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Research Article

Effect of bio-fertilizer on growth, development and nutrient content (leaf and soil) of *Stevia rebaudiana* Bertoni

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Abstract: The aim of this study was to evaluate the effect of bio-fertilizers on growth and development of plants in pots containing (V) vermicompost and (M) cow manure: (soil amendments) and (S) garden soil separately and in combination. In the first experiment, treatments included S (control), SM (soil + 15%M + 0%V), SMV10 (soil + 15%M + 10%V), and SMV20 (soil + 15%M + 20%V) by dry weight (w/w). In the second experiment, mycorrhizae (*Glomus mosseae* and *Piriformospora indica*) were also added to the treatments. Plants and soils were evaluated 90 days after inoculation with fungi. At maturity, plant height and stem diameter, leaf area index (LAI), chlorophylls (a + b) content, root length, fresh and dry weights (aerial parts and root), and mineral nutrient content in plant leaves were measured. The obtained results indicated significant differences between treatments. Among all the treatments, interaction of soil amendments and fungus was significant for all traits. Almost the maximum amount of morphological traits was found in the combined applications of soil amendments and fungi. In particular, the highest weights were obtained in the SMV20 treatment inoculated with *G. mosseae*. Nutrient analysis of leaves, revealed maximum amounts of N, P and K in SMV10 and SMV20 treatments, and the highest amount of Ca and Mg was obtained in plants treated with SM and inoculated with *P. indica* in comparison to control plants. Also, the highest amount of phosphorous in the soil was obtained when SMV20 treatment was inoculated with *P. indica*. It seems that the combination of biofertilizers is the best result for the stevia plant growth..

Keywords: *Stevia rebaudiana* Bertoni, *Glomus mosseae*, *Piriformospora indica*, inoculation, cow manure

Introduction

Stevia rebaudiana Bertoni is a perennial plant that belongs to the Asteraceae family. The Origin of this plant is America (Brandle *et al*., 1998). About 33 types of steviol glycosides (SVglys) are detected in the leaves (Ohta *et al*., 2010; Chaturvedula *et al*., 2011). Also some are found within the stem and flowers (Darise *et al*., 1983). In recent years, the natural sweetener steviol glycoside is replaced with sucrose. In addition to their high sweetness, steviosid have significantly medicinal effects for e.g., diabetes-type 2, atherosclerosis and hypertension (Geuns, 2010).

Researchers have shown active constituents specifically steviosid in *Stevia* herb are very much dependent on the agricultural operations and the use of organic fertilizers in a technical way (Geuns, 2003) and water management (Fronza and Folegatti, 2003). Nowadays, seedlings of this plant are cultivated on a large scale in north of Iran (Nadri, 2012). Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs

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belonging to the phylum Glomeromycota (Schussler *et al*., 2001) which prepare a lot of benefits to their host (Brundrett *et al*., 1996).

In recent years organic matter as a suitable source of nutrients to the soil has been replaced by unfavorable chemical fertilizers (Ntanos and Koutroubas, 2002). One of the best organic materials for boosting crops yield is vermicompost. Vermicomposts are the products of the degradation of organic matter through interactions between earthworms and microorganisms. Vermicomposts are finely divided materials with high porosity, aeration, drainage and water-holding capacity and usually contain nutrients in the available forms (Atiyeh *et al*., 2002; Arancon *et al*., 2004). Organic fertilizers and especially manure, compared to chemical fertilizers contain high amounts of organic matter and could be a source of nutrients, especially nitrogen, phosphorus and potassium (Fernandez *et al*., 1993). These elements gradually are placed at the disposal of plant (Eghball *et al*., 2004) but cannot provide all that plants need (Mallanagouda *et al*., 1995). However, improving the soil physical structure will cause the balance of chemical characteristics of the soil (Chaudhry *et al*., 1999). In this study, we examined what combinations of biofertilizers influence the growth and nutrient content in stevia plant and p content in soil.

Materials and Methods

Pot culture experiments

In order to identify the best combination of bio-fertilizers on *stevia rebaudiana* growth and development, nutrient content in stevia leaves and soil, an experiment was carried out in factorial format based on randomized complete block design with twelve treatments and three replications. The greenhouse experiments were conducted at Faculty of Agriculture of Tarbiat Modares University in Iran. The vermicompost was produced from poultry manure with the earthworm *Eisenia fetida*.

The soil texture was loamy clay and bed of pots included garden soil (control or S), (M) cow manure and (V) vermicompost: (soil amendments). So treatments consisted of S, SM (S + 15%M + 0%V), SMV10(S + 15%M + 10%V) and SMV20(S + 15%M + 20%V) by dry weight in pots (w/w), then *Glomus mosseae* (*G. mosseae*) and *Piriformospora indica* (*P. indica*) inoculated. Garden soil had been fumigated with a 7% formaldehyde solution. *Stevia rebaudiana* seedlings (seedlings of tissue culture) were obtained from Saba Sekkhe Institute of Research and Technologies (Isfahan) and planted in pots. After 30 days when *Stevia* seedlings were established they were inoculated with *G. mosseae* and *P. indica*.

