JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2020, VOL. 3 (SPECIAL ISSUE: ABIOTIC AND BIOTIC STRESSES IN HORTICULTURAL CROPS), 11-28

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Effect of some Yew endophytes on vegetative growth and phytochemical variation of *Stevia rebaudiana* B.

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ARTICLE INFO

Original Article

Article history:

Received 1 September 2019

Revised 1 October 2019

Accepted 23 October 2019

Available online 15 February 2020

Keywords:

Endophytes

Medicinal plant

Stevia

Sugar

Secondary metabolite

DOI: 10.22077/jhpr.2019.2783.1090

P-ISSN: 2588-4883

E-ISSN: 2588-6169

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ABSTRACT

Purpose: Due the importance of taxol and based on the confirmed role of certain endophytes of Yew tree in the production of this vital compound, the present study was designed to investigate the effect of isolated endophytic fungi on vegetative and qualitative characteristics of stevia plant. **Research method:** The experiment included five different endophytic fungi isolated from Yew tree. In order to do that, rooted cuttings of stevia were transferred to the pot after being inoculated with different isolated endophytic fungi (TB2, TB2-3, TB20, TB55) of Yew tree in four replications as a completely randomized design and were kept in outdoor conditions. To isolate endophytic fungi, root samples were collected from the yew trees in the Ziyarat forest located in Golestan province, Iran. **Main findings:** The results of the study revealed the positive effect of Yew tree endophytes as biotic agents on the growth and physiological parameters of stevia. Also, the fungal pathogenicity effect on stevia was not observed. In addition, the main stem diameter, internode spacing, leaf area and other measured parameters were affected by studied endophytes. Among of the five used isolates fungi, three were classified as stevia plant grows stimulator. Also, due to the highest total sugar and antioxidant activity of stevia, rest two isolates were classified as "bio-elicitor". **Limitations:** There was no limitation to report. **Originality/Value:** The findings of the present study suggest that stevia, as a medicinal and economical herb, is capable of coexistence with yew endophytes. If taxol producer endophytes successfully transfer to stevia, it is possible to study the production of taxol in herbaceous plants, thereby opening the new door to easier and cheaper access to Taxol.

INTRODUCTION

Endophytes are found in almost all of the plants. These creatures are located in the living tissue of the host plant and are classified in a wide range of interactions with the plant from symbiosis to pathogenicity (Sikora et al., 2007). Endophytes may contribute to sustainability and prolong the host life by providing food. Finally, these compounds, when isolated and marketed, may even have value for use in modern medicine. New antibiotics, anti-mycoses, immunosuppressive compounds, and anti-tumor compounds are just a few examples of compounds found in endophyte culture (Naupaka et al., 2012). Considering the extensive efforts of researchers in recent decades to increase the yield of agricultural products, providing human food is still a major challenge (Naupaka et al., 2012). Research has shown that endophytic fungi, in addition to having a direct effect on plant growth through growth stimulation and defense system stimulation, increase plant resistance to live and abiotic stresses (Waller et al., 2005). The main causes of product degradation are biotic and abiotic environmental stresses due to adverse climatic conditions and plant pests and diseases (Waller et al., 2005). Fungi are physically attached to the soil particles, increase water holding capacity of soil, soil porosity, organic matter decomposition and preventing harmful pathogens of root zone as a useful biological control agent (Ansari et al., 2013). Endophytes increase plant resistance to abiotic stresses such as drought, heavy metals and salinity (Waller et al., 2005). Definition of endophyte in published papers and books has been diverse. But often this term is used for fungi (microbes) that can be observed without visible symptoms in the living tissue of the host (Bacon & White, 2000). The absence of apparent symptoms of endophytes in the host plant has been obscured because of their way of life, whether they are coexisting or parasitic with their host. However, it can be concluded from the high bioavailability of endophytes that these microorganisms are both saprophytic and symbiotic. Endophytes have been shown to play an important role in the biosynthesis of secondary metabolites in plants. Plant endophytes, especially fungi, play an important role in controlling and reducing the damage caused by pathogenic agents to host plants (Zaiyou et al., 2017). Due to the importance of the yew trees (*Taxus bacata*) many studies have been done on the endophytic relationship of this kind of plant (Ajikumar et al., 2010). *Stevia rebaudiana* Bertoni is a perennial herb of the Asteraceae family. Due to the presence of water-soluble di-terpene glycosides, which is 250-300 times as sweet as conventional sugar, stevia is a valuable medicinal plant (Ramesh et al., 2006). Stevioside, as the most important glycoside, contains 5 to 10% of the dry weight of stevia leaves (Ramesh et al., 2006). The most important bioactive compounds of stevia include alkaloids, flavonoids, tannins and phenolic compounds which have antimicrobial properties (Sharma et al., 2015). Today, there is a high demand for secondary metabolites of stevia in the world (Otroshy et al., 2015). In the context of increasing the performance of secondary compounds such as steviol glycoside and phenolic agents affected by endophytic relationship under stress conditions, studies are underway (Otroshy et al., 2015). If it be possible to inoculate taxol producing endophytes of yew trees to the herbal plants, new way will be opened in taxol production. The purpose of this study was to investigate the possibility of symbiotic relation of the stevia plant with endophytic fungi isolated from the yew tree, to improve growth patterns and metabolite production of the stevia plant.

