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## Growth, yield index, and photosynthesis traits of sweet pepper grown in vermicompost inoculated with *Arbuscular mycorrhiza*

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**ABSTRACT-** The aim of this study was to investigate the effects of “Arbuscular mycorrhizal fungi” (AM fungi) density and different growing media on the growth, photosynthesis parameters and yield of sweet pepper under greenhouse conditions. The experiment was conducted as a factorial plan based on control randomized design (CRD ) by using three growing media, Perlite (PR) and cocopeat (Co) (PR50:Co50 V:V) (C), PR25:Co50: vermicompost (V) 25 (C+V25) and PR25:Co25: V50 (C+V50), and three levels of AM fungi inoculation (0, 1000 and 2000 spores) with three replications. Results indicated that AM fungi inoculation and mixture of vermicompost increased shoot and root fresh weights, Total Suspended Solids (TSS), fruit fresh and dry weights in the C+V50 compared to other treatments. Fruit yield increased more than 100 and 94.95% with AM-fungi inoculation with 2000 spores at C+V25 and V50 treatments, respectively. Mycorrhiza dependency decreased with high application rate of vermicompost, and vermicompost dependency was the highest in non-inoculated plants. With AM-fungi inoculations, the chlorophyll level (SPAD values) was increased in C+V25 by 100%. Photosynthesis rate was increased in C+V25×M1 significantly compared to other treatments. Nitrogen, phosphorus, and potassium concentrations significantly increased by mycorrhiza inoculation in the high vermicompost ratio.

### INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is one of the most important vegetable crops with antioxidant properties widely cultivated in the field and greenhouse in both soil and soilless substrates. Two critical factors for vegetable production are the growth substrate and fertilization method; on the other hand, in organic horticulture, the use of chemical fertilizer is discouraged (Perner et al., 2007). Recently, the aim of the producers are reducing chemical fertilization and using organic ones to provide healthy food and improve soil physical properties (Gutierrez-Miceli et al., 2008). Vermiculite and cocopeat are limited natural recourses which are not available everywhere. The alternative organic materials such as compost and vermicompost can be used instead of cocopeat and perlite. Bhagat et al. (2013) demonstrated that a combination of growing media is preferred to a single form. Furthermore, cost and environmental risks can be more when using just chemical fertilizers compared with when using organic soil amendments such as plant residues, manure, and compost less (Duong et al., 2012). Vermicompost could affect plant growth indirectly through an effect on soil microorganisms (Cavender et al., 2003). *Arbuscular mycorrhiza* fungi are among the most important soil microorganisms (Sylvia et al., 1998). Since soilless culture has been developed and soilless substrates are used for plant propagation (Martinez et al., 2013;

Chavez et al., 2008), the beneficial effects of soil microorganisms have been ignored. Arbuscular mycorrhizal fungi are known to stimulate host plant growth, enhance nutrient uptake, especially phosphorus, enhance water uptake and affect soil physical properties (Ustuner et al., 2009). The beneficial effects include helping plants to mobilize and acquire nutrients from the substrate (Perner et al., 2007). Compost improves soil fertility and affects a number of phytopathogenic fungi (Reuveni et al., 2002). Yang et al. (2002) suggested that the combination of organic material such as compost with mycorrhiza inoculation is an appropriate approach in ensuring high yield in agricultural production. The effect of organic materials on mycorrhiza inoculation may be due to their effect on a substrate structure, microbial activity, chemical exudates from organic matter, and water holding capacity (Ryan et al., 1994). The result of Cavender et al. (2003) indicated that vermicompost increased mycorrhiza colonization of roots, particularly in peat growth medium. For healthy production, good quality and also high yield in plant production, using mycorrhiza and organic growing media are very important (Ortas & Ustuner, 2014). Ustuner et al. (2009) indicated that to conclude maximal yield in organic culture by using *arbuscular mycorrhiza* fungi technology, the selection of organic substrate and

mycorrhiza fungi is critical. In the present experiment, the common substrate used in Iran (compost and vermicompost) was chosen as a media and one of the most popular soilless products, sweet pepper, was used as a plant. The aim of the present study was to evaluate a) increasing the rate of compost application on substrate of soilless culture containing cocopeat and perlite b) studying the effect of mycorrhizal fungi inoculation on soilless culture to use its benefits in soilless media c) studying the effect of mycorrhiza inoculation in organic substrate on plant growth, photosynthesis traits and harvest index of sweet pepper in a greenhouse pot experiment.

## MATERIALS AND METHODS

### Experimental Design and Growth Condition

The experiment was conducted in a plastic greenhouse in the Department of Horticultural Science at Isfahan University of Technology, Isfahan, Iran, with temperature 30-35°C between July and October 2013. The study was carried out as the factorial experiment based on complete randomized design (CRD) with 3 replications. Treatments were AM fungi inoculation and different substrates including non-inoculation AM fungi (M0), AM fungi with 1000 spores per pot (M1) and AM fungi with 2000 spores per pot (M2). The substrates included perlite (PR) and cocopeat (Co) (PR50: Co50 V:V%) (C), PR25:Co50: vermicompost (V) 25 (C+V25) and PR25:Co25: V50 (C+V50). All pots were irrigated with half strength Johnson Nutrient Solution in the first month and afterward with about 100 ml complete Johnson Nutrient Solution every day (Jones, 2004).

### Production of Seedlings

Sweet pepper seeds (*Capsicum annum* cv. Gold flame) were surface sterilized in 70% ethanol for 5 min, washed four times and sown into peat moss: perlite (1:1) at 25 °C and the seedlings were allowed to grow for 40 days. The plants with uniform size were transplanted to 3 L pots subjected to different treatments.

### Growth Media and Properties

Vermicompost was produced from organic waste by Goldasht Company (Isfahan, Iran) which was made from the composting activity of earthworms (*Eisenia fetida*) on the municipal solid waste compost. Properties of vermicompost were as follows: available N: 2.3%; available P: 1%; available K; 1.12%, EC: 2.56 dSm<sup>-1</sup>,

pH: 7.25; OC: 29%, and C/N 12.6 (Mobli & Aghdak, 2011). Substrate treatments included Perlite (PR) and Cocopeat (Co) (PR50:Co50 V:V) (C), PR25:Co50: vermicompost (V25) (C+V25), and PR25:Co25:V50 (C+V50). Chemical properties of the growth media are given in Table 1 (Mobli & Aghdak, 2011).

### AM Fungi Inoculation

The Arbuscular mycorrhizal fungi (*Glomus intraradices*) (mycorrhizal root, soil containing spores and extra radical mycelium) were obtained from Touran Biotech Company (Shahroud, Iran). The inoculated dosage was approximately 50 spores per g counted by microscope. According to the treatments, the inoculums were supplied 20 g of *Arbuscular mycorrhiza*, including 1000 spores (M1) and 40 g inoculums with 2000 spores (M2) and non-mycorrhizal plants as the control (M0). The inoculums were mixed into potting substrates before transplanting the plants to the pots.