For *G. mosseae* inoculation, inoculum material 18g (was provided by Institute of organic botanical clinic, Hamadan, Iran) and for *P. indica* inoculation, inoculum material 20 ml (was prepared at TMU) was added to pot soils (Mohammadi Goltapeh *et al*., 2013). The efforts were made to keep the root system in somewhat need direct contact with the fungal inoculum. The controls were also maintained without inoculation. Plants were kept in greenhouse at 24-30:18 °C day: night and photoperiod of 16: 8 h light: dark and 40-60% humidity. Plants grown in pots were removed from bed and were analyzed 90 days after inoculation. Roots were washed thoroughly under running tap water and cut into 1cm pieces for microscopic observation. Segments were stained following the techniques described in literature (Dickson *et al*., 1998; Phillip *et al*., 1970) the root-pieces were examined under microscope at magnifications of 10-40 X.

During harvest, the dried stevia leaf samples were prepared by a grinding machine with a sieve spacing of o.5mm to determine nutrient content in the leaf. Available N was determined using Kjeldahl method (Walinga *et al*., 1989), available P was determined by spectrophotometry (Chapman and Pratt, 1961), available K was determined by flame photometer (Whanig *et al*., 1989), Calcium and magnesium was determined by atomic absorption (Walinga *et al*., 1989). P content in soil was measured by Olsen method (ISRIC, 1986).

Statistical analysis

The collected data were statistically computed using software SAS 9.2. Data were subjected to analyses of variance and treatment means were compared by an approximate Duncan's multiple-range tests and main effective Interaction was found significant at $p < 0.05$

Results

Soil and fertilizers analysis results are shown in (Tables 1 and 2).

Plant height and stem diameter

Growth of plants was measured after 120 days. Plants treated with amendments and inoculated with *G. mosseae* and *P. indica* were more vigorous than control plants, had extended leaves, thicker stems and numerous shoots than those of the control plants.

Results (Table 3) indicate that plant height and stem diameter was significantly affected by the application of amendments and fungus ($P < 0.01$). The greatest plant height and stem diameter were attained by SM, SMV10 and SMV20 treatments in comparison to control plants (Table 4). *G. mosseae* and *P. indica* significantly increased height (0.97%, 2.97%) and stem diameter (3.9%, 2.91%) over those of non-inoculated control plants. Results also (Table 3) indicated that the interaction of amendments (M and V) and *G. mosseae* and *P. indica* fungi was significant ($P < 0.05$, $P < 0.01$). The Maximum height was found in SM, SMV10 and SMV20 with (or without) *G. mosseae* and *P. indica* inoculation over that of controls. Also maximum stem diameter was obtained in SM*P. indica*, SMV10*G. mosseae* and SMV20*G. mosseae* in comparison to control plants (Table 4).

Leaf area index (LAI)

The results presented in (Table 3) have revealed that various levels of biofertilizers had significant effects on LAI ($P < 0.01$). The mean plant LAI ranged from 11.7 to 16.7 mm^2 in amendments treatment. The maximum was obtained by using 20% V (SMV20) in comparison to control plants 11.0 and 12.8 (Table 4).

G. mosseae and *P. indica* increased LAI in comparison to controls. LAI in plants inoculated with *G. mosseae* was 16.91% more than that of controls and 5.26% more than that of inoculated with *P. indica*. The interaction of amendments and fungus was also significant (Table 3). The highest LAI was obtained with application 20%V (SMV20) inoculated with *P. indica* and was 91.4% higher than non-inoculated control plants (S).

Root length

The results indicate that root length was affected by the inoculation with fungus alone (Table 3) and tallest roots were recorded 18.19 cm and 17.3 cmfor inoculations of *G. mosseae* and *P. indica* respectively in comparison to non-inoculated control plants (15.6 cm) (Table 4).

Chlorophylla (a + b) content

The results (Table 3) indicate that chlorophyll (a + b) content was not affected by amendments and mycorrhizae. But the interactions of amendments with *G. mosseae* and *P. indica* were significant ($P < 0.05$). Maximum chlorophyll content (17.6%) was obtained by using SM together with *G. mosseae* in comparison to control plants (Table 4).

