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Article

Synergistic Effects of Mycorrhizal Inoculation and Nutrient Management on Stevia Yield and Quality in Hydroponic Culture

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Abstract: *Stevia rebaudiana* Bertoni, a perennial herb, has garnered significant attention for its diterpene glycosides, natural sweeteners approximately 250 to 300 times sweeter than sucrose. These glycosides, concentrated in the plant's leaves, not only serve as a low-calorie alternative to traditional sweeteners but also exhibit notable antimicrobial properties, making Stevia a promising candidate for both the food and pharmaceutical industries. In order to study the effect of mycorrhiza fungi on some growth characteristics of the stevia herb under inoculum conditions with Arbuscular mycorrhizal fungi (AMF) Arbuscular mycorrhizal fungi, a factorial experiment based on randomized complete block design with four replications was conducted at University of Mohaghegh Ardabili. The first factor was Imma & Angel and Novella nutrient solutions; the second was planting bed including leaf composts, vermicompost, and biolan (peat & perlite); and third factor involved inoculation with mycorrhiza (*Glomus Musea*). The control fungi indicated that the highest percentage of stevioside (16.45%) was related to vermicompost planting bed under conditions of invocation with mycorrhizal in harvest for the Imma and Angel nutrient solution. The maximum percentage of Rebaudioside A (7.57%) was related to vermicompost planting bed under conditions of invocation with mycorrhizal in harvest for the Novella nutrient solution. Further, the greatest levels of Rebaudioside C were obtained under the same conditions, with the difference that in the case of this glycoside, the leaf composts planting bed showed a higher percentage. Then, the highest leaf fresh weight, leaf dry weight, and number of main branches were obtained for the Novella nutrient solution in vermicompost planting bed under inoculation with mycorrhiza. Finally, Mycorrhizal inoculation in vermicompost beds with the Novella nutrient solution resulted in the greatest plant height. Conversely, the Imma & Angel nutrient solution in leaf compost beds, when combined with mycorrhizal inoculation, produced the tallest plants. The positive impact of AMF on plant growth was evident in the quantitative and qualitative improvements observed across various measured parameters.

Keywords: medicinal plant; cultivation substrate; biofertilizers; nutrient use efficiency

Introduction

Stevia rebaudiana is an herbaceous, perennial herb with steviol glycoside which can be used as a sugar substitute for diabetic patients. The importance of stevia lies in its potential as a natural alternative to artificial sweeteners (Talevi, 2021). It is assumed that the extract of Stevia leaves containing major glycosides of stevioside, Rebaudioside A and C, and isosteviol, in addition to having sweetening property, have abundant medical properties including lowering blood pressure, regulation of the immune system, anti-inflammatory and anti-tumor properties, anti-diarrhea, and diuretic properties (Lemus-Mondaca, 2012).

The best known and the most abundant Steviol glycosides in *S. rebaudiana* Bertoni are stevioside and rebaudioside A. Their concentration varies from 5 to 22% for stevioside and from 22 to 61.6% for rebaudioside A, depending on the genotype and cultivation conditions (Ohta et al. 2010; Wolwer-Rieck 2012). Studies have shown that arbuscular mycorrhizal fungi affect the secondary metabolic

processes of plants, including flavonoids and terpenoids. Most terpenoids and alkaloids are the active ingredients of medicinal plants and have anti-inflammatory, antibacterial, cardiac and anti-cancer effects (Zhang et al, 2015). Arbuscular mycorrhizal fungi in the soil create symbiosis with most terrestrial plants (Meng et al, 2020). The hyphae of arbuscular mycorrhizae on the external surface of the root can spread with other areas, otherwise they become unavailable for the roots to absorb water and nutrients and provide them to the host to accelerate the acquisition of important food resources by the host in plants.(Cheng et al., 2021).