### Photosynthesis Properties Assay

Leaf chlorophyll content was measured by using a nondestructive dual-wavelength chlorophyll meter (SPAD-502, Minolta Corp, USA). Photosynthesis properties were determined from the youngest fully expanded leaf for 3 replications per treatment by a portable photosynthesis meter (Li-Cor Li-3000, USA) from 10:00 to 11:00 am on a clear day (without clouds). The measurements were conducted with photosynthetically active radiation (PAR) intensity of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and reference CO<sub>2</sub> concentration of 350  $\mu\text{mol mol}^{-1}$ . Mesophyll conductance ( $\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was calculated by dividing the photosynthetic rate by the sub-stomatal CO<sub>2</sub> concentration (Ahmadi and Siosemardeh, 2005). Photosynthetic Water Use Efficiency (PWUE) was calculated by dividing the photosynthesis rate by the transpiration rate (Haghighi et al., 2012).

### Plant Growth

After 124 days from transplanting the seedlings, inoculated and non-inoculated plants (3 plants per replication) were carefully uprooted. The roots were dipped in water to remove substrate particles 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. 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).

**Table 1.** Chemical and physical properties of substrates used in this study

	N %	K %	Ca %	Mg (mg Kg <sup>-1</sup> )	Mn (mg Kg <sup>-1</sup> )	Cu (mg Kg <sup>-1</sup> )	Zn (mg Kg <sup>-1</sup> )	Fe (mg Kg <sup>-1</sup> )	pH	EC (dSm <sup>-1</sup> )	Bulk density (g cm <sup>-3</sup> )	Particle density (g cm <sup>-3</sup> )	Total porosity %	Moisture capacity %	Air filled porosity %
Cocopeat	1.8	0.5	3.5	0.5	108	36	64	1324	5.5	0.11	0.26	1.46	0.82	0.8	0.02
Perlite	0.3	0.01	0.5	0.3	10	4	5.3	250	5.6	0.04	0.12	0.43	0.72	0.3	0.42

## Harvest and Yield Index Analysis

During the experiment and finally at 124 days after transplanting, fruits were harvested and washed using tap water. Fruits diameter was measured using a digital caliper (Mitutoyo Corp, Japan) at the end of the experiment. Total soluble solids (TSS) were measured using refractometer (PAL-1 Brix, Japan). Then, the fruits were oven dried at 70°C to a constant weight. Both fresh and dry weights of the fruits were measured by an analytical balance. Water Use Efficiency Index (IWUE) was calculated as the ratio of the plant yield to the amount of water used during the experiment (Oweis et al., 2000). Mycorrhizal dependency (MD) was calculated using the following formula (Planchette et al., 1983) with some modification which shows the dependency of yield to mycorrhiza inoculation (M1 and M2) compared with the non-inoculation media:

$$MD = \frac{\text{mycorrhizal plant yield} - \text{non-mycorrhizal plant yield}}{\text{mycorrhizal plant yield}}$$

Vermicompost dependency (VD) was calculated using the following formula which shows the dependency of yield to vermicompost application (V25 and V50) compared with the non-vermicompost application (control) (Planchette et al., 1983):

$$VD = \frac{\text{Yield with vermicompost application} - \text{Yield without vermicompost application}}{\text{Yield with vermicompost application}}$$

## Nutrient concentration assay

N was analyzed by Kjeldahl (ZDDN-II, China) method, P by colorimetry, and K by flame photometry in sweet pepper leaves following Haghighi et al.'s (2014) procedure.

## Statistics Analysis

All data were subjected to a two-way ANOVA using Statistix 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

### Main Effect of AM Fungi Inoculation and Different Media

Shoot and root fresh weights, and root volume were not affected by the media. Shoot dry weight, TSS, fruit fresh and dry weights significantly increased in the C+V50 treatment. Fruit earliness was accelerated (decreased) with vermicompost application. Yield increased with V25 and reached the highest in the C+V50 treatment. IWUE increased by vermicompost application at both levels (Table 2).

Chlorophyll content increased in the C+V50 treatment. Photosynthesis rate and P concentration increased by vermicompost application and reached the highest level in the C+V50 treatment. Transpiration and PWUE as well as N concentration increased when vermicompost was applied. Stomata conductance and K concentration did not differ between the treatments (Table 3).

Shoot FW and DW, root FW, root volume, fruit FW, fruit diameter, yield, and IWUE increased in M1. Root DW, TSS, and fruit DW were not affected by AM fungi inoculation. Fruit earliness decreased in M1 and M2 treatments (Table 4).

Chlorophyll and photosynthesis rate, PWUE and mesophyll conductance increased in M1. Transpiration, stomata conductance, P and K concentrations did not change significantly between the treatments (Table 5).

In the media with just compost (C), the use of AM fungi could not show any significant impact on the yield. At the high level of vermicompost (i.e., C+V50), yield was dependent on vermicompost and mycorrhiza with the same weights, but at the lower level of AM fungi (C+V25), vermicompost had more influence than AM fungi (Table 6).

**Table 2.** The main effect of different media on growth and some fruit characteristics of sweet pepper

Treatment	Shoot FW (g/plant)	Shoot DW (g/plant)	Root FW (g/plant)	Root DW (g/plant)	Root volume (cm <sup>3</sup> )	TSS (Brix)	Fruit FW (g/plant)	Fruit DW (g/plant)	Fruit diameter (cm)	Fruit Earliness (day)	Yield (g dry w/plant)	IWUE (kg/m <sup>3</sup> )
C	105.80 <sup>a</sup>	13.75 <sup>b</sup>	36.71 <sup>a</sup>	2.47 <sup>b</sup>	41.11 <sup>a</sup>	6.11 <sup>c</sup>	46.28 <sup>b</sup>	4.16 <sup>c</sup>	5.63 <sup>a</sup>	94.11 <sup>a</sup>	62.49 <sup>b</sup>	0.61 <sup>b</sup>
C+V25	102.37 <sup>a</sup>	12.61 <sup>b</sup>	36.21 <sup>a</sup>	3.31 <sup>a</sup>	46.66 <sup>a</sup>	7.34 <sup>b</sup>	52.30 <sup>b</sup>	5.49 <sup>b</sup>	5.47 <sup>a</sup>	79.44 <sup>b</sup>	73.25 <sup>ab</sup>	0.73 <sup>a</sup>
C+V50	95.62 <sup>a</sup>	15.20 <sup>a</sup>	31.45 <sup>a</sup>	3.80 <sup>a</sup>	41.66 <sup>a</sup>	8.22 <sup>a</sup>	78.08 <sup>a</sup>	6.79 <sup>a</sup>	5.00 <sup>b</sup>	81.55 <sup>b</sup>	77.44 <sup>a</sup>	0.70 <sup>a</sup>