Fresh and dry weight

The fresh and dry weight of aerial parts and root were significantly affected by amendments and inoculation with *G. mosseae* and *P. indica* ($P < 0.01$), except aerial dry weight that was affected by amendments alone. Also root fresh weight/root dry weight that was affected by amendments and interaction of amendments and inoculation ($P < 0.01$) and was not affected by fungi (Table 5). The increase resulted from the higher mean number of shoots and leaves per plants, as well as increase in total aerial parts fresh and dry weight (Fig 1). Three months after co-culture, treatments differed in plant fresh and dry weight. *G. mosseae* and *P. indica* increased aerial fresh weight (20.04%, 19.11%) respectively, also *G. mosseae* increased.

Table 1 Physical and chemical properties of garden soil used as potting medium.

Characteristics	C (%)	CEC (cmol/Kg)	Mn (ppm)	Cu (ppm)	Zn (ppm)	Fe (ppm)	PH	EC (dS/m)	K (ppm)	P (ppm)	N (%)
Soil sample	0.75	13.4	4.84	1.3	0.58	9.6	7.55	1.45	107.6	2.2	0.07

Table 2 Chemical characteristics of vermicompost and manure.

Soil amendments	OC (%)	pH	EC (dS/m)	N (%)	P (ppm)	K (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Ca (%)	Mg (%)
Vermicompost	7.44	7.67	12	0.74	96	2667	1820	210	108	1.52	0.22
Manure	7.83	7.98	6.75	0.78	88	1630	-	-	-	-	-

Table 3 Analysis of variance of growth parameters of *Stevia*, under greenhouse conditions.

Sources of variation	df	Mean square				
		Plant height (cm)	Stem diameter (mm)	Leaf area index (mm^2)	Root length (cm)	Chlorophylla (a + b) $\mu g.ml^{-1}$
Fertilizer (soil amendments)	3	8.24**	34.30**	40.50**	8.42^{ns}	4.83^{ns}
Inoculation(inoculated with Fungi)	2	23.10**	0.44**	13.90**	20.20*	2.35^{ns}
Fertilizer × Inoculation	6	4.10*	5.32**	18.40**	4.30^{ns}	6.73*
Error	24	2.92	0.32	4.63	3.06	2.00
CV		7.20	8.20	12.50	9.80	17.12

Table 4 Effect of different combinations of soil amendments and mycorrhiza on some growth parameters of *Stevia rebaudiana*.

Treatment	Plant height (cm)[1]	Stem diameter (mm)[1]	Leaf area index (mm^2)[1]	Root length (cm)[1]	Chlorophylla (a + b) ($\mu g.ml^{-1}$)[1]
Fertilizer (Soil amendments)					
S (control)	76.556b	5.32b	11.07c		
SM	95.62a	7.45a	13.91b		
SMV10	98.80a	7.66a	14.95ab		
SMV20	95.31a	7.68a	16.17a		
Inoculation					
G. mosseae	91.26b	7.14a	15.00a	18.19a	
P. indica	93.07a	7.07a	14.25ab	17.30a	
control	90.38c	6.87b	12.83b	15.60b	
Fertilizer × Inoculation					
S × *G. mosseae*	78.66b	5.27c	15.16b		8.65ab
S × *P. indica*	78.33b	5.44c	7.96d		5.80cd
S	72.66b	5.26c	10.08cd		8.84ab
SM × *G. mosseae*	97.55a	7.26ab	14.80b		10.40a
SM × P. indica	98.55a	8.34a	14.36b		4.61d
SM	90.77a	6.75b	12.58bc		6.21cd
SMV10 × G. mosseae	99.10a	7.91a	15.88ab		8.46ab
SMV10 × *P. indica*	97.32a	7.25ab	15.38b		9.08ab
SMV10	100a	7.82ab	13.6bc		8.27b
SMV20 × *G. mosseae*	89.72a	8.13a	14.16b		7.30bc
SMV20 × *P. indica*	98.10a	7.27ab	19.30a		8.64ab
SMV20	98.11a	7.64ab	15.04b		8.31b

S: garden soil, M: cow manure, V: vermicompost.

[1]: Means followed by the same letters in a column are not significantly different (Duncan's multiple range test, $P < 0.05$).

root fresh and dry weight (27% and 27.6%) in comparison to non-inoculated control plants, respectively (Table 6) but there were no significant differences among plants inoculated with *P. indica* and control. Also in soil amendments, a significant increase was observed in (aerial parts and root) fresh and dry weight in SMV20 treatment as compared to control (Table 6).

Also the interaction of amendments and mycorrhizae was significant (Table 5). The results of present study, demonstrated that plants weight was significantly influenced by the application of amendments and *G. mosseae* (Figs. 1, 2). Among various treatments, almost the application of SMV20 has indicated maximum increase in fresh and dry weight per plant. (The relation of root and aerial part is shown in Table 6).

Microscopic inspection of roots inoculated with *G. mosseae* and *P. indica* detected heavy colonization and abundant production a large number of chlamydospores in root tissue (Fig 3).

