13b	36.71a	3.80ab	41.11ab	7.68a	97.38 ab	8.17ab	11.69a	6.64a	98.44ab
AA3	05.80a	13.75a	31.15ab	4.01a	48.88a	8.06a	118.37a	9.39a	5.80a	6.66a	94.11b

C: Control TSS: Total soluble solids. Treatments with the same letters are not significantly different ($P < 0.05$).

Table 2. Effect of different amino acid concentrations on photosynthesis trait and nutrient concentration

Chlorophyll content (SPAD value)	Chlorophyll content (SPAD value)	Photosynthesis rate (µmol m^{-2} s^{-1})	Transpiration ($mmolm^{-2}$ s^{-1})	Stomata conductance (mmol m^{-2} s^{-1})	Mesophyll conductance ($mmolm^{-2}$ s^{-1})	PWUE (mol $CO_2mmol^{-1}H_2O$)	N (%)	P (mg/kg DW)	K (mg/kg DW)
C	11.99b	10.86b	1.14b	0.03b	353.29 a	0.61bc	0.24b	6.33E-03b	11.65b
AA1	18.84b	14.74b	2.73a	0.06ab	498.14 a	0.55c	0.51a	6.10E-03b	13.98a
AA2	19.63b	23.11ab	3.62a	0.10a	460.35 a	0.70ab	0.591a	5.77E-03b	14.88a
AA3	40.69a	34.89a	2.48a	0.06ab	232.41 a	0.72a	0.67a	9.46E-03a	14.64a

C, AA1, 2 and 3: control, 3g, 4.5g and 6 g amino acid per pot, respectively.Treatments with the same letters are not significantly different ($P < 0.05$).

Table 3. Effect of mycorrhiza inoculation on photosynthesis trait and nutrient concentration

	Shoot fresh weight (perplantg)	Shoot dry weight (per plantg)	Root fresh weight (per plantg)	Root dry weight (per plantg)	Root volume (ml)	TSS	Fruit fresh weight(g)	Fruit dry weight (g)	IWUE	Fruit diameter (cm)	Day to harvest (day)
AMF1	50.53a	4.85a	27.76a	3.52ab	35.83a	7.55a	91.99 b	7.52b	6.37b	5.95b	101.00a
AMF2	52.73a	5.51a	29.28a	3.48b	35.83a	7.45a	92.41b	7.76b	7.85b	6.30ab	94.83 b
AMF3	54.49a	6.94a	25.92a	4.18a	37.08a	8.05a	113.43a	9.55a	13.90a	6.72a	94.75 b

AMF1: non-mycorrhiza inoculation, AMF2 and AMF3, plant inoculation with 1000 and 2000 spores in pot, respectively. Treatments with the same letters are not significantly different ($P < 0.05$).

Table 4. Effect of mycorrhiza inoculation on plant growth and fruit properties of pepper

	Chlorophyll content (SPADvalue)	Photosynthesis rate (µmol m^{-2} s^{-1})	Transpiration (mmol m^{-2} s^{-1})	Stomata conductance (mmol m^{-2} s^{-1})	Mesophyll conductance ($mmolm^{-2}$ s^{-1})	PWUE ($molCO_2mmol^{-1}$ H_2O)	N (%)	P (mg/kg DW)	K (mg/kg DW)
AMF1	20.15a	16.18b	2.61a	0.07a	250.98 b	0.61b	0.47ab	6.71a	13.85a
AMF2	22.61a	28.73a	2.45a	0.06a	319.87b	0.60b	0.41b	6.83 a	13.97a
AMF3	25.60a	17.79ab	2.42a	0.06a	587.29a	0.74a	0.63a	7.21a	13.54a

AMF1: non-mycorrhiza inoculation, AMF2 and AMF3, plant inoculation with 1000 and 2000 spores in pot, respectively. Treatments with the same letters are not significantly different ($P < 0.05$).