Vesicular-arbuscular mycorrhiza (VAM) fungus is one of the biofertilizer types with a symbiosis relationship with the root of the majority of agricultural plants. The fungi could increase intake of food materials such as phosphorus, nitrogen, and some micronutrients, enhance water absorption, and improve resistance against pathogenic factors. It can also promote the growth and function of inoculated plants in sustainable agricultural systems. In a study conducted by Mandal (2013), it was found that inoculation of plants with *Rhizophagus fasciculatus* mycorrhizal could improve the biomass of stems and increase the concentration of glycosides of the medicinal plant compared to non-mycorrhiza plants. It has also been reported that use of phosphate solubilizing bacteria (PSB) could increase the stem length, root length, stem and leaf dry weight, and stem biomass as well as the concentration of glycosides compared to control plants (Mamta et al., 2010). Wu et al. (2005) showed in a research that the use of biological fertilizers improves the physical structure of the soil as well as the content of organic matter and nitrogen available for the symbiotic plant. Mycorrhizal fungi increase the ability of the host plant to absorb phosphorus and mineral elements from the soil, especially from their inaccessible sources.

In the research of Makizadeh Tafti et al. (2011) reported that the use of biological and chemical nitrogen fertilizers caused a significant increase in the harvest index, yield and the amount of dill essential oil compared to the control.

In another study, the use of different species of mycorrhizal fungi increased the amount of essential oil of the basil medicinal plant, Zulfiqari et al. (2013). Another biofertilizer is vermicompost, which could be gained through processing organic waste materials such as animal manure and crop residues by earthworm. The material has high porosity, high adsorption power of minerals, appropriate ventilation and drainage, high capacity for water storage with no bad smell and pathogenic agents. It is currently under usage in sustainable agriculture to increase the growth and improve the quality of agricultural produce (Arancon et al., 2004). Vermicompost is a fertilizer gained from microbial analysis and the stability of organic materials is affected by the reaction between earthworms and different microorganisms. This product is in the form of tiny particles like peat with high porosity, sufficient air, high capacity for water storage, and great microbial activity, which could also provide appropriate conditions for the growth of plants in combination with soil. Vermicompost could increase the production of plant growth regulators and resistance of plants against pathogenic agents. Humic substances play a key role in ventilation, water storage capacity of soil, and its permeability. Accordingly, the effect of vermicompost on physical properties of soil is more than that of compost (Weber, 2007). Perlite is an aluminosilicate with a volcanic origin. The material does not have a high cation exchange (Ronaghi and Maftoon, 2006). Perlite could increase drainage of the culture medium and improve its ventilation. Perlite is mainly used by itself without being combined with another material. Alternatively, it is used in combination with Vermiculite as 1:1 volume (Malakooti et al., 2008). The material has appropriate conditions for growth of plants such as ventilation, drainage, and access to nutrients, especially in combination with other materials. Peat moss is composed of decomposed particles of organic materials and is produced in wet and cold areas. The type of combination and components of peat moss vary in different types. Specifically, peat moss is an herbal substance which is decomposed to a low extent and is formed in moss lands and anaerobic conditions such as swamps and marshes with an acidic pH. The cation exchange capacity of this material is very high and has low EC (about 0.5ds/m) (Samiei et al., 2004). Organic fertilizers positively affect soil structure, increase the nutrient availability, and improve the quality and function of the products, with lower costs than synthetic fertilizers. (Thy and Buntha 2005; Rekha et al., 2013). According to the importance and position of Stevia and the desirable effects of

biofertilizers on plants, this study has been conducted to investigate plant growth, leaf yield, and steviol glycosides in *Stevia (rebaudiana Bert)* under the influence of Arbuscular mycorrhizal fungi and nutrient solutions in a hydroponic system. The research hypothesis was that the applied treatments affect the yield of active substances and the amount of natural sugars in stevia. This study provides important information about the potential benefits and effects of mycorrhizal fungi and different culture media under conditions of nutrition with Imma, Angel and Novella nutrient solutions on stevia.