There were significant differences at 1% probability level between nutrient in plant leaves and p content in soil in various levels of biofertilizers (Table 7).

Table 5 Analysis of variance of the *Stevia* plant growth parameters under greenhouse conditions.

Sources of variation	df	Mean square						
		APFW (g)	APDW (g)	RFW (g)	RDW (g)	RFW/APFW (g)	APFW/APDW(g)	RFW/RDW (g)
Fertilizer (soil amendments)	3	17459.7^{**}	920.80^{**}	106.60^{**}	92.30^{**}	0.50^{**}	0.70^{ns}	2.80^{**}
Inoculation	2	868.6^{**}	52.01^{ns}	315.37^{**}	32.70^{**}	0.03^{*}	0.95^{ns}	0.02^{ns}
Fertilizer × Inoculation	6	609.6^{**}	22.90^{ns}	205.15^{**}	25.38^{**}	0.07^{**}	0.99^{ns}	2.73^{**}
Error	24	106.1	18.90	41.90	1.12	0.007	0.57	0.17
CV		12.1	20.60	17.30	11.46	16.20	18.66	10.08

**: significant at $P < 0.01$, *: significant at $P < 0.05$, ns: non significance.
APFW: aerial parts fresh weight, APDW: aerial parts dry weight, RFW: root fresh weight, RDW: root dry weight.

Figure 1 Effect of different combinations of soil amendments and mycorrhiza on growth of *Stevia rebaudiana Bertoni* in greenhouse condition.

Table 6 Effect of different combinations of soil amendments and mycorrhiza on fresh & dry weights of shoot and root.

Treatment	APFW (g)[1]	APDW (g)[1]	RFW (g)[1]	RDW (g)[1]	RFW/ APFW (g)[1]	RFW/ RDW (g)[1]
Fertilizer (soil amendments)						
S (control)	25.4d	6.6c	22.30c	4.99c	0.87a	4.40a
SM	82.4c	22.3b	34.60b	9.20b	0.42b	3.75b
SMV10	102.5b	25.3b	34.75b	9.90b	0.34c	3.50b
SMV20	129.3a	29.9a	57.23a	12.70a	0.45b	4.68a
Inoculation						
G. mosseae	90.2a		43.10a	11.13a	0.58a	
P. indica	89.5a		34.60b	8.54b	0.51ab	
Control	75.1b		33.92b	8.05b	0.47b	
Fertilizer × Inoculation						
S × *G. mosseae*	28.6g		29.39de	4.88ef	1.01a	5.91a
S × *P. indica*	21.9g		22.90e	6.32e	1.04a	3.50de
S	25.9g		14.54f	3.78f	0.55b	3.82de
SM × *G. mosseae*	75.2ef		30.70de	8.85cd	0.40bcd	3.45de
SM × *P. indica*	100.8cd		37.66c	10.54bc	0.37cd	3.57de
SM	71.2f		35.51cd	8.39d	0.49bc	4.23cd
SMV10 × *G. mosseae*	102.4bcd		38.39c	11.87b	0.37cd	3.20e
SMV10 × *P. indica*	114.1bc		33.60d	9.10cd	0.29d	3.68de
SMV10	91.2de		32.26de	8.95cd	0.35cd	3.62de
SMV20 × *G. mosseae*	154.5a		74.01a	18.91a	0.53b	3.93de
SMV20 × *P. indica*	121.1b		44.28bc	8.20d	0.36cd	5.30ab
SMV20	112.2bc		53.40b	11.10b	0.47bc	4.80bc

S: garden soil, M: cow manure, V: vermicompost, APFW: aerial parts fresh weight, APDW: aerial parts dry weight, RFW: root fresh weight, RDW: root dry weight.

[1]: Means followed by the same letters in a column are not significantly different (Duncan's multiple range test, $P < 0.05$).