Columns with different letters across treatments are significantly different at  $P < 0.05$  according to the LSD test. PR50:Co50 (C); PR25:Co50: vermicompost25 (C+V25); PR25:Co25: V50 (C+V50)

**Table 3.** The main effect of different media on photosynthesis and nutrient characters of sweet pepper

Treatment	Chlorophyll content (SPAD value)	Photosynthesis rate (μmol m <sup>-2</sup> s <sup>-1</sup> )	Transpiration (mmol m <sup>-2</sup> s <sup>-1</sup> )	Stomata Conductance (mmol m <sup>-2</sup> s <sup>-1</sup> )	Mesophyll conductance (m <sup>2</sup> s <sup>-1</sup> mmol)	PWUE (mol CO <sub>2</sub> m mol <sup>-1</sup> H <sub>2</sub> O)	N (%)	P (μg/kg DW)	K (mg/kg DW)
C	28.40 <sup>b</sup>	12.47 <sup>b</sup>	1.31 <sup>b</sup>	0.03 <sup>a</sup>	346.97 <sup>a</sup>	5.95 <sup>b</sup>	10.10 <sup>b</sup>	6.48 <sup>b</sup>	0.40 <sup>a</sup>
C+V25	25.09 <sup>b</sup>	13.40 <sup>ab</sup>	2.35 <sup>a</sup>	0.05 <sup>a</sup>	290.86 <sup>a</sup>	9.65 <sup>a</sup>	11.65 <sup>a</sup>	7.87 <sup>ab</sup>	0.37 <sup>a</sup>
C+V50	40.69 <sup>a</sup>	15.25 <sup>a</sup>	2.56 <sup>a</sup>	0.06 <sup>a</sup>	274.73 <sup>a</sup>	9.19 <sup>a</sup>	11.34 <sup>a</sup>	9.46 <sup>a</sup>	0.24 <sup>a</sup>

Columns with different letters across treatments are significantly different at  $P < 0.05$  according to the LSD test. PR50:Co50 (C); PR25:Co50: vermicompost25 (C+V25); PR25:Co25: V50 (C+V50)

**Table 4.** The main effect of *Arbuscular mycorrhiza* (M) on plant growth and some fruit characteristics of sweet pepper

Treatment	Shoot FW (g/plant)	Shoot DW (g/plant)	Root FW (g/plant)	Root DW (g/plant)	Root volume (cm <sup>3</sup> )	TSS (Brix)	Fruit FW (g/plant)	Fruit DW (g/plant)	Fruit diameter (cm)	Fruit Earliness (day)	Yield (g dry w/plant)	IWUE (kg/m <sup>3</sup> )
M0	92.67 <sup>b</sup>	12.05 <sup>c</sup>	31.50 <sup>b</sup>	3.08 <sup>a</sup>	45.00 <sup>ab</sup>	7.24 <sup>a</sup>	55.24 <sup>b</sup>	5.27 <sup>a</sup>	5.51 <sup>b</sup>	94.55 <sup>a</sup>	61.87 <sup>b</sup>	0.61 <sup>b</sup>
M1	112.27 <sup>a</sup>	15.67 <sup>a</sup>	41.58 <sup>a</sup>	3.47 <sup>a</sup>	50.55 <sup>a</sup>	7.20 <sup>a</sup>	77.00 <sup>a</sup>	6.09 <sup>a</sup>	6.31 <sup>a</sup>	82.77 <sup>b</sup>	94.98 <sup>a</sup>	0.91 <sup>a</sup>
M2	98.84 <sup>b</sup>	13.84 <sup>b</sup>	31.29 <sup>b</sup>	3.02 <sup>a</sup>	33.88 <sup>b</sup>	7.23 <sup>a</sup>	44.42 <sup>b</sup>	5.08 <sup>a</sup>	4.28 <sup>c</sup>	77.77 <sup>b</sup>	56.34 <sup>b</sup>	0.51 <sup>c</sup>

Columns with different letters across treatments are significantly different at  $P < 0.05$  according to the LSD test. M0: un-inoculated plant; M1: mycorrhiza with 1000 spore; M2: mycorrhiza with 2000 spore

**Table 5.** The main effect of *Arbuscular mycorrhiza* (M) on photosynthesis and nutrient character of sweet pepper

Treatment	Chlorophyll content (SPAD value)	Photosynthesis rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Transpiration ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	Stomata Conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	Mesophyll conductance ( $\text{m}^{-2} \text{s}^{-1} \text{mmol}$ )	PWUE ( $\text{mol CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$ )	N (%)	P (mg/kg DW)	K (mg/kg DW)
M0	26.41 <sup>b</sup>	10.78 <sup>b</sup>	1.88 <sup>a</sup>	0.04 <sup>a</sup>	315.87 <sup>b</sup>	7.17 <sup>b</sup>	10.94 <sup>a</sup>	7.52e-03 <sup>a</sup>	0.40 <sup>a</sup>
M1	39.14 <sup>a</sup>	18.92 <sup>a</sup>	2.53 <sup>a</sup>	0.06 <sup>a</sup>	405.30 <sup>a</sup>	9.59 <sup>a</sup>	8.23 <sup>b</sup>	8.61e-03 <sup>a</sup>	0.32 <sup>a</sup>
M2	31.63 <sup>b</sup>	11.42 <sup>b</sup>	1.80 <sup>a</sup>	0.04 <sup>a</sup>	301.40 <sup>b</sup>	8.02 <sup>b</sup>	10.92 <sup>a</sup>	7.68e-03 <sup>a</sup>	0.29 <sup>a</sup>

Columns with different letters across treatments are significantly different at  $P < 0.05$  according to the LSD test. M0: un-inoculated plant; M1: mycorrhiza with 1000 spore; M2: mycorrhiza with 2000 spore.