Shoot fresh weight increased with AMF1 and AMF2 at all mycorrhiza levels. The lowest shoot fresh weight was seen in the treatment in which mycorrhiza was not applied (i.e. C×AMF1, AA1×AMF1 AA2 ×AMF1 AA3×AMF1) (Figure 1a). Shoot dry weight increased in AA3×AMF2 and AMF3 (Fig. 1b). Root fresh weight increased in C×AMF3 and AA3×AMF2 and AMF3 while the lowest was seen in AA2×AMF1 (Figure 1 c). Root dry weight increased with AMF2 and AMF3 in AA1and AA3 (Fig. 1d).

The interactive effect of mycorrhiza inoculation and different amino acid concentrations on TSS did not show any significant difference (data were not shown).Root volume was the highest in AA3 with AMF (Fig. 2).

Fruit fresh and dry weights were the highest in AA2 and AA3 with AMF2 (Fig. 3).Fruit diameter did not change between tretaments and earliness of friut accelarate in all treatments compared with C×AMF1 (Fig. 4).

SPAD value decreased at all AA concentrations and AMF2 sharply increased SPAD value at all AA levels (Fig. 5a). Photosynthesis rate significantly increased in AA2 and AA3 and mycorrhiza inoculation with AMF2 signicficantly increased photosynthesis at all AA concentrations and control (Fig. 5b). Transpiration and stomata conductance signicifanty increased in AA2 as comparedto control (Fig. 5c and 5d). The highest mesophyll conductance was in AA2 combination with AMF2 (Fig. 5f).

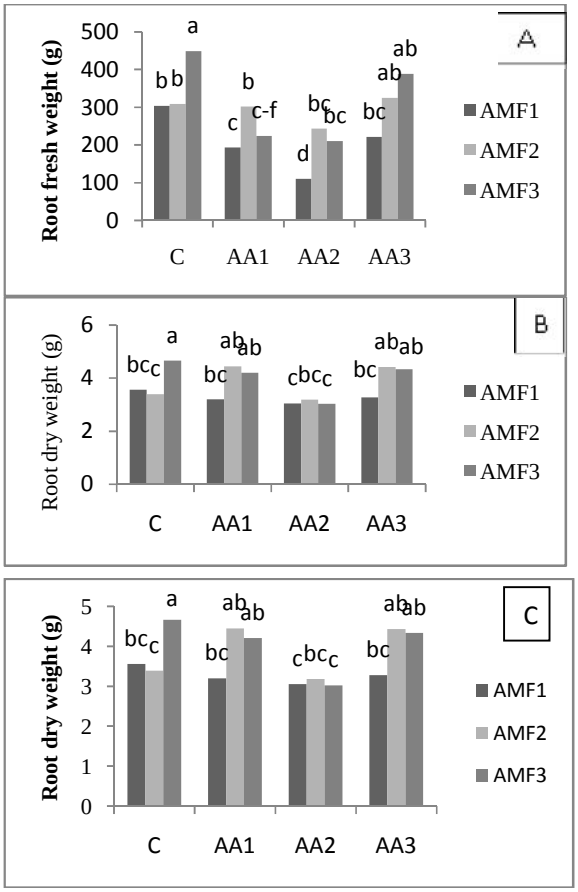


Fig. 1. Interactive effect of mycorrhiza inoculation and different amino acid concentrations on shoot fresh weight (a), dry weight (b), root fresh weight (c) and dry weight (d). (AMF1: non mycorrhiza inoculation, AMF2 and AMF3 plant inoculation with 1000 and 2000 spores in pot, respectively. C, AA1, 2 and 3: control, 3 g, 4.5 g and 6 g amino acid per pot, respectively).Treatments with the same letters are not significantly different ($P < 0.05$)