1. Materials and Methods

To investigate the effect of different planting beds and inoculation of Arbuscular mycorrhizal fungi on the concentration of stevioside, Rebaudioside A and C in *Stevia rebaudiana Bertoni*, a factorial based plan was implemented based on completely random blocks with four repetitions in the greenhouse of Mohaghegh Ardabili. The greenhouse temperature was about 27-28 °C. The first factor of planting bed involved peat moss and perlite (peat and perlite), vermicompost, and peat in 3 levels. The second factor dealt with inoculation with mycorrhizal fungi in glomus type and control treatment at two levels. For mycorrhizal inoculation, Javari inoculum mixture of mushroom spores, external mycelium and colonized fragments was used in the amount of 35 to 40 spores per gram of culture medium..The inoculation with mycorrhiza fungus was performed such that the mycorrhiza fungus was placed in the root zone of 15 grams. Vermicompost and Arbuscular mycorrhizal fungi were used in different treatments, where Arbuscular mycorrhizal fungi can use organic material for the culture of humic acid and humus culture. After culturing the plants in the flower pots, the *Stevia rebaudiana Bertoni* was constantly fed in the mentioned media and in dropped mode using Imma and Angell as well as novella nutrient solutions. Measurements were performed based on four replications, with two observations for each replicate. The leaf fresh weight was measured after the harvesting using digital balance scale per gram with accuracy of 0.1g. Further, the dry weight of leaves was measured after drying the bushes in an oven at 70°C for 72 min. In this experiment, to measure the sugar percentage, sampling was done from different parts of the plant. Initially, 0.2g of the plant was removed to which 10ml of ethanol 70% was added which was then placed under vertex. Then, the extraction was done using ultrasonic device for 60 min, and at the end it was centrifuged for 4 min with speed of 5000 rpm. Next, the surface solution was diluted with 1:10 ratio with mobile phase and was used for analysis by HPLC (Ravikumar et al, 2004). The measurement methods was as follows (Equation 1, 2, and 3): the standard values of 100 µg/ml of standard Rebaudioside A were injected to the device and then, according to the standard sub-peak level, the percentage of other contents of sugar in the plant was calculated as follows (Ursula *et al.*, 2010):

% Rebaudioside A= [Ws/W] x [Aa/As] x 100 1.

% stevioside= [Ws/W] x Astv x [0.83/As] x100 2.

% Rebaudioside C =[Ws/W] x AC x [0.98/As] x 100 3.

Ws= standard weight of Rebaudioside A (per mg) in the standard solution

W=weight of plant treatment (per mg)

As= standard sub-peak level of Rebaudioside A

Aa= sub-peak level of Rebaudioside A in the unknown sample

Astv= sub-peak level of stevioside in the sample

Ac= sub-peak level of Rebaudioside A in the sample

Initially, the sub-peak level of the sample representing the molar value of the sample was multiplied by 10. Indeed it was diluted to 10 times and then multiplied by 10 ml of the examined solution to obtain the sub-peak level of the desired sugar content. Then, based on the formula, the percentage of the desired sugar content could be measured. In this test, first, 50 µl of standard and sample diluted to 10 times was injected to the device. For measurement, the unknown samples were firstly obtained from the standard sample and then multiplied by 10. Next, it was multiplied by 10

ml of solution to obtain the weight of the desired sugar content in 10 ml. Thereafter, the result was divided by the plant value and further multiplied by 100 (Ravikumar et al, 2004).

Statistical Analysis

The analysis of data variance was performed using SAS 9.2 software and the mean values were compared at the probability levels of 1% and 5% through Duncan Test. The information of HPLC device used in this research is presented in Table 1.

Table 1. information of HPLC device.