**Table 6.** Comparison of the effectiveness of VD and MD on yield in different vermicompost levels

	C		C+V25		C+V50	
	VD%	MD%	VD%	MD%	VD%	MD%
Mean	-----*	0.34	0.15	0.43	0.34	0.19
T-test		0.9		0.3		0.16

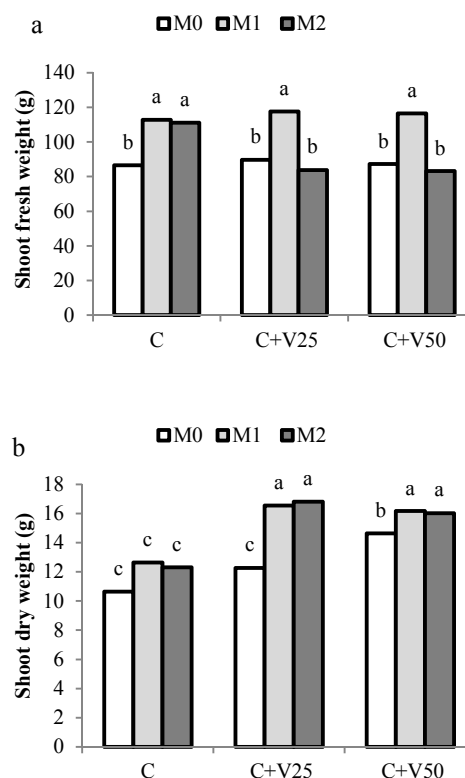
PR50:Co50 (C); PR25:Co50: vermicompost25 (C+V25); PR25:Co25: V50 (C+V50)

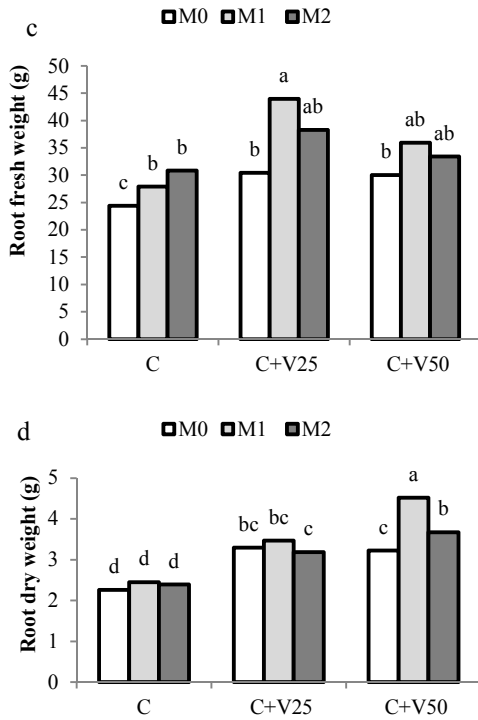
\*there were not any vermicompost in this treatment

### Interactive effect of AM fungi and media

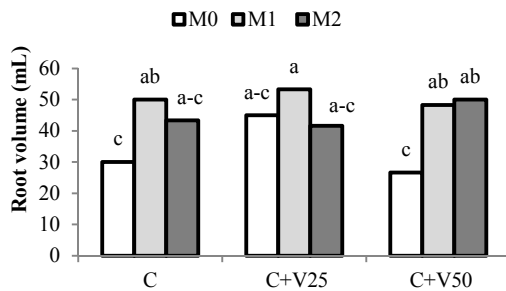
Shoot fresh weight increased significantly with M1 in all substrates, and with M2 in the C treatment compared to the control treatment (M0). There were not any significant differences between other treatments (Fig. 1a). Shoot dry weight increased with M1 and M2 in C+V25 and C+V50 treatments (Fig. 1b). AM fungi application increased root fresh weight significantly in C+V25 with M1 compared to the non-AM fungi application. However, the root fresh weight improved with M1 and M2 and vermicompost adding to compost, although, this improvement was not statistically significant (Fig. 1c). Root dry weight increased with vermicompost was the highest in C+V50×M1 treatment (Fig. 1d).

The lowest root volume was observed in C and C+V50 treatments in non-inoculated plants (M0). However, there was not significant different between these treatments and C and C+V25 treatments in M2 plants and C+V25 treatment in M0 plant (Fig. 2).



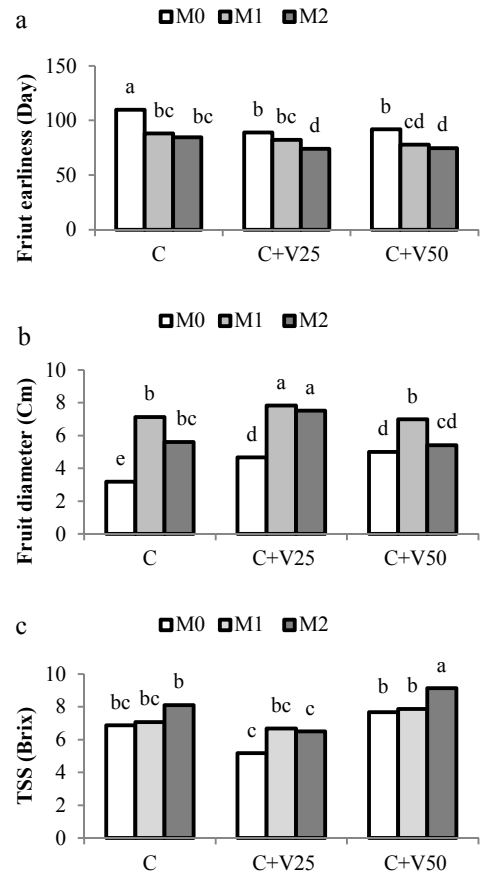


**Fig. 1.** The effect of different levels of vermicompost and mycorrhiza on shoot fresh weight (a), shoot dry weight (b), root fresh weight (c) and root dry weight (d) of sweet pepper. PR50:Co50 (C); PR25:Co50:vermicompost25 (C+V25); PR25:Co25:V50 (C+V50) M0: non-inoculated plant; M1: mycorrhiza with 1000 spores; M2: mycorrhiza with 2000 spores. Columns with different letters across treatments are significantly different at  $P < 0.05$  according to the LSD test.



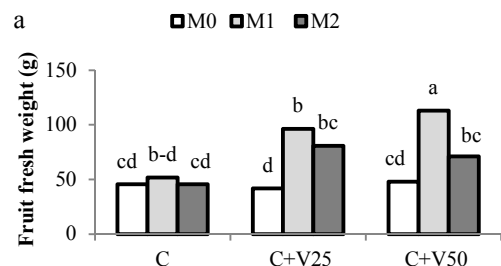
**Fig. 2.** The effect of different levels of vermicompost and mycorrhiza on root volume of sweet pepper. PR50:Co50 (C); PR25:Co50:vermicompost25 (C+V25); PR25:Co25:V50 (C+V50) M0: non-inoculated plant; M1: mycorrhiza with 1000 spores; M2: mycorrhiza with 2000 spores. Columns with different letters across treatments are significantly different at  $P < 0.05$  according to the LSD test.