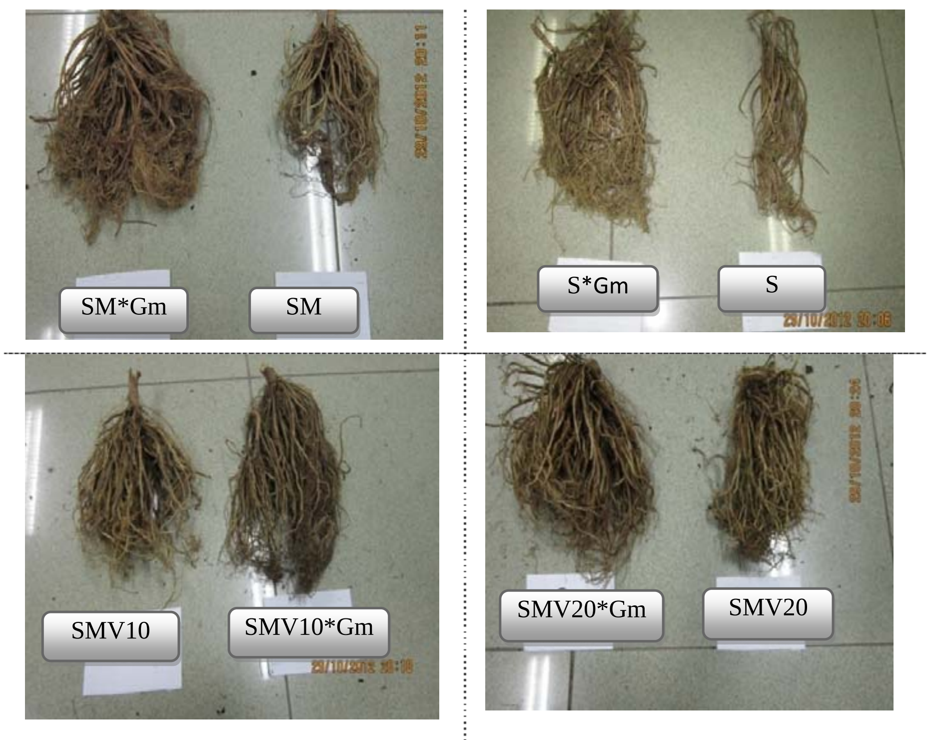

Figure 2 Effect of different combinations, soil amendments and mycorrhiza on root growth in *Stevia rebaudiana* in greenhouse condition.