Model of HPLC device	Waters
Column type	Teknokroma-NH2
Column length	25cm
Interior column diameter	0.46cm
Pump	Waters- 600E
Detector	Waters- 486: 210nm
Flow rate	1.0ml/min

2. Results and Discussion

2.1. Steviol glycoside Content

2.1.1. Stevioside

According to the results of analysis of variance on the measured traits, the content of glycoside stevioside showed a significant difference between different nutrient solutions, different planting beds, and inoculation with Arbuscular mycorrhizal fungi as well as first and second impressions, at the probability level of 1% (Table 2). The interactions between the first and second harvest, the nutrient solutions, planting bed and inoculum with mycorrhizal fungi were significant. Also, the highest amount of stevioside was applied to the Imma and Angel and vermicompost planting bed under inoculum with Arbuscular mycorrhizal fungi in the second harvest (16.45%)(Table 4). The boron element results in a higher content of glycoside stevioside (Sheu et al., 1987), where the Imma and Angel nutrient solutions indicated higher levels of stevioside with a greater content than other nutrient solutions. The use of vermicompost led to a significant increase in the percentage of stevioside, which is due to its positive effect on photosynthesis and carbohydrates. The cause of the rise in the percentage of stevioside in inoculum conditions with Arbuscular mycorrhizal fungi was to improve the absorption of nutrients and other carbon hydrates in the plants under treatment. Probably, the fungus stimulates the photosynthesis system by increasing the ability of the plant's photosynthetic system, facilitating electron transfer and increasing the density of photosynthetic units. Therefore, the positive and reciprocal effect of the plant with the root mycorrhizal fungus leads to the photosynthetic performance of plants. Bhuyan and colleagues (Bhuyan et al, 2015) have reported an increase in the number of molasses in plants treated with Mycorrhizal fungi.

The reason for the effect of mushrooms in increasing the content of soluble sugars is the increase in the levels of plant hormones such as cytokinin and gibberellin in mycorrhizal plants. An increase in the amount of these hormones, especially cytokinin, can increase the rate of photosynthesis by transferring ions that are effective in opening the stomata and regulating the level of chlorophyll, and ultimately increase the content of carbohydrates in applied fertilizers (Zare Hassanabadi et al., 2020). Biological plants with the origin of mycorrhizal fungi caused an increase in soluble sugars and anthocyanin content of the plant (Valinezhad et al, 2019)..

بحث؟؟؟

Table 2. analysis of variance of the effect of studied treatments on stevioside, Rebaudioside A, and Rebaudioside C.

		Mean squares		
Source of variances	df	Stevioside	Rebadioside C	Rebadioside A
Repetition	3	0/836	0/234	0/098
Nutrient solution	1	6/314**	2/898**	47/388**
Planting bed	2	119/085**	17/51**	40/66**
Mycorrhiza inoculation	1	6/648**	16/884**	0/67**
Harvest	1	0/112**	2/47**	42/24**
Nutrient solution*Planting bed	2	10/525**	1/642**	10/55**
Nutrient solution* Mycorrhiza inoculation	1	14/362**	13/03**	0/017**
Nutrient solution*Harvest	1	0/16**	0/767**	26/423**
Planting bed* Mycorrhiza inoculation	2	14/72**	1/684**	23/488**
Planting bed*Harvest	2	27/444**	21/474**	25/974**
Mycorrhiza inoculation*Harvest	1	73/116**	31/622**	4/705**
Nutrient solution*Planting bed* Mycorrhiza inoculation	2	25/8**	0/398**	1/531**
Nutrient solution*Planting bed*Harvest	2	85/312**	10/51**	10/225**
Planting bed* Mycorrhiza inoculation*Harvest	2	32/526**	6/49**	0/588**
Nutrient solution* Mycorrhiza inoculation*Harvest	1	0/265**	15/18**	8/731**
Nutrient solution*Planting bed* Mycorrhiza inoculation*Harvest	2	39/68**	20/06**	0/886**
Error		0/176	0/526	0/078
%CV	-	0/46	0/466	0/894

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

Table 3. comparing the mean of simple effect of the studied treatments on concentration of Stevioside, Rebaudioside A and Rebaudioside C.