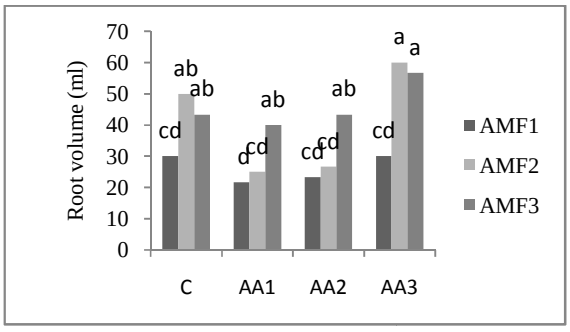


Fig. 2. Interactive effect of mycorrhiza inoculation and different amino acid concentrations on root volume(AMF1: non mycorrhiza inoculation, AMF2 and AMF3 plant inoculation with 1000 and 2000 spores in pot, respectively. C, AA1, 2 and 3: control, 3g, 4.5g and 6 g amino acid per pot, respectively). Treatments with the same letters are not significantly different (P=0.05)

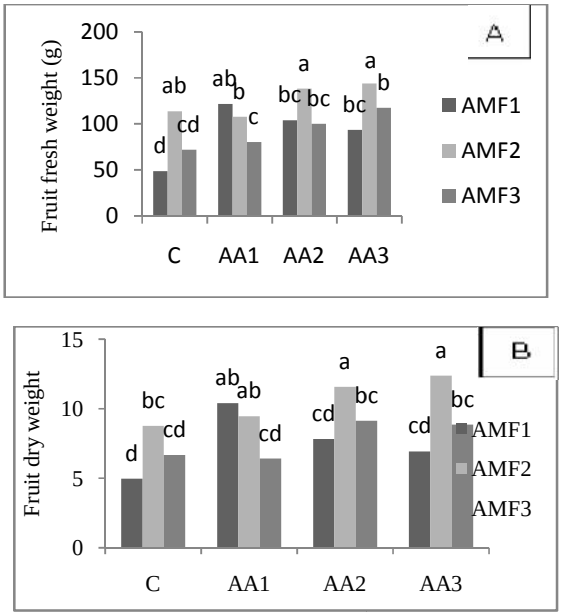


Fig. 3. Interactive effect of mycorrhiza inoculation and different amino acid concentrations on fruit fresh weight (a), fruit dry weight (b).(AMF1: non mycorrhiza inoculation, AMF2 and AMF3 plant inoculation with 1000 and 2000 spores in pot, respectively. C, AA1, 2 and 3: control, 3g, 4.5g and 6 g amino acid per pot, respectively). Treatments with the same letters are not significantly different ($P < 0.05$)

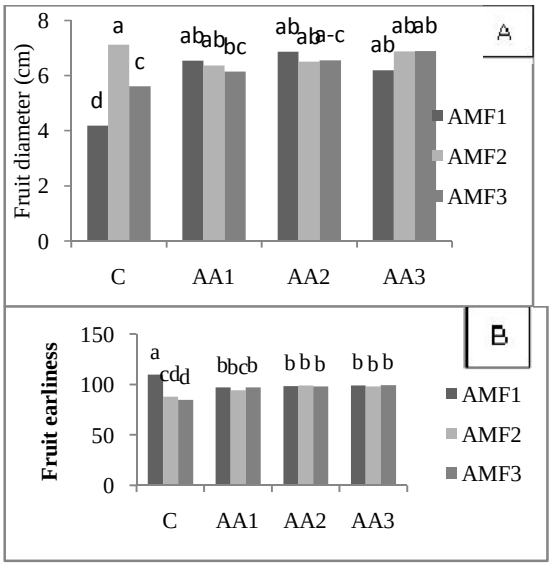


Fig. 4. Interactive effect of mycorrhiza inoculation and different amino acid concentrations on fruit diameter (a) and fruit earliness (b). (AMF1: non mycorrhiza inoculation, AMF2 and AMF3 plant inoculation with 1000 and 2000 spores in pot, respectively.C, AA1, 2 and 3: control, 3g, 4.5g and 6 g amino acid per pot, respectively). Treatments with the same letters are not significantly different ($P < 0.05$)

