EFFECT OF NUTRIENT SOLUTIONS AND DIFFERENT PLANTING BED ON SOME GROWTH CHARACTERISTICS OF STEVIA REBAUDIANA IN INOCULUM CONDITIONS WITH MYCORRHIZAL FUNGUS

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Abstract. Stevia is a perennial plant and is well-known for its high content of steviol glycoside in dryleaf matter that is responsible for providing the non-caloric value sweet taste. Stevia rebaudiana leaves contain diterpen glycosides, which are about 250 to 300 times more sweetened than sucrose, and their sweeteners are widely used today in the food and pharmaceutical industries. In order to study the effect of mycorrhiza fungi on some growth characteristics of the stevia herb in inoculum conditions with mycorrhizal fungus, a factorial experiment based on randomized complete block design with four replications was conducted in greenhouse of Mohaghegh Ardabili University in 2016. The first factor was Imma & Angel and Novella nutrient solutions, the second factor was planting bed including leaf composts, vermicompost and biolan (peat & perlite) and third factor Inoculation with mycorrhiza and control fungi showed that the highest fresh and dry weight of leaves and number of number of leaves to the Imma & Angel nutrient solution in vermicompost planting bed under inoculation with mycorrhiza. The highest plant height was related to the Imma & Angel nutrient solution in leaf composts planting bed was observed under inoculum with mycorrhiza and the highest chlorophyll content was observed for Novella nutrient solution in vermicompost planting bed under inoculation conditions with mycorrhiza.
Keywords: *Stevia, biofertilizers, hydroponic, mycorrhiza*

Introduction

Stevia rebaudiana Bertoni (Asteracea family) is a herbaceous, perennial and indigenous herbaceous plant in Paraguay and Brazil and its leaf is used as a calorie-free natural sweetener for diabetic patients and for the prevention of obesity (Garana et al., 2010). Stevia has high nutrient requirements, especially N, P and K, and the lack of these elements has a major constraint on the quantity and quality of biomass of this plant (Pramanik and Singh, 2003). A proposal to prevent the effects of chemical fertilizers on the plant, which may overwhelm the good properties of the plant, is to examine the impact of bio-fertilizers that are present in mycorrhizal fungi or bacteria on the growth of this plant. Growth stimulating microorganisms include mycorrhizal fungi and a group of bacteria, and the most important glomus mycorrhizal fungi can be mentioned (Cocking, 2003). In hydroponic system, all essential elements should be given as soluble salts to the plant. A proper nutritional solution is a solution that

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contains all the nutrients in certain concentrations, and the experts, based on the research components and the conditions of cultivation and plant species studied, have changed from standard solutions or different concentrations of it (Tabatabaei et al., 2006). Proper media, in addition to the desired physical, chemical and biological properties, should be accessible, sustainable and economically feasible (Davidson et al., 1998). The planting bed used in this qualification were listed (*Table 1*).

Table 1. *Concentration of nutrient solution elements (mg / l)*

Elements	Imma & Angel (2006)	Novella et al. (2008)
KNO_3	40.4	505
$Ca(NO_3)_2$	254.2	656
NH_4NO_3	352	47
KH_2PO_4	598.4	240
$MgSO_4$	96	180
Na_2MoO_4	0.033	0/055
H_3BO_3	2.21	1.56
$CuSO_4$	0.481	0.912
$ZnSO_4$	0.645	1.07
$MnSO_4$	5.15	8.5
Fe-EDDHA	13.25	4

Vermicompost has substances such as plant growth hormones and enzymes that increase the microbial population of the soil and preserve the nutrients for longer periods without negative effects on the environment (Padmavathiamma et al., 2008). Perlite has a slight weight, is chemically neutral and has a high porosity and water holding capacity. Vermiculite also has a high cation exchange capacity and is chemically active (Malakouti et al., 2005). Perlites are often used alone or in combination with vermiculite 1: 1 volumes alone (Malakouti et al., 2008). This material provides suitable conditions for plant growth, such as ventilation, drainage and access to nutrients, especially in combination with other substrates (Martinez and Abad, 1992). Peat Moss is a degraded particle that is produced in humid and cold areas. The type of composition and its constituent varies in different types (Rafiei and Akbarzadeh, 2007).