Treatment		Mean		
		Stevioside	Rebadioside C	Rebadioside A
Novella	10/7 b		6/09 a	4/46 a
Imma& Angel	11/21 a		5/74 b	3/06 b
Leaf composts	8/77 c		5/79 b	3/11 b
Vermi compost	12/44 c		6/707 a	5/06 a
Peat moss& Perlite	11/65 b		5/24 c	3/1 b
Mycorrhiza inoculation	11/22 a		6/33 a	3/84 a
Control	10/69 b		5/49 b	3/67 b
First harvest	10/92 b		5/75 b	3/09 b
Second harvest	10/99 a		6/07 a	4/42 a

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

Table 4. comparing the mean values of the interaction effects of studied treatments on the rate of stevioside, Rebaudioside A and Rebaudioside C.

Mean		Treatments		Stevioside	Rebadioside C	Rebadioside A
Novella	Leaf composts	Mycorrhiza inoculation	First harvest	i12/08	v3/06	e5/46
			Second harvest	s6/34	a8/78	b6/69
		Control	First harvest	r7/17	q4/58	m2/98
			Second harvest	m10/26	f7/14	c6/05
	Vermicompost	Mycorrhiza inoculation	First harvest	b15/8	c7/92	n2/52
			Second harvest	L10/5	l5/76	a7/57
		Control	First harvest	o9/18	o5/3	r1/07
			Second harvest	k10/67	d7/82	b6/67
	Peat moss& Perlite	Mycorrhiza inoculation	First harvest	q3/35	t4/11	L3/2
			Second harvest	e12/87	h6/57	q1/81
		Control	First harvest	f12/58	n5/41	j4/11
			Second harvest	d13/6	h6/58	g5/1
Imma & Angel	Leaf composts	Mycorrhiza inoculation	First harvest	J11/4	k5/82	f5/31
			Second harvest	t5/04	s4/25	d5/91
		Control	First harvest	P8/62	g7/04	k3/29
			Second harvest	n9/28	m5/63	h4/77
	Vermicompost	Mycorrhiza inoculation	First harvest	n9/35	r4/39	s1/012
			Second harvest	a16/45	e7/7	q1/824
		Control	First harvest	h12/3	j6/08	p1/88
			Second harvest	c15/27	b8/69	o2/34
	Peat moss& Perlite	Mycorrhiza inoculation	First harvest	d13/62	r4/42	p1/89
			Second harvest	p8/69	u3/13	q1/81
		Control	First harvest	g12/43	p5/18	j4/11
			Second harvest	i12/09	i6/54	n2/34

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

2.1.2. Rebaudioside C Glycoside Content

The results of analysis of variance on the measured traits showed a significant difference in terms of the content of Rebaudioside C glycoside between different nutrient solutions, different planting beds and inoculation with Arbuscular mycorrhizal fungi as well as first and second harvest, at a probability level of 1% (Table 2). The interactions between the four treatments were significant and the highest percentage of Rebaudioside C glycoside was observed in irrigated Novella nutrient solution and leaf composts soil planting bed under inoculation with Arbuscular mycorrhizal fungi and in the second harvest (8/78%).

2.1.3. Rebaudioside A Glycoside Content

Based on the results of analysis of variance, there was a significant difference in the content of Rebaudioside A glycoside, between different nutrient solutions, different planting beds, and inoculation with Arbuscular mycorrhizal fungi as well as first and second impressions (Table 2). The