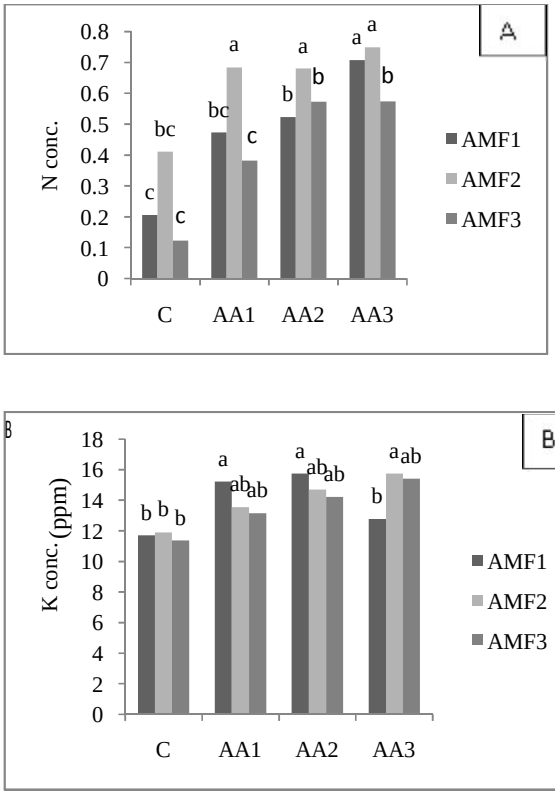


Fig. 5. Interactive effect of mycorrhiza inoculation and different amino acid concentrations on SPAD value (a), photosynthesis rate (b), transpiration (c), stomata conductance (d) and mesophyll conductance of pepper.(AMF1: non mycorrhiza inoculation, AMF2 and AMF3 plant inoculation with 1000 and 2000 spores in pot, respectively. C, AA1, 2 and 3: control, 3g, 4.5g and 6 g amino acid per pot, respectively). Treatments with the same letters are not significantly different (P=0.05)

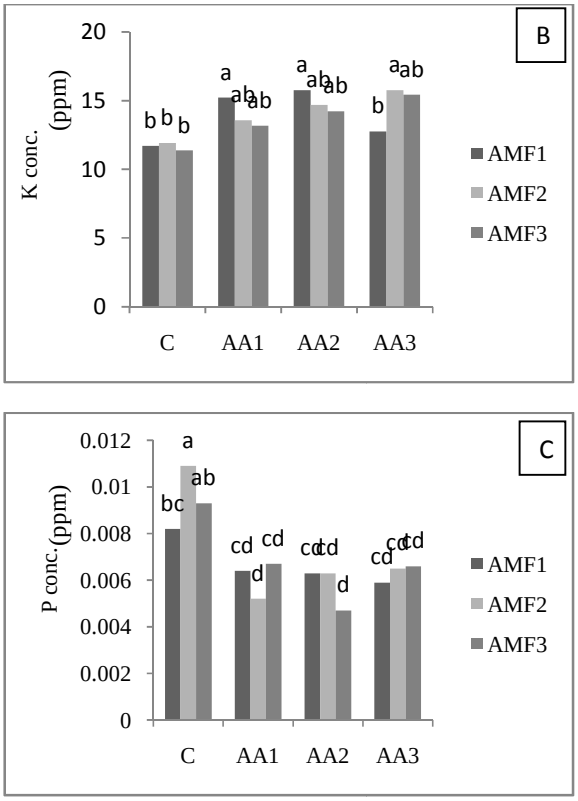
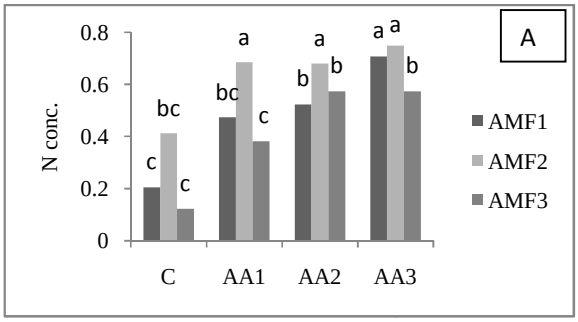


Fig. 6. Interaction effect of mycorrhiza inoculation and different amino acid concentrations on N (a), P (b) and K (c) concentration. (AMF1: non mycorrhiza inoculation, AMF2 and AMF3 plant inoculation with 1000 and 2000 spores in pot, respectively. C, AA1, 2 and 3: control, 3g, 4.5g and 6 g amino acid per pot, respectively). Treatments with the same letters are not significantly different ($P < 0.05$)

K concentration did not change significantly between different AMF levels in different AA (Fig.6-a). P concentration was the highest in AMF2× AA1 and did not change significantly between other treatments (Fig. 6-b). N concentration increased in AMF2 with AA2,AA3 and AA4 (Fig.6-c).