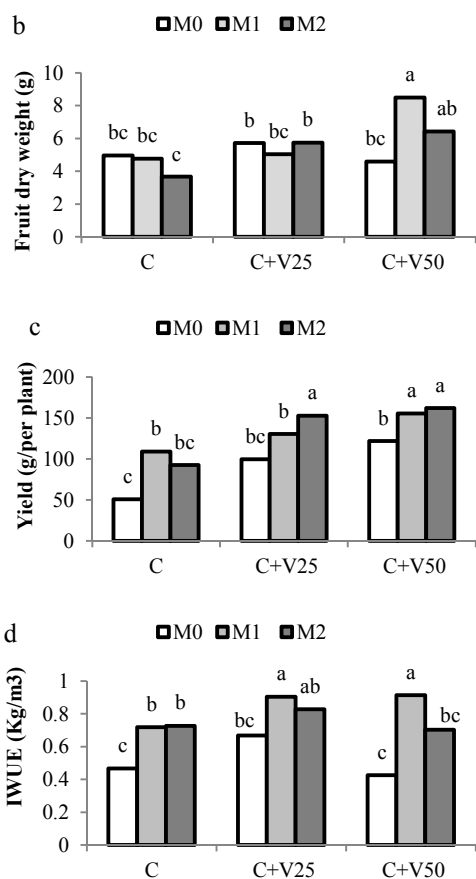
Fruit earliness was accelerated with AM fungi application in C+V50 with M1 and M2, and C+V25×M2 treatment. The first harvest was in C+V50 and C+V25 treatments with M2 (Fig. 3a). Fruit diameter increased with M1 and M2 in all substrates and it was the highest in C+V25 with M1 and M2 (Fig. 3b). The highest TSS was seen in C+V50×M2 treatment (Fig. 3c).



**Fig. 3.** The effect of different levels of vermicompost and mycorrhiza on fruit earliness (a), fruit diameter (b) and TSS (c) of sweet pepper PR50:Co50 (C); PR25:Co50:vermicompost25 (C+V25); PR25:Co25:V50 (C+V50) M0: un-inoculated plant; M1: mycorrhiza with 1000 spores; M2: mycorrhiza with 2000 spores. Columns with different letters across treatments are significantly different at  $P < 0.05$  according to the LSD test.

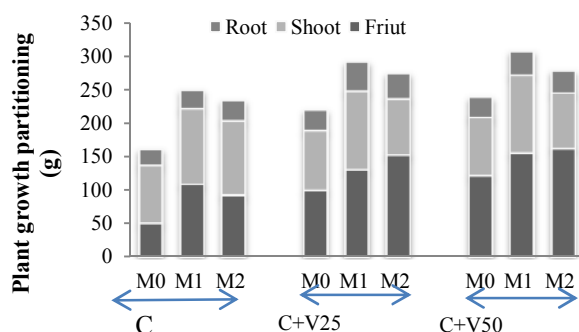
The fruit fresh weight was the highest in C+V50×M1 treatment compared to other treatments (Fig. 4a). Whereas, the fruit dry weight of sweet pepper was in the highest level in C+V50×M1 treatment compared to other treatments (Fig. 4b). Yield increased significantly with M2 in C+V25 and in with M1 and M2 C+V50 compared to other treatments and the yield was the lowest in C×M0 treatment (Fig. 4c). IWUE increased in the most application of both mycorrhiza (M1 and M2) levels compared to no mycorrhiza (M0) application in all substrates (Fig. 4d).





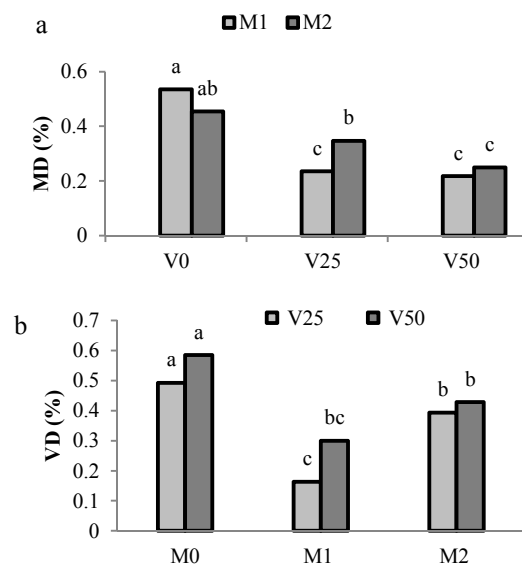
**Fig. 4.** The effect of different levels of vermicompost and mycorrhiza on fruit fresh weight (a), fruit dry weight (b), yield (c) and IWUE (d) of sweet pepper PR50:Co50 (C); PR25:Co50: vermicompost25 (C+V25); PR25:Co25: V50 (C+V50) M0: un-inoculated plant; M1: mycorrhiza with 1000 spores; M2: mycorrhiza with 2000 spores. Columns with different letters across treatments are significantly different at  $P < 0.05$  according to the LSD test.

Plant growth increased with application of AM fungi, and this increase seems to be more related to the growth, in order, of fruit  $\geq$  shoot  $\geq$  root (Fig. 5).



**Fig. 5.** The effect of different levels of vermicompost and mycorrhiza on different plant parts PR50:Co50 (C); PR25:Co50: vermicompost25 (C+V25); PR25: Co25: V50 (C+V50) M0: non-inoculated plant; M1: mycorrhiza with 1000 spores; M2: mycorrhiza with 2000 spores. Columns with different letters across treatments are significantly different at  $P < 0.05$  according to the LSD test.

Mycorrhiza dependency (MD) was the highest in the media without vermicompost and there was not significant different in this trait between two mycorrhiza applications (M1 and M2) in V0. When vermicompost was used, the M1 was more effective in this trait than M2 in V25. MD was the lowest in the V2×M1 and in the high vermicompost (V50) application at both AM fungi levels (Fig. 6a). Vermicompost dependency (VD) was the highest in the non-AM fungi inoculation at both vermicompost levels, decreased in M1 and M2 at both vermicompost levels, and was the lowest in M1×V25 treatment (Fig. 6b).