interaction between the four treatments was significant and the highest percentage of Rebaudioside A was found in nourished treatments with Novella nutrient solution and vermicompost planting bed under inoculum with Arbuscular mycorrhizal fungi and in the second harvest (7.568%)(Table 4). Due to the effect of mycorrhiza on stomatal conductance, the magnitude of photosynthesis of mycorrhizal plants was higher than that of non- plants inoculated with mycorrhiza. The production of plant hormones such as cytokines with these fungal organisms can also correlate with their effects on the metabolic processes of the plant, which can increase the growth, leaf area, and the photosynthesis in inoculated plants. This increase in photosynthetic capacity allows the production of carbohydrates for fungi and enhances the plant's yield. Mycorrhizal fungi increase the level of contact with soil and moisture by 10-1000 times through forming networks around the plant roots, thus making the plant more capable of using the resources in its surroundings (Sharma, 2002). A study conducted on Stevia, Patil (2010), found that the use of vermicompost increased the content of carbohydrates in the leaves and the percentage of sugars. Mojtaba et al. (2015) observed that application of vermicompost fertilizer increased sugar content in sugar beets. The results of Borda-Molina et al. (2011) suggested a positive correlation between vermicompost application and glycoside production in stevia. By examining the effect of mycorrhizal fungi on the stevia plant, Mandal et al. (2013) stated that the plants inoculated with mycorrhiza exhibited higher concentrations of stevioside glycosides compared to the control treatments. They stated that the concentration of stevioside glycosides was higher due to the increase in the biomass of the plant's air organs. On the other hand, the biomass increased directly due to improved absorption of nutrients (nitrogen, potassium, copper, iron, manganese, and zinc), chlorophyll content, and carbon hydrates in plants treated with mycorrhiza.

2.2. Biomass Production

2.2.1. Leaf Fresh and Dry Weight:

The results obtained from analysis of variance for measuring the leaf fresh weight and leaf dry weight showed a significant difference between different nutrient solutions, inoculation with Arbuscular mycorrhizal fungi and different planting beds. Comparison of the mean showed that Novella nutrition solution in vermicompost culture under inoculation with Arbuscular mycorrhizal fungi had the highest leaf fresh weight (666.8 g) and highest leaf dry weight(128.1g) (Table 7) Vermicompost had a positive effect on photosynthesis and production of biomass of stevia and led to the growth of the bush through increasing water absorption power and favorable access to macro and micronutrients. The results obtained by other researchers on a type of plant in urban green space (*Sesbania emerus*) and *Pennisetum glaucum* L. confirmed this (hameeda, 2006). Use of suitable amounts of vermicompost through improving the microbial activities of soil and producing plant growth regulators by the organisms and greater intakes of nutrients can lead to increased photosynthesis and enhanced growth of shoots and plant dry matter (Ravi Kumar *et al.*, 2004, Singh *et al.*, 2008). Azarpoor et al. (2013) conducted a study on the use of vermicompost in stevia farming and reported that the use of vermicompost could have a positive and significant effect on fresh and dry weight of leaf and biomass. In the case of nutritious treatments with Novella nutrient solution, due to the availability of more nitrogen than the Imma and Angel nutrient solution, we observed more leaf fresh weight and leaf dry weight of leaves. Further, inoculation with mycorrhizal fungi had a significant effect on the leaf fresh weight and leaf dry weight compared to the control treatment. In general, increase in the nutrient and water intake could be mainly because of release of mycelium in mycorrhizal fungi associated with the interior tissues of the root, in the soil around the root, and formation of an additional adsorption system as a complement of the root system. It is assumed that the symbiosis of Arbuscular mycorrhizal fungi with the root of stevia, through absorption of water and nutrients, increases the photosynthesis level, thereby producing more products and enhancing growth in the form of increased fresh and dry weight of the stem. The results obtained from studying *Mentha arvensis* and *Cymbopogon spp* have confirmed this result (ratti *et al.*, 2011). In this way, mycorrhizae could increase the concentration of phosphor and enhance the speed of photosynthesis in the host plant. Relevant studies have suggested that symbiosis of plants with mycorrhizal fungi could

improve the growth properties of the plant such as development of growing parts and elevation of fresh and dry weight of plant tissues (Silveria *et al.*, 2006). It has also been noted that *G. mosseae* type of mycorrhizae could increase the dry weight of root and stem of acacia through raising the adsorption of nitrogen, phosphorus, and potassium (Kaushik *et al.*, 2005). Increased dry weight of shoot by *Arbuscular mycorrhizal fungi* has been reported in Chinese chive (Perner *et al.*, 2011) onion (Asgharzad *et al.*, 2001), tomato (Al-Karaki and Hammeeda, 2001), and pepper (Sensoy *et al.*, 2007).