Peat Moss is a plant material that breaks down slightly in mossy soils, as well as anaerobic conditions such as swamps and marshes, and has acidic pH. The cation exchange capacity of this substance is very high and the EC has a low (about ds.m 0.5) (Samiei et al., 2004). Mycorrhizal fungi in direct ways such as improving plant nutrition by increasing the absorption of water and nutrients, in particular, low-mobility elements such as phosphorus (Smith and Read, 1997), as well as the production of hormones such as auxin, cytokinin and abscisic acid in stimulating plant growth (Awotoye et al., 2009) and indirectly, such as reduction of biological stresses of plant diseases (Calvet et al., 1995) and non-toxic (salinity, drought, heavy metals, etc.) increase growth and yield Plants are hosted (Raiesi and Ghollarata, 2006). Research has shown that bio-fertilizers significantly provide plant nutrients such as nitrogen, phosphorus and potassium, and The other side protects the plant against various environmental stresses and increases plant resistance under stress conditions (Smith and Read, 1997).

The purpose of this study was to investigate the effect of nutrient solutions and planting bed (Peat moss and Perlite, leaf compost and vermicompost) simultaneously on Glomus mosseae mycorrhiza inoculum on some growth characteristics of stevia herb.

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Methods and materials

In order to investigate the effect of Nutrient solutions and different planting bed on some growth characteristics of Stevia rebaudiana under inoculation with mycorrhizal fungus, a factorial design based on randomized complete block design with 4 replications in greenhouse of Mohaghegh Ardebili University in 2016 was conducted. The first factor was two levels: two different Nutrient solution solutions including Imma & Angel and Novella et al. (*Table 1*), the second factor was planting bed including Peat Moss and Perlite (Biolan), Vermicompost and Leaf composts in three levels and the third factor were inoculated with mycorrhizal fungus from Glomus mosseae and control treatment at two levels. After planting seedlings in pots, they were fed continuously with drops in nutrient solutions. Height of plant with ruler, number of leaves with counting, vegetation rate with cost meter (chlorophyll meter) Konica Minolta 502 according to SPAD index, fresh and dry weight of leaf was measured by digital scale. The analysis of data variance was done using SAS 9.2 software and the mean values were compared at the probability level of 1% and 5% using LSD Test.

Results and discussion

Leaf fresh weight

The results of analysis of variance of trait weight of leaf in stevia were significantly affected by the treatments at 1% level (*Table 2*). The results of analysis of variance of interaction between Nutrient solution solutions, different planting bed and inoculation with mycorrhizal fungus showed a significant difference. Mean comparison showed that the Imma & Angel solution in the planting bed of vermicompost under the conditions of mycorrhizal fungus (666.8 g) had the highest leaf fresh weight (*Table 3*).

Table 2. *Analysis of variance of the effects of treatments on Leaf fresh weight, Leaf dry weight, Number of leaves, Plant height and Chlorophyll*

	Mean squares					
Source of variances	**df**	**Leaf fresh weight**	**Leaf dry weight**	**Number of leaves**	**Plant height**	**Chlorophyll**
Repetition	3	6341/66	458/33	631637/5	712/423	18/57
Nutrient solution	1	88648/83**	732/57**	2643285/33**	1656/75*	147/17*
Planting bed	2	124957/51**	7237/26**	7710277**	347/078^{ns}	38/52^{ns}
Inoculation	1	46800/03**	2215/17**	99372**	2976/75**	0/574^{ns}
Planting bed×nutrient solution	2	78939/07**	727/6**	640164/33**	316/797^{ns}	31/48^{ns}
Nutrient solution×Inoculation	1	79283/76**	430/08**	2033633/33**	500/52^{ns}	30/16^{ns}
Planting bed×Inoculation	2	144228/91**	9578/57**	10889749**	1773/109*	116/122*
Planting bed×nutrient solution×Inoculation	2	11865/36**	1929/62**	4705210/33**	602/036^{ns}	182/967**
Error	33	520/04	19/17	4705210/33	386/62	24
%CV	-	14	10	9/5	20/23	12/88