MATERIALS AND METHODS

Experiment was done during 2016-2017. In order to do the experiment, rooted cuttings in which obtained from 10-15 cm of annual and matured branches of Stevia plant were transferred to the mist system containing cocopite and perlite (1:1) for rooting. Then, rooted cutting inoculated with different endophytic fungi and were planted in prepared pots and kept in outdoor conditions during June to end of August, in Gorgan weather conditions.

To isolate endophyte from Taxus tree, during spring and early morning young roots from different directions of plant were collected from the 5 matured yew trees located in Hircanian forest of Ziyarat in Golestan province, Iran (latitude 36° N and Longitude 54° E). All used trees grown in the situation of North slope. Collected root samples were transferred in the paper envelopes to the laboratory of department of horticultural sciences of Gorgan University of Agricultural Sciences and Natural resources. In the laboratory the healthy root samples without any extra injuries and any other contamination symptom were separated and the Schultz method (Schulz et al., 1993) with a slight modification was used to isolate the root endophyte fungi. Isolated endophytes where cultured in PDA to prepare spore suspension. After preparing suspensions by distilled water and tween 20%, the spore concentration of suspension was calculated using homocytometer technique approximately 5×10^7 spore/ml (Drüge et al., 2007). In order to inoculate the fungi, immediately after rooting (two weeks after planting), the rooted cuttings were immersed in container containing fungi suspension for 5 hours. After this time, for good establishment of spores in root tissue, seedlings were placed on filter paper for 8 hours and then cultivated in pots containing of field soil and leaf compost (2:1). In order to determine the acceptability and percentage of coexistence, after two weeks the roots of infected seedlings were stained according to the method of Vierheilig et al. (1998) and the samples were photographed (Figure 1) with a fluorescence microscope OLYMPUS BX51 (DICimaging, USA). The measured morphological traits were included plant height, number of branches, main and sub-main stem diameter, number of nodes, number of leaves per plant, internally spaced, leaf length and width, leaf area index, total plant weight, leaf fresh and dry weight, leaf relative humidity, fresh and dry weight of root (Digital scale), root volume (difference between initial and final water volume difference) and root length (ruler). Also phytochemical parameters including total phenol, total flavonoids, antioxidant activity, leaf chlorophyll content, total and reduced sugars and the auxin content of the root were measured.