2.3. Biometric Characteristics

2.3.1. Number of Main Branches

The number of main branches in the stevia plant was not affected by different nutrient solutions. However, the different planting beds and the main effect of application or non-application of mycorrhizal fungi were significant at 1% probability level (Table 5). The results of analysis of variance of the interaction between nutrient solutions, different planting beds, and inoculation with *Arbuscular mycorrhizal fungi* also revealed a significant difference. Comparison of the mean showed that Novella nutrition solution in vermicompost culture under inoculation with *Arbuscular mycorrhizal fungi* (main branch of 5.87) had the highest number of main branches (Table 7). Rashid *et al.* (2013) examined the effect of different levels of nitrogen on the growth and yield of stevia. The results of this study showed that the plants grown under treatments of 40 and 60 kg N ha⁻¹ had significantly more branches and leaves than other levels. Nitrogen increases the growth rate, thus the number of branches also grows in return. In this experiment, we also saw a larger number of branches nutritionally treated with Novella nutritional solution due to the higher nitrogen content than the other nutrient solutions. Touati *et al.* (2014) found that all *Arbuscular mycorrhizal fungi* plants had higher heights and more branches, compared with plants not inoculated with mycorrhizal fungi.

2.3.2. Plant Height

Plant height was significantly affected by the different nutrient solutions at 5% level. Also, the planting bed and inoculation with *Mycorrhiza* fungus showed a significant difference at the level of 1% (Table 5). The mean comparison indicated that the Imma and Angel nutrient solutions in the leaf composts planting bed under the conditions of inoculation with *Arbuscular mycorrhizal fungi* (123 cm) had the highest plant height (Table 7). 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. Also, greater photosynthetic extraction is expected to be yielded by the plant, which is a good condition required for elongation of the stem (Nourmohammadi *et al.*, 2011). Saber hameshagi *et al.* (2012) reported a study on the effects of nitrogen and potassium on the *Stewia* plant. The highest stem length (altitude) was observed in 60 kg/ha treatment and the minimum nitrogen application was obtained. The higher percentage of nitrogen in the Imma and Angel nutrient solutions could be attributed to the effect of nitrogen on the height of the plants treated with this solution. According to the results, *arbuscular mycorrhizal fungi* had a positive effect on the growth traits (plant height and branch number), which was consistent with the results of Piedra *et al.* (2005) and Soriano *et al.* (2009). Vafadar *et al.* (2014) in a study on the culture of stevia found that the plants cultivated by the fungus were much taller than the cases of non-inoculation with mycorrhizal fungi. During a research, the use of mycorrhizal fungi and biosphere biofertilizer caused an increase in plant height traits, growth and yield indicators, and yield components of cumin medicinal plant, and they stated that probably some of the filaments entered the root system and caused a decrease in concentration. Absciscic acid and cytokinins increase, which expands the beard system and increases water and food absorption (Haghir Ebrahimabadi *et al.*, 2018). In general, one of the important reasons that can be mentioned for the effect of biological fertilizers on increasing the height of the plant is that the consumption of these fertilizers led to an increase in the length of the internodes. Regarding the effect of beneficial microorganisms on increasing the height of the plant, it should be

stated that this was probably due to the increase in the absorption of nutrients by mycorrhiza and their effect on photosynthesis and as a result plant growth.

Table 5. analysis of variance of the effect of the studied treatments on leaf dry weight , number of main branches, and plant height.