**and * were significant at level of probability of 1% and 5% and without significant differences

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Table 3. *Comparison of the average effect of different treatments on Leaf fresh weight, Leaf dry weight, Number of leaves, Plant height and Chlorophyll*

Treatments			Mean				
			Leaf fresh weight	**Leaf dry weight**	**Number of leaves**	**Plant height**	**Chlorophyll**
Novella	Leaf composts	Mycorrhiza inoculation	145b	46/8c	2332b	99/75abcd	36/45bc
		Control	81/5de	18/2g	993h	65/63d	41/838ab
	Vermicompost	Mycorrhiza inoculation	142/5b	77/5b	2080c	78/5de	45/513a
		Control	140/8b	38/6de	1736d	102/5abcd	37/2bc
	Peat moss & Perlite	Mycorrhiza inoculation	151/7b	39/7de	1480ef	109/63abc	39/388ab
		Control	84/7de	26/1f	687i	91/88bcde	38/213bc
Imma & Angel	Leaf composts	Mycorrhiza inoculation	150/3b	43/6cd	1646de	123a	36/225bc
		Control	123/6bc	35/2e	1060gh	81/5cde	41/538ab
	Vermicompost	Mycorrhiza inoculation	666/8a	128/1a	4653a	112ab	31/4cd
		Control	105/1cd	27/18f	1012h	96/25abcd	41/113ab
	Peat moss & Perlite	Mycorrhiza inoculation	96/7cde	26f	1270fg	107/5abc	38/463b
		Control	65/2e	18/3g	550i	98/13abcd	28/85d

Similar letters to the averages in each column indicate no significant difference at the 5% level (LSD test)

Leaf dry weight

The results of this experiment showed that this trait was affected by different nutrient solutions, different planting bed and inoculation with mycorrhizal fungus at a one percent significance level (*Table 2*). The results of analysis of variance of interaction between Nutrient solution solutions, different planting bed and inoculation with mycorrhizal fungus showed a significant difference. Mean comparison showed that the Imma & Angel nutrient solution in the planting bed of vermicompost had the highest leaf dry weight under inoculation with mycorrhizal fungus (128.1 g) (*Table 3*).

Research by Silveira et al. (2006) showed that the symbiosis of plants with mycorrhizal fungi improves plant growth characteristics, including the development of vegetative parts and the increase of fresh and dry weight of plant tissues. (Hawkesford et al., 2012) stated that phosphorus deficiency impairs plant growth and affects its various aspects of metabolism. Deficiency of phosphorus disrupts the transfer of water through the root, and because of the lack of adequate water for expansion of the cells, the leaves remain small and the growth of the air organs is prevented, thus, in the present experiment, Mycorrhiza fungus, with the availability of more phosphorus for the plant, increased the growth of the plant's airways. Also, vermicompost planting bed caused more vegetative growth because of the presence of more amounts of precipitated elements.

The trait of leaf number in Stevia plant was affected by the treatments at 1% level (*Table 2*). The results of analysis of variance of interaction between Nutrient solution solutions, different planting bed and inoculation with mycorrhizal fungus showed a significant difference. The mean comparison showed that the Imma & Angel diet in the planting bed of vermicompost had the highest number of leaves in inoculation with mycorrhizal fungus (4653) (*Table 3*). The growth of Imma & Angel, due to providing more suitable conditions for vegetative growth, resulted in more leaves. Vermicompost