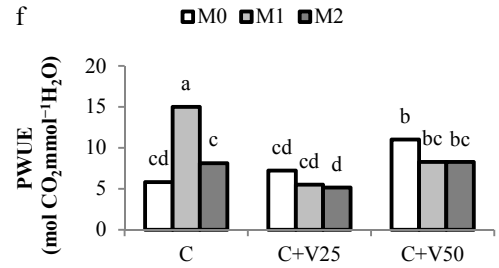
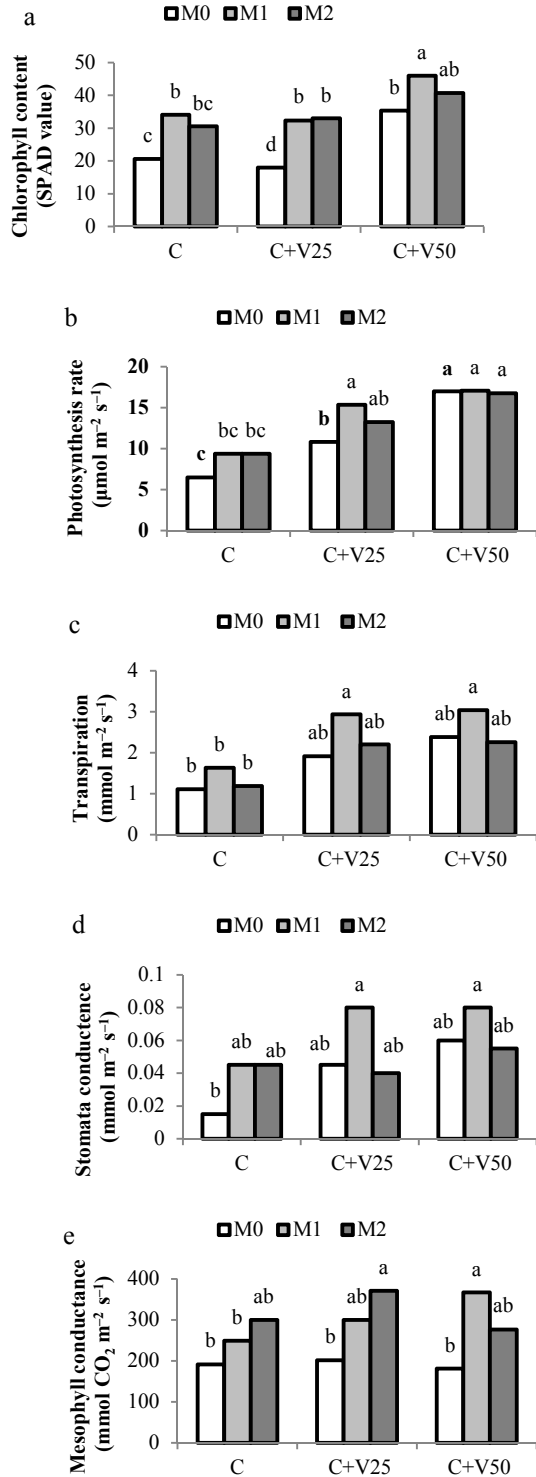


**Fig. 6.** The effect of different vermicompost and mycorrhiza levels on MD (a) and VD (b) PR50:Co50 (C); PR25:Co50: vermicompost25 (C+V25); R25:Co25: V50 (C+V50) M0: un-inoculated plant; M1: mycorrhiza with 1000 spores; M2: mycorrhiza with 2000 spores. Columns with different letters across treatments are significantly different at  $P < 0.05$  according to the LSD test.

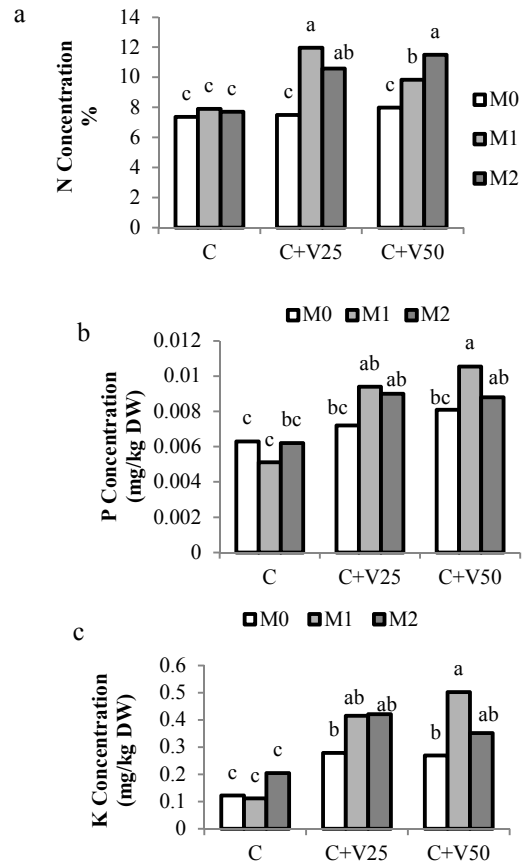
The highest and lowest chlorophyll content was observed in C+V50 × M1 and C+V25 × M0 treatments, respectively (Fig. 7a). Photosynthesis rate was the highest in C+V50 treatment at all mycorrhiza levels as well as in C+V25 × M0 treatment. Photosynthesis rate was the lowest in C×M0 treatment (Fig. 7b). Transpiration rate increased significantly with M1 in both vermicompost treatments (C+V25 and C+V50) compared to C treatment in at all levels of AM fungi inoculations in which the lowest rate of transpiration was observed (Fig. 7c). C+V25 × M1 and C+V50× M1 treatments showed significant difference with C × M0 treatment when stomata conductance (Fig. 7d) was measured. No significant difference was observed between other treatments in stomata conductance trait (Fig. 7d) Also, no significant difference was observed between all treatments when mesophyll conductance (Fig. 7e) was measured. PWUE was the highest in C×M1 treatment (Fig. 7f).

Nitrogen concentration of the leaves increased with M1 and M2 in C+V25 and C+50 treatments compared to the non-AM fungi inoculation in C+V25 and C+50

treatments and with all mycorrhiza levels in C treatment. (Fig. 8a). The highest P concentration of the leaves was observed in C+V50 treatment when M1 was applied. However, there was not significant difference in P concentration between M1 and M2 levels in C+V50 treatment (Fig. 8b). The highest K concentration was found in C+V50 treatment with M1, although it was statistically the same in C+V25 treatment with M1 and M2 and in C+V50 treatment with M2. The lowest K concentration was observed in C treatment with and without AM fungi applications (Fig. 8c).



**Fig. 7.** The effect of different levels of vermicompost and mycorrhiza on chlorophyll content (a) photosynthesis rate (b), transpiration (c), stomata conductance (d), mesophyll conductance (e) and PWUE (f) of sweet pepper. PR50:Co50 (C); PR25:Co50:vermicompost25 (C+V25); PR25:Co25:V50 (C+V50). M0: un-inoculated plant; M1: mycorrhiza with 1000 spores; M2: mycorrhiza with 2000 spores. Columns with different letters across treatments are significantly different at  $P < 0.05$  according to the LSD test.



**Fig. 8.** The effect of different levels of vermicompost and mycorrhiza on N (a), P (b) and K (c) concentrations of sweet pepper's leaves PR50:Co50 (C); PR25:Co50:vermicompost25 (C+V25); PR25:Co25:V50 (C+V50) M0: un-inoculated plant; M1: mycorrhiza with 1000 spores; M2: mycorrhiza with 2000 spores. Columns with different letters across treatments are significantly different at  $P < 0.05$  according to the LSD test.