Mean					
Source of variances	df	Leaf fresh weight	Leaf dry weight	Number of main branches	Plant height
Repetition	3	6341/66	458/33	1/29	712/423
nutrient solution	1	88648/83**	732/57**	0/63	1656/75*
Planting bed	2	124957/51**	7237/26**	5/81**	347/078 ^{ns}
Inoculation	1	46800/03**	2215/17**	18/13**	2976/75**
Planting bed × nutrient solution	2	78939/07**	727/6**	4/41*	316/797 ^{ns}
nutrient solution× Inoculation	1	79283/76**	430/08**	4/38*	500/52 ^{ns}
Planting bed × Inoculation	2	144228/91**	9578/57**	0/56	1773/109*
Planting bed × nutrient solution× Inoculation	2	11865/36**	1929/62**	20/036**	602/036 ^{ns}
Error	33	520/04	19/17	0/899	386/62
%CV	-	14	10	33/1	20/23

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

Table 6. comparing the mean of simple effect of the studied treatments on leaf dry weight, leaf dry weight, number of main branches and plant height.

Mean								
Planting bed	Leaf fresh weight (g plant ⁻¹)			Leaf dry weight(g plant ⁻¹)		Number of main branches		Plant height(cm)
Peat	164/617	a		47/63	a	2/75	b	91/31 b
Vermicompost	119/85	b		39/66	b	2/97	a	103/06 a
Peat & perlite	124/975	b		35/88	b	2/187	b	92/47 a
Mycorrhizal fungi	202/15	a		67/845	a	3/062	a	97/31 b
Inoculation by fungi	99/575	c		27/225	c	3/343	a	101/78 a
Control	152/95	a		36/938	b	3/479	a	105/06 a

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

Table 7. comparing the mean values of the interaction effects of studied treatments on the leaf dry weight, leaf dry weight, number of main branches and plant height.

Treatments			Mean			
			Leaf fresh weight (g plant ⁻¹)	Leaf dry weight (g plant ⁻¹)	Number of main branches	Plant height(cm)
Novella	Leaf composts	Mycorrhiza inoculation	145b	46/8c	1e	99/75abcd
		Control	81/5de	18/2g	2cde	65/63d
	Vermicompost	Mycorrhiza inoculation	666/8a	128/1a	5.87a	78/5de
		Control	105/1Cd	27/18f	3bc/12	102/5abcd
	Peat moss& Perlite	Mycorrhiza inoculation	151/7B	39/7de	3bcd	109/63abc
		Control	84/7De	26/1f	1.5 e	91/88bcde
	Leaf composts	Mycorrhiza inoculation	150/3B	43/6cd	1/75de	123a
		Control	123/6Bc	35/2e	2cde	81/5cde
		Mycorrhiza inoculation	142/5B	77/5b	2/25cde	112ab
	Imma & Angel Vermicompost					

Peat moss& Perlite	Control	140/8B	38/6de	3/87b	96/25abcd
	Mycorrhiza inoculation	96/7Cde	26e	4b	107/5abc
	Control	65/2E	18/3g	2cde	98/13abcd

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

Table 8. Concentration of nutrient solution (mg / l).

Elements	KNO3	Ca(NO3)2	NH4NO3	KH2PO4	MgSO4	Na2MoO4	H3BO3	CuSO4	ZnSO4	MnSO4	Fe- EDDHA
Imma & Angel	20/4	254/2	352	598/4	96	0/033	2/21	0/481	0/645	5/15	13/25
Novella	505	656	47	240	180	0/055	1/56	0/912	1/07	8/5	4

3. Conclusions

At the end of the experiment, it was observed that the application of planting bed and mycorrhiza fungus had a significant effect on Stevioside in stevia. Stevia can have symbiosis with the mycorrhiza fungus and grow to a large extent, as symbiosis with mycorrhiza fungus increases fresh and dry leaf yield. Accordingly, mycorrhizal fungi can be used as a useful biologic fertilizer for the cultivation of stevia. The recommended application of mycorrhiza fungus and vermicompost planting bed to farmers can lead to increased leaf yield and stevioside percentage. It is also suggested to use Novella's nutritional solution to increase the yield and glycosidic percentages.

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