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has a positive effect on the amount of photosynthesis and production of biomass of stevia, by increasing the water absorption capacity and the optimum access to high-consumption and low nutrient elements, resulting in more plant growth and increased leaf number. Also, according to Yousefi Shayade et al. (2015), the planting bed containing vermicompost increased the number of leaves in the stevia. (Bachman and Metzger, 2008) reported that adding vermicompost to soil increases leaf number, leaf area. The amount of photosynthesis in the French spring rose. Today it is known that direct mycorrhizal fungi increase plant growth by direct methods such as improving plant nutrition through absorption of nutrients, as well as increasing plant and inbound water absorption, such as decreasing biological stresses (Tahat and Sijam, 2012). The increase in the number of leaves in plants was reported by inoculation with mycorrhizal fungus in comparison with non-inoculated plants in tomato (Khalied and Elkhider, 1993) and Citrus tangering seedlings (Wu and Xia, 2006). An increase in the number and level of leaf associated with mycorrhizal fungi has also been reported by Copetta et al. (2006) in Basil, which is consistent with the results of this experiment.

Plant height

The height of plant was affected by different nutrient solutions at 5% level, and the highest number of leaves belonged to Imma & Angel (91.313 cm). Inoculation with mycorrhizal fungus (105.063 cm) also showed a significant difference in the level of one percent (*Table 2*). The results of analysis of variance of interaction between different substrates of culture and inoculation with mycorrhizal fungi showed a significant difference at 1% level. The highest plant height was related to the culture of leaf soil culture under inoculation with mycorrhizal fungus with 111.375 (cm). The comparison of the mean showed that the Imma & Angel nutrient solution in the soil planting bed under inoculum conditions with mycorrhizae (123 cm) had the highest plant height (*Table 3*). Utumi et al. (1999) conducted a stevia test The effect of nitrogen nutrient deficiency on the absorption of high-energy elements by the plant was that they said that the deficiency of this nutrient component would reduce the absorption of high-energy nutrients in this plant and decrease the plant's height. Studies by Sheelavanta (1993) showed that with increasing nitrogen fertilization, the length of safflower stem increased. Principally, the cause of increasing altitude due to the use of urea can be attributed to the effect of nitrogen escalation on vegetative growth and cell division in the plant, especially the stem, and more photosynthetic extraction is expected to be produced by the plant, which is a good condition Provides for elongation of the stem (Nourmohammadi et al., 2001). Saberhamishiegi et al. (2012) reported a study on the effects of nitrogen and potassium on the Stevia plant. The highest stem length (altitude) was observed in 60 kg / ha treatment and the least nitrogen application was obtained. Due to the fact that the percentage of nitrogen in the Imma & Angel sugars is higher, it can be attributed to the effect of nitrogen on the height of plants treated with this solution. According to the results, arbuscular mycorrhizal fungi had a positive effect on growth traits (plant height and branch number), which was consistent with the results of Piedra et al. (2005) and Soriano et al. (2009).

Chlorophyll

The results of analysis of variance of interaction between Nutrient solution solutions, different planting bed and inoculation with mycorrhizal fungus showed significant differences. Comparison of the mean showed that Novella nutrient solution in

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vermicompost was in inoculated with mycorrhizal fungus at 45.513 (*Table 3*). With the presence of nitrogen in the chlorophyll components, it can be expected that with increasing nitrogen levels, it will have a significant effect on the chlorophyll content of leaves. The presence of chlorophyll as a source of light absorption and the synthesis of essential elements for the growth of plants associated with this element is critical (Saberhamishi et al., 2014). Sedeghi Moghadam and Mirzaie (2008) reported that adding vermicompost to soil causes nitrogen absorption by the roots, increasing vegetative growth and producing more leaves, which in turn increases the level of absorption of light, the level of photosynthesis, the creation and b, a hydrocarbon substances in leaves and total chlorophyll content increase.

Increasing chlorophyll content of leaves due to mycorrhizal coexistence can be due to increased phosphorus absorption from the soil by these fungi. Demir (2004) showed that mycorrhizal coexistence increases the concentration of chlorophyll in pepper plant leaves. According to the results of Hoseini et al. (2015), mycorrhizal inoculated plants contain more vegetation than control. Also, Tang et al. (2009) observed in their study of maize that insemination with the G.mossea mushroom improved the chlorophyll synthesis in the plant and increased the photosynthesis of the plant. They attributed this to increasing nitrogen absorption by mycorrhizal plants.

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