### Plant Growth, Yield and Reproductive Traits of Sweet Pepper

Beneficial plant microbe interactions in rhizosphere were sufficient for plant health and soil fertility when organic matter was used (Hameeda et al., 2007). Increasing pelargonium growth by compost may have been due to the increase in P supply as evidenced by increased level of shoot P concentration of the plants grown in 40% compost compared with 20% (Perner et al., 2007). Similarly, in this study, shoot dry weight, TSS, fruit fresh and dry weights of sweet pepper increased in C+V50. Yield increased with V25 and reached the highest in C+V50 treatment. One of the reasons can be increased P concentration by vermicompost application as previously was reported by Perner et al. (2007). Also, Perner et al. (2007) reported that the higher water holding capacity of peat based substrate with higher compost addition caused increased plant growth. Furthermore, Fernández-Gómez et al. (2012) indicated that increase in shoot and root biomass of *Trifolium repens* by vermicompost application may have been due to the ability of organic material to stimulate plant growth by increasing available nutrients for crop. In contrast, in our study, AM fungi inoculation alone or combined with vermicompost did not affect plant biomass production. The result of Ortas and Ustner (2014) showed that root dry weight of citrus plant increased by mycorrhiza in andesitic tuff+ peat + compost. In line with previous results, in pepper plants, it was seen that shoot FW and DW, root FW, root volume, fruit FW, fruit diameter, yield and IWUE increased in M1. Ortas and Ustner (2014) stated that growth media play an important role in plant growth as required air, moisture, and nutrients. Nemeč (1992) reported that shoot and root growth increased by mycorrhiza inoculation with *G. intradicas* in different growth media. In the present study, in the substrate without vermicompost, both M1 and M2 increased shoot fresh weight, but when vermicompost was applied in the substrate, the low level of AM fungi (M1) was more effective than the higher level (M2) (Fig. 1a). In the present experiment, it was concluded that mycorrhiza dependency was the highest in the media without vermicompost, and with M1 application. When vermicompost was used, the M1 was more effective than M2. MD was the lowest in the high vermicompost (V50) application at both mycorrhiza levels (Fig. 6a). It is assumed that in poor media of organic matter, AM fungi has an important role on plant growth, but when vermicompost is applied, it can partially compensate for the role of mycorrhiza on plant growth, and the dependency of growth to mycorrhiza is decreased. The effectiveness of vermicompost can be explained by Canellas et al.'s (2002) results that humic acid isolated from earthworm compost enhanced root elongation, lateral root emergence, and H<sup>+</sup>-ATPase activity of the plasma membrane of maize roots. In the present experiment, it seems that the high level of vermicompost (V50) together with AM fungi at both levels were more effective on dry weight of root, but the lower vermicompost content (V25) and AM fungi (M1) had more influence on the root fresh weight and root

volume (Fig. 1c, 1d & Fig. 2). All growth parameters of sorghum increased by inoculation with mycorrhiza (*Glomouss* Spp) along with bacterial isolated with plant (Hameeda et al., 2007). Conversa et al. (2013) showed that 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. Conversa et al. (2013) stated that greater fruit yield in mycorrhizal plants was correlated with the highest flower production and larger inflorescence compared with the non-mycorrhizal plants. Poulton et al. (2001) found that the increase in pollen quality and quantity enhanced fruit setup and increased flower numbers in mycorrhizal plants that resulted in higher pepper yield. Also, Salviooli et al. (2012) reported that AM fungi inoculation improved fruit load of tomato plants and caused enhanced total yield of the plants. In the present experiment, the sooner harvest was observed at mycorrhiza inoculation with 2000 spores in the media contained vermicompost and resulted in higher yield in this treatment than the other media and the non-inoculated plants (Fig. 3a & Fig. 4c). Chatterjee et al. (2014) reported that root volume, root length, root weight of carrot increased by using vermicompost. Vermicompost improved substrate environment and encouraged proliferation of roots that drew more water and nutrients and resulted in higher yield in carrot (Padmavathiamma et al., 2008). Vermicompost with high porosity is an excellent soil or substrate conditioner and was reported to increase the growth and yield of vegetables such as tomatoes (Gutierrez-Miceli et al., 2007) and peppers (Arancon et al., 2004). Gutierrez-Miceli et al. (2007) stated that yield enhancement by vermicompost may be due to plant growth regulators and humic acids present in the vermicompost. Similar results were observed by Atiyeh et al. (2002) and Arancon et al. (2004) who found that humic extracted from vermicompost could produce hormone like effects on plant growth and yield.

Flower and bud numbers of pelargonium were unaffected by compost additions, but increased by inoculation with mycorrhiza (Perner et al., 2007), and this increase may have been due to increased plant nutrients combined with a possible hormonal effect induced by mycorrhiza colonization (Perner et al., 2007). Mycorrhizal plants accumulated nutrients in a shorter time, so that earlier in life, they were sufficiently supplied with nutrients to initiate flower development in pelargonium (Perner et al., 2007). In the present study, it seems that vermicompost accompanied by AM fungi had the most influence in earliness of sweet pepper fruits and shortened the harvest time more effectively, although it was observed that even when the vermicompost was not applied, the mycorrhiza itself accelerated fruit harvest in C treatment (Fig. 3a). Vermicompost increased TSS of Chinese cabbage in the experiment conducted by Wang et al. (2010), and this may have been due to improvement in the nutrient condition of the growth media. Liu et al. (2008) reported that N, P, K, and organic fertilization increased sugar content of plants. Similar results were observed in the present experiment that may have been due to the



chemical properties of the vermicompost or higher nutrient uptake of plants by AM fungi inoculation. In the present study, TSS increased in C+V50 treatment compared to the other media that together with AM fungi with 2000 spores enhanced TSS of sweet pepper fruits compared to the non-inoculated plants (Fig. 3c). In our experiment, yield improved with AM fungi application in all substrates, but it increased more when accompanied with vermicompost application in the media and reached the highest yield in C+V50 in M1 and M2 and C+V25×M2 treatments (Fig. 4c). Yield increase was more associated with the fresh weight of the fruits and the IWUE than the dry weight. For clarification, the impacts of vermicompost and AM fungi on the MD and VD were measured and compared with T-test. It was observed that when vermicompost was more available to the roots, AM fungi did not have a high impact on yield. On the other hand, when the vermicompost was in moderate amounts, yield increased accompanied with AM fungi. Lastly, when vermicompost was not applied, the AM fungi alone could not significantly change the yield (Table 6, Fig. 4). At the moderate level of vermicompost, the high level of AM fungi (C+V25×M2) was more effective (Fig. 4c) and resulted in the same yield as the high level of vermicompost with AM fungi at both levels (C+V50 in M1 and M2) (Fig. 4c).