Root inoculation increased with AMF3 compared with AMF2 at all amino acid levels (Fig. 7).



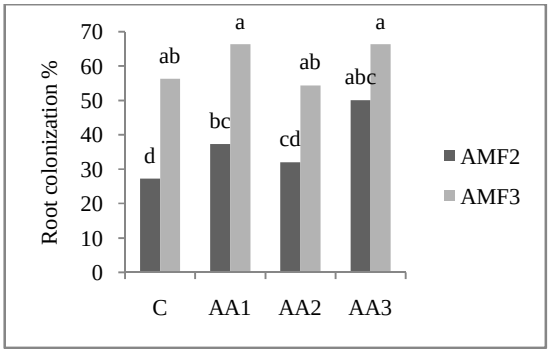


Fig. 7. Interaction effect of mycorrhiza inoculation and different amino acid concentrations on sweet pepper root colonization.(AMF1: non mycorrhiza inoculation, AMF2 and AMF3 plant inoculation with 1000 and 2000 spores in pot, respectively. C, AA1, 2 and 3: control, 3g, 4.5g and 6 g amino acid per pot, respectively).Treatments with the same letters are not significantly different ($P < 0.05$)

The Main Effect of Different Amino Acid Concentrations and Mycorrhiza Inoculation on Plant Growth and Fruit Properties of Pepper

In line with the results of the present study, amino acid application has been shown to have positive effects on the Chinese cabbage (Cao et al., 2010) yield and quality of flowering as well as on leafy radish biomass production (Liu et al., 2008). Moreover, the role of amino acids to act as bio stimulants in plants under a biotic and biotic stress conditions has been demonstrated (Maini and Bertucci, 1999; Heuer, 2003). Furthermore, foliar application of amino acid with concentrations of 1% and 2% on lettuce significantly increased the plant height, and number and weight of the leaves (Bassiouny, 1993; Bassiouny et al., 1993). On the other hand, amino acid application in the tomato hydroponic cultivation nutrient solution has been demonstrated to have negative effects on the plant growth (Garcia et al., 2006). Also, similar to what we found for pepper, in a piece of research, it was shown that by using AMF in *Medic ago sativa* L., root dry weight and length increased (Klironomos, 2003).

In the present study, some photosynthetic traits like chlorophyll index and transpiration were not affected by AMF application but Mesophyll conductance, PWUE and N concentrations increased in AMF3. Mycorrhiza inoculation increased nutrient elements mobilization under drought stress so that stress damages decreased. Many reports demonstrated that by applying AMF to the soil substrate nutrient, absorption increased; likewise, plant growth and crop productivity under sub optimal conditions improved and lower yield losses were observed (Rooney et al., 2009; Al-Karaki et al., 2001). Mycorrhiza inoculation increased chlorophyll by enhancing light harvesting; however, in the present study, chlorophyll index did not show any significant difference under different AMF concentrations (Zhu et al., 2011).

The Main Effect of Different Amino Acid Concentrations and Mycorrhiza Inoculation on Photosynthesis Trait and Nutrient Concentration

Positive interactions between amino acids and some mineral nutrients have been observed in nature. Plants are able to increase nutrient availability in the rhizosphere (Dakora and Phillips, 2002) by exuding amino acids through the roots. Besides, nutrient translocation through the vascular system has been shown to be facilitated by the enhancement of their permeability in cell membranes (Franco et al., 1994). The foliar application of amino acids improved uptake efficiency of N from the soil and prevented N loss through leaching (Liu et al., 2006; Junxi et al., 2010). In line with the results of the present study, in tomato hydroponic grown-plants, growth improved by adding amino acids to the nutrient solution (Liu et al., 2008). Neeraja et al. (2005) found that amino acids application increased the number of flowers, fruit setting and fruit yield of tomatoes.