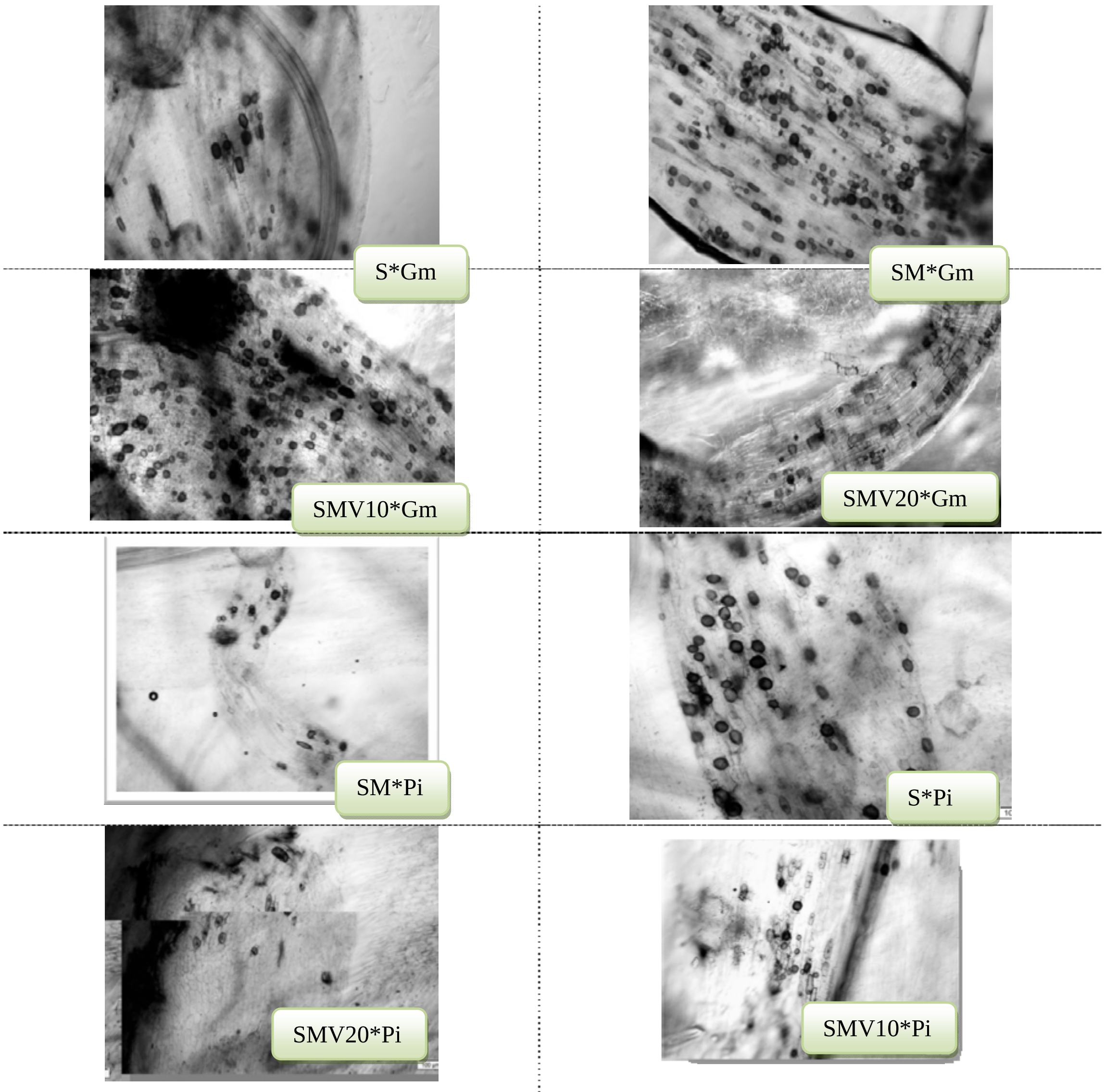

Figure 3 Detection of clear chlamydospores of *G. mosseae* and *P. indica* in roots of *Stevia rebaudiana* Bertoni.

Nitrogen, phosphorus and potassium content in plant leaves

The results presented in Table 7 have revealed that various levels of amendments had significant effect on N, P and K content in plant leaves. The maximum amounts of N, P and K were obtained by using 20% vermicompost treatment and were 36.6%, 29.6%, and 18.18% higher than control (S) respectively. In inoculation with *G. mosseae* and *P. indica*, significant increase in N, P and K content was observed in control plants, but N and K content in plants inoculated with *P. indica* was higher than that of plants inoculated with *G. mosseae* and P content was higher in inoculation with *G. mosseae* (Table 8). In addition in the interaction of bio-fertilizers the highest N, P and K contents (27.3%, 37.14%, and 16.29%) were obtained with application of 20%, 10% and 20% V respectively without *G. mosseae* and *P. indica* inoculation as compared to the control plants. (Table 8)

Calcium and magnesium content in plant leaves

The results (Table 8) showed that the maximum amount of Ca and Mg content in plant leaves was obtained in SM treatment compared to control plants. Ca and Mg content in plants inoculated with *P. indica* were higher than those of control and inoculated plants with *G. mosseae* (Table 8). The present results showed that the interaction of bio-fertilizers was significant (Table 8) and the highest amount Ca and Mg was obtained by SM* *P. indica* treatment in comparison to control plants (S).

Changes in available p content of soil

The results (Table 8) showed that the maximum available P was in 10%V (SMV10 treatment). However, the magnitude of such changes varied with treatments, the available P content with *G. mossea* inoculation was the same as control plants after 4 months of crop growth and was higher than that of plants inoculated with *P. indica*. In addition, in the interaction of bio-fertilizers, the highest amount P in soil was obtained when SMV20 treatment with *P. indica* was inoculated with *P. indica* (without significant difference with SM*G. mosseae).

Table 7 Analysis of variance for leaf elements and soil phosphorus.

Sources of variation	df	Mean square					
		N ($mg.g^{-1}$dwt)	P ($mg.g^{-1}$ wt)	K ($mg.g^{-1}$ dwt)	Ca ($mg.g^{-1}$ dwt)	Mg ($mg.g^{-1}$ dwt)	P soil (ppm)
Fertilizer (soil amendments)	3	0.360**	0.017**	0.10**	1.62**	0.37**	11616.5**
Inoculation	2	0.216**	0.028**	0.0018**	1.24**	0.35**	3.28**
Fertilizer × Inoculation	6	0.038**	0.006**	0.0011**	0.75**	0.14**	71.90**
Error	24	0.00002	0.00003	0.000026	0.0001	0.0002	0.19
CV		0.40	0.47	0.11	0.95	2.66	0.65

**: significant at $P < 0.01$, *: significant at $P < 0.05$, ns: non significance.

Table 8 Effect of different combinations of soil amendments and mycorrhiza on nutrient content of *stevia* plant leaves and in soil.