#### Photosynthesis Character

AM fungi inoculation or P application alone did not influence chlorophyll and carotenoids contents, but AM fungi and P together significantly increased chlorophyll a and b compared to the control in *Artemisia annua* L. (Kapoor et al., 2008). According to Kapoor et al. (2008), this was probably due to the non-significant Cu concentration in the AM fungi and non-AM fungi inoculated plants. Photosynthesis rate, stomatal conductance, and transpiration rate in maize leaves were enhanced by AM fungi inoculation (Zhu et al., 2011). The result of Doan et al. (2013) showed that Chlorophyll (SPAD values) of maize and tomato were unaffected by vermicompost with and without earthworm compared to the control plants, and they explained that this may have been due to high nitrogen concentration in vermicompost than the control. In the present experiment, the highest Chlorophyll (SPAD values) was observed in C+V50 treatment with M1. Chemical properties of the growth media given in Table 1 and nutrient concentration analysis in the leaves (Fig. 8) showed that the vermicompost media had high N level and also plants grown in vermicompost had high N concentrations that resulted in high Chlorophyll (SPAD values) in C+M50 treatment with M1 (Fig. 7a).

#### Nutrient Concentration

All AM fungi inoculation levels increased N concentration in C+V50 treatment and mycorrhiza with 1000 spores improved N concentration in C+V25 treatment (Fig. 8a). In the present study, the highest P concentration was observed at the highest vermicompost level together with AM fungi inoculation with 1000 spores (Fig. 8b). It seems that AM fungi inoculation did

not influence K concentration in the growth media without vermicompost, but AM fungi with 1000 spores significantly increased K concentration of the sweet pepper leaves in the media contained 50% vermicompost (C+V50). In general, AM fungi inoculation had no influence on nutrient concentration in the growth media without applying vermicompost, and AM fungi inoculation caused higher nutrient uptake in the media containing vermicompost (Fig. 8). AM fungi inoculated plants accumulated more P in their shoots than the non-AM fungi inoculated ones in *Artemisia annua* L. (Kapoor et al., 2008). In agreement with the present study, Pelargoniums shoot P and K concentrations increased by AM fungi inoculation in 20 and 40% compost, but no AM fungi effect on Zn and N concentrations accrued (Perner et al. 2007). This indicated that the contribution of AM fungi in nutrient uptake showed a deficient in a certain nutrient in plant. Also, Ortas and Ustuner (2014) reported that citrus AM fungi inoculated plants had greater P and Zn concentrations compared to the non-AM fungi ones in all growth media (peat, compost, peat+compost, peat+soil). However, the substrate containing peat was more effective on plant growth, P and Zn contents of citrus, and this may have been related to physical and chemical properties of the media. Perner et al. (2007) stated that compost and peat remained acidic and hypoxanthine entered in this acidic media aggregated and exploited additional K source. Microorganisms' activities lead to increase in humic acid and consequently decrease in pH and increase in K availability. In our experiment, AM fungi inoculation increased K concentration in the media containing high vermicompost with mycorrhiza inoculation (Fig. 8c). Properties of vermicompost in Table 1 show that the pH of vermicompost was more than that of the other growth media, and this may have resulted in high K concentration in the leaves.

#### CONCLUSIONS

Shoot fresh weight increased by both AM fungi inoculations in the media without vermicompost, while AM fungi with 1000 spores enhanced shoot fresh weight of the plants grown in vermicompost. Both AM fungi inoculation levels increased shoot dry weight of the plants in the media containing vermicompost. Root fresh weight was stimulated by both AM fungi inoculations (M1 and M2) and mycorrhiza with 1000 spores in C and C+V25 treatments, respectively. Root dry weight increased by AM fungi inoculation with 1000 spores in C+V50 treatment. Root volume improved by AM fungi with 1000 spores and both AM fungi inoculations (M1 and M2) in C and C+V50 treatments, respectively, compared to the non-inoculated plants. Increases in growth with AM fungi and vermicompost are due to increasing nutrient uptake and photosynthesis traits which resulted in more growth as well as hormone like effect of vermicompost. Yield and fruit quality (diameter and TSS) improved too. It seems that when vermicompost is applied, there is no need to use high AM fungi level, and moderate amount can result in a sufficient growth. The highest fruit fresh and dry weights were observed at high vermicompost

level and AM fungi inoculation with 1000 spores. Plant yield increased by AM fungi inoculation with 2000 spores in the media containing vermicompost. It can be concluded that a moderate amount of AM fungi inoculation, 1000 spores per pot, as well as

vermicompost (25%) are recommended for soilless production of sweet pepper which results in proper quality and quantity of sweet pepper yield.

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## رشد، شاخص عملکرد و فتوسنتز فلفل دلمه‌ای رشد یافته در ورمی- کمپوست تلقیح شده با *Arbuscular mycorrhiza*

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غلظت نیتروژن

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**چکیده** - هدف اصلی این پژوهش بررسی اثرات غلظت‌های قارچ میکوریز (*Arbuscular mycorrhiza*) و محیط کشت‌های مختلف در فاکتورهای رشد، صفات فتوسنتز و عملکرد فلفل دلمه‌ای تحت شرایط گلخانه ای بود. این آزمایش بصورت فاکتوریل در قالب طرح کاملاً تصادفی با استفاده از ۳ محیط کشت، پرلایت (PR) و کوکوپیت (Co) با نسبت حجمی ۵۰ به ۵۰ (C)، نسبت حجمی ۲۵ پرلایت به ۵۰ کوکوپیت و ۲۵ ورمی کمپوست (V)، (V25) و نسبت حجمی ۲۵ پرلایت به ۲۵ کوکوپیت به ۵۰ ورمی کمپوست (V50) و سه سطح میکوریز (گیاه بدون میکوریز (M0)، میکوریز با ۱۰۰۰ (M1) و ۲۰۰۰ (M2) اسپور و سه تکرار اجرا شد. نتایج نشان داد که تلقیح میکوریز و مخلوط ورمی-کمپوست وزن تر ریشه و شاخه را افزایش داد. تلقیح میکوریز با ۲۰۰۰ اسپور به طور معنی داری حجم ریشه را در تیمار C+V50 و C+V25 افزایش داد. برداشت میوه با M2 نسبت به M1 در C+V25 و V50 سریعتر بود. عملکرد میوه با تلقیح ۲۰۰۰ اسپور میکوریز در محیط کشت V25 و C+V50 افزایش داشت. وابستگی میکوریز با کاهش کاربرد ورمی کمپوست کاهش یافت و بیشترین وابستگی ورمی کمپوست در گیاهان بدون تلقیح میکوریز بود. هر دو سطح میکوریز تلقیح شده شاخص کلروفیل را افزایش داد و بیشترین افزایش با تلقیح M1 در تیمارهای C و C+V50 بود. M1 به میزان قابل توجهی سرعت فتوسنتز را در C+V25 افزایش داد و سرعت فتوسنتز در سایر محیط‌ها اختلاف معنی دار نداشت. میزان نیتروژن، فسفر و پتاسیم توسط تلقیح میکوریز در تیمارهای با ورمی-کمپوست بالا نسبت به بقیه تیمارها افزایش یافت.