In low fertile root media, the AMF application increased plant ability to survive under this condition. In a *Medicago sativa* L. plant by AMF application, nutrient absorption ability and P and Zn uptake increased (Klironomos, 2003). In the present study, N and K absorption also increased with AMF application. Under salinity stress, stomata conductivity increased by AMF application in lettuce and also had higher photosynthesis rate observed. These microorganisms helped plants to overcome stress condition with less damages (Aroca et al., 2013). Also, Giri and Mukerji (2007) reported that with AMF application under stress condition, photosynthetic capacity increased and plants produced more biomass.

The Interactive Effects of Amino Acid and AMF Application on Evaluated Parameters

Nemec (1992) reported that shoot and root growth increased by mycorrhiza inoculation with *Glomusintradicas* in different growth media. In the present study, root fresh and dry weight were affected more by AMF application and increased in C×AMF3 treatment. Also, potassium concentration was not affected by different AMF and AA levels. It was concluded that mycorrhiza dependency was the highest in the media without amino acid. All growth parameters of sorghum increased by inoculation with mycorrhiza (*Glomus* spp) along with bacterial isolated with plant (Hameeda et al., 2007). Mycorrhizal plants had higher aerial dry weight and fruit fresh weight, and also produced larger inflorescence, total and marketable fruit numbers compared with non-mycorrhiza plants. Also, greater fruit yield in mycorrhizal plants was correlated with the highest flower production and larger inflorescence compared with the non-mycorrhizal plants (Conversa et al., 2013). Furthermore, in tomato plants, mycorrhiza inoculation improved fruit load and caused an enhancement in total yield of the plant.

CONCLUSIONS

As a natural nitrogen source, amino acids could be used in plant growth media and mycorrhiza could improve nutrient elements and water absorption under deficiency conditions. In the present study, increases in growth with mycorrhiza and amino acid are due to increasing nutrient uptake and photosynthesis traits which resulted in more growth. It can be concluded that a moderate amount of mycorrhiza inoculation, 1000 spores per pot, as well as 6 g amino acid are recommended for soilless production of pepper.

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اثر آمینواسید و تلقیح مایکوریز بر رشد فلفل دلمه در گلخانه

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همزیستی

چکیده-تولید فلفل دلمه‌ای در سالهای اخیر به دلیل افزایش تقاضای مصرف کننده افزایش چشمگیری داشته است. خصوصیات فیزیولوژیکی فلفل دلمه‌ای در گلخانه‌های تحقیقاتی دانشگاه صنعتی اصفهان تحت تیمارهای قارچ arbascularmycorrhiza گونه *lomusintraradices*(AMF) و سطوح مختلف آمینواسید (AA) ارزیابی شدند. تیمارها شامل AMF1 (بدون مایه زنی میکوریزا) به عنوان شاهد، AMF2 با۱۰۰۰ اسپور و AMF3 با ۲۰۰۰ اسپور مایکوریز و ۴ غلظت شاهد (C، بدون امینو اسید)، ۳ گرم(AA1)، ۴/۵گرم (AA2) و ۶ گرم(AA3) امینواسید بودند. نتایج نشان داد که تلقیح مایکوریز و مخلوط آمینواسید، وزن تر و خشک ریشه و شاخساره را افزایش می‌دهد. کاربرد قارچ، تعرق، میزان کلروفیل و غلظت فسفر و پتاسیم را تحت تاثیر قرار نداد اما فتوسنتز با کاربرد AMF بهبودیافت. همچنین کیفیت میوه تحت تیمارهای AMF2 و AMF3 بهبود یافت. در نهایت با افزایش غلظت آمینواسید و ۱۰۰۰ اسپور قارچ تاثیرات مثبتی در رشد فلفل دلمه ای مشاهده شد.