Treatment	N ($mg.g^{-1}$ dwt)[1]	P ($mg.g^{-1}$ dwt)[1]	K ($mg.g^{-1}$ dwt)[1]	Ca ($mg.g^{-1}$ dwt)[1]	Mg ($mg.g^{-1}$ dwt)[1]	P soil (ppm)[1]
Fertilizer (soil amendments)						
S (control)	1.20d	0.33d	1.32d	0.92b	0.56b	12.89d
SM	1.22c	0.42b	1.50c	1.73a	0.88a	82.04c
SMV10	1.36b	0.40c	1.53b	0.88c	0.45c	86.43a
SMV20	1.64a	0.428a	1.56a	0.85d	0.46c	85.47b
Inoculation						
G. mosseae	1.21c	0.38b	1.48c	0.81c	0.41c	66.92a
P. indica	1.39b	0.35c	1.48b	1.44a	0.75a	66.11b
control	1.48a	0.40a	1.50a	1.03b	0.60b	67.09a
Fertilizer × Inoculation						
S × *G. mosseae*	1.03j	0.38e	1.31i	0.91f	0.41h	11.46h
S × *P. indica*	1.23g	0.25i	1.29j	1.11c	0.83c	12.13h
S	1.39f	0.35f	1.35h	0.74j	0.45f	15.09g
SM × *G. mosseae*	1.22h	0.43c	1.50f	0.85g	0.44gh	88.86ab
SM × *P. indica*	1.23gh	0.35gh	1.49g	2.89a	1.31a	79.10e
SM	1.23gh	0.48b	1.52e	1.46b	0.90b	78.16f
SMV10 × *G. mosseae*	1.07i	0.34h	1.52e	0.76i	0.36i	88.16b
SMV10 × *P. indica*	1.48e	0.38e	1.55c	0.97e	0.48e	84.10d
SMV10	1.53d	0.48a	1.53d	0.92f	0.50e	87.03c
SMV20 × *G. mosseae*	1.55c	0.38e	1.55c	0.73g	0.42gh	79.20e
SMV20 × *P. indica*	1.62b	0.42d	1.56b	0.81h	0.38i	89.13ab
SMV20	1.77a	0.47b	1.57a	1.01d	0.58d	88.10b

S: garden soil, M: cow manure, V: vermicompost.

[1]: Means followed by the same letters in a column are not significantly different (Duncan's multiple range test, $P < 0.05$).

Discussion

Data on growth clearly showed that *G. mosseae, P. indica*, vermicompost and cow manure, had a stimulating effect on the growth. In this study, both fungi increased growth parameters as compared to control plans. Maximum plant height, stem diameter, LAI, root length and aerial part fresh and dry weights were attained by inoculated plants. Differences in growth may be because of the greater absorption of water and mineral nutrients due to extensive colonization of roots (Harrier and Sawczak, 2000). The strong stimulation of *stevia* root system extension by *G. mosseae* almost paralleled the growth promotion of the above ground plants. Perhaps the more intense root proliferation inoculated plants has attributed to the synthesis phytohormones to be the reasons for superior growth (Singh *et al*., 2000; Varma *et al*., 2001). It is well known that Arbuscular mycorrhizal-like fungi enhance growth and biomass production in symbiotic plants (Varma *et al*., 1999; Sahay and Varma, 1999; Rai *et al*., 2001; Kumari *et al*., 2003; Waller *el al*., 2005). Rai *et al*. (2001) reported that shoot and root length, biomass, basal stem, leaf area, overall size, number of inflorescences and seed production in *Spilanthes calva* and *Withania somnifera* were increased in the presence of *P. indica* which corresponds with results of this investigation. In this study, we saw the effect of bio-fertilizers on the fresh biomass yield and NPK content in *Stevia*, it's possibility the NPK content in *stevia* plant leaves gradually decreased with the progress of plant growth up to 90 days after *G. mossea* inoculation with the combined amendments. This is due to their ability to fix atmospheric nitrogen (symbiotic and a symbiotic) and transform native soil nutrients likely phosphorus and other nutrient from the non-usable (fixed) to usable form and decomposed organic wastes through biological processes which releases nutrients in a form which can be easily assimilated by plants and, thus increased uptake of soil phosphorus and potassium by the *stevia* plant and resulting in an increase in biomass production and decreased NPK of stevia inoculated plant that is confirmed by Das *et al*. (2007). On the basis of these results, it is clear that the soil amendments had significant effect on the morphological characteristics and nutrients in the soil and subsequently in plant leaves especially 20% vermicompost had the most effect in total biomass and macro elements. It seems that vermicompost, with high water-holding capacity and proper supply of macro-and micro-nutrients (Edwards and Burrows, 1988; Atiyeh *et al*., 2002; Arancon *et al*., 2004), has a positive effect on biomass production and subsequently the enhanced plant height. It has been hypothesized that the increases in growth, flowering and crop yields are due to earthworms as they enhance microbial populations that produce plant growth hormones (Paterson. 2003). Also, vermicompost has high microbial activity due to containing fungi, bacteria and actinomycetes (Tomati, U *et al*., 1988) that can produce plant growth regulators (PGRs) such as auxins, gibberellins, cytokinins, ethylene and abscisic acid (Frankenberger and Arshad, 1995). Improved growth, development and height of medicinal plants and other crops have previously been reported in the presence of optimal amounts of vermicompost (Vadiraj *et al*., 1998; Arguello *et al*., 2006; Darzi *et al*., 2007; Azizi *et al*., 2008). Vermicompost has been found to effectively enhance root formation, elongation of stem and production of biomass in vegetables, ornamental plants etc (Grappelli *et al*., 1985; Kale and Bano, 1986; Kale *et al*., 1987; Kale, 1998; Bano *et al*., 1993; Atiyeh *et al*., 1999) These earlier findings are in accordance with the results of present experiment, and the observations on the *Fragaria ananasa* (Arancon *et al*., 2004). Similar, results were also obtained for several other plants such as *Artemisia pallens, Foeniculum vulgare* (Pandey 2005; Roy and Singh 2006; Darzi *et al*., 2007). Also, Joshi and Adarsh (2010) reported plant dry biomass was to be significantly higher in vermicompost treatment as compared control tomatoes plant. A significant increase in mean stem diameter and mean plant height of tomato plant was observed by the addition of different concentrations of sheep manure and vermicompost in soil (Gutiérrez-Miceli *et al.*, 2007) that confirms present results. It was also reported that the increased N, P and K content of the leaves of tomato plants was due to foliar application of a liquid extract from vermicompost (Tejada *et al*., 2007). Also, Tejada and Gonzalez (2006) found that increased macronutrients in the leaves of rice and maize were due to foliar application of vermicompost extracts high in humic substances that confirms our results. All the earlier reports and present results support the fact of positive and synergistic effect on interaction between two or more factors, which is highly dependent on the effect of organic matter, contained in vermicompost or organic manures, on the activity of AM fungi. Many reports have shown that the interaction between bio-fertilizers can be beneficial for plant growth and yield (Hazarika *et al*., 2000; Ratti *et al*., 2001; Kumar *et al*., 2002; Darzi *et al*., 2008; Padmapriya and Chezhiyan, 2009). In this study, we showed that plants treated with bio-fertilizers were superior in development to control plants.

Conclusion

Because, *S. rebaudiana* Bert is a unique medicinal plant and importance of aerial biomass production in Gelicozid extraction, so we recommended combination of amendments with *G. mossea* that resulted from their strong synergistic relationship among themselves. However such increased effects have been found to be further enhanced significantly due to single or dual biofertilizers. Use of biofertilizers is an efficient approach to replace chemical fertilizers for sustainable cultivation of *Stevia rebaudiana*.

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تأثیرکودهای زیستی بر رشد، توسعه و محتوای عناصر غذایی (در برگ و خاک) در گیاه دارویی استویا

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چکیده: هدف از این آزمایش ارزیابی اثر کودهای زیستی بر رشد و توسعه گیاهان در گلدان‌هایی شامل کودهای اصلاحی (ورمی کمپوست و کود گاوی) و خاک باغچه جدا و در ترکیب با هم بود. در شروع آزمایش، تیمارها شامل S (شاهد)، SM (خاک باغچه + ۱۵٪ کود گاوی+۰٪ ورمی کمپوست)، SMV10 (خاک باغچه + ۱۵٪ کود گاوی + ۰٪ ۱۰ ورمی کمپوست) و SM20 (خاک باغچه + ۱۵٪ کود گاوی +۰٪۱۰ ورمی کمپوست) بر پایه وزن خشک (w/w) بود. در قسمت دوم آزمایش، قارچ‌های مایکوریز (*Glomus mosseae, Piriformospora indica*) به تیمارها اضافه شد. خاک و گیاهان ۹۰ روز پس از تلقیح با قارچ ارزیابی شدند. در زمان بلوغ رویشی، ارتفاع گیاه، قطر ساقه، شاخص سطح برگ، محتوای کلروفیل (a + b)، طول ریشه، وزن تر و خشک قسمت‌های هوایی و ریشه و محتوای مواد معدنی گیاه اندازه‌گیری شد. نتایج به‌دست آمده، تفاوت‌های چشم‌گیری را بین تیمارها نشان داد. در میان همه تیمارها، تنها تعامل بین کودهای اصلاحی و قارچ‌ها برای تمامی صفات معنی‌دار بود. تقریباً بیش‌ترین میزان صفات مورفولوژیکی در کاربرد ترکیبی کودهای اصلاحی و قارچ به‌دست آمد. به‌خصوص بالاترین میزان وزن‌ها در تیمار SMV20 تلقیح شده با قارچ *G. mosseae* به‌دست آمد. آنالیز عناصر غذایی در برگ‌ها نشان داد که میزان N، P و K در تیمارهای SMV10 و SMV20 و بالاترین میزان Ca و Mg در گیاهان تلقیح شده با تیمار SM و در تلقیح با *P. indica* در مقایسه با گیاهان شاهد به‌دست آمد. هم‌چنین بیش‌ترین میزان P خاک هم زمانی به‌دست آمد که گیاهان تیمار شده با SMV20 با قارچ*P. indica* تلقیح شدند. به‌نظر می‌رسد تیمار ترکیب کودهای زیستی بهترین نتیجه برای رشد گیاه استویا است.

واژگان کلید: استویا ریبائودیانا برتونی، *Piriformospora indica*، *Glomus mossea*، تلقیح، کود گاوی