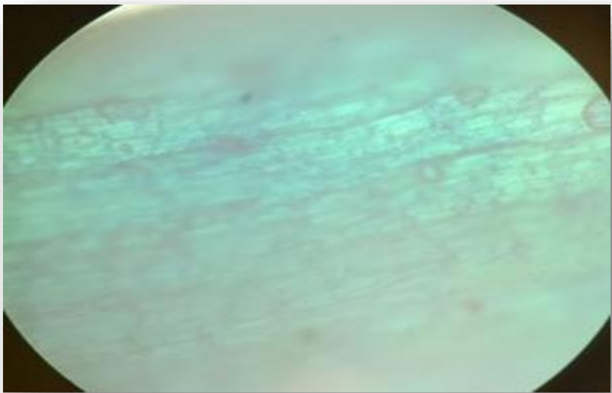

Fig. 1. Illustration fungal spores in Stevia root.

To determine the relative humidity of leaves (RWC), the proposed method of Srivastava and Sairam (2002) was used. The levels of leaf chlorophyll and carotenoid content were also measured by the proposed method of Hiscox and Israelstam (1979) and the amount of auxin was determined using He et al. (2002) method. In order to determine the total phenol content, total flavonoid and free radical scavenging ability methanolic extract was obtained from the experimental plants. For this purpose, one gram of each sample was homogenized with 10 milliliters of 80% methanol and incubated for 24 hours at room temperature. The sample contents were then passed through filter paper and the filtered solution was used as a methanolic extract. In order to measure total phenol, total flavonoid, free radical scavenging ability, total and reduced sugar, the proposed methods of Ragazzi and Veronese (1973), Chang et al. (2006), Brand-Williams et al. (1995), Maccready et al. (1950) and Miller et al. (1959) were used, respectively. Analysis of the data obtained was done as a completely randomized design with 5 different treatments of endophytic fungi in 5 replicates. The LSD test was performed at the probability level of 5% using SAS. 9.

RESULTS AND DISCUSSION

Morphological traits of stevia plant affected by endophyte fungi treatments

According to the analysis of variance (Table 1), the fungi used had significant effect on the number of nodes and main stem diameter at 1% probability level. Also, plant height, leaf number per plant and number of branches were significantly affected by endophytes at 5% probability level. Except leaf width, in other measured parameters, a significant difference was observed in endophytes at 1% probability level (Table 1). The effect of fungal treatments on height and number of branches of stevia plants was significant. The highest plant (57.7 cm) was observed as treated with TB55 (Fig. 1). However, there was no significant difference between control and treated plants with other endophytes fungi. Under the influence of TB3, TB2 and TB55 fungi, the number of stevia branches increased compared to the control plants. The maximum number of branches (4.5) was observed in plants inspected with TB55 and the lowest one (0.2) in plants treated with TB2-3 and TB20 (Fig. 2). Researchers showed that the symbiosis of mycorrhizal fungi with mint root increased photosynthesis via increasing water and nutrient elements absorption, resulting in a higher product yields and improved the plant vegetative growth (Gupta et al., 2002). It was also shown that tangerine seedlings (from Citrus) inoculated with mycorrhizal arbuscular fungus had higher height than non-contaminated seedlings under irrigated conditions and stress (Wu & Xia, 2006). Inoculation with mycorrhizal fungus increased the height of the tomato plant due to adequate absorption of water and nutrients (Alkaraki & Hamnad, 2001). Saleh and Al-Garni (2006) reported an increase in the plant height and number of lateral branches in the presence of mycorrhiza. It was also reported that in olive plants, inoculated with endophytic fungi, both the over ground and underground systems development were significantly improved compared with the control (Chliyeh et al., 2014). In previous studies, it was found that mycorrhiza increased the number of foliage, leaf dry weight, leaf area and stem dry weight of the plant by providing more phosphorus, manganese and iron accumulation in *Thymus vulgaris* (Dolatabadi et al., 2012).

Table 1. Analysis variance of effects of fungal treatments on morphological parameters of stevia plant

Sources of changes	df	Leaf length	Leaf area	Internally spaced	Number of leaves per	Number of nodes	Branch diameter
Treatment	5	0.184^{ns}	3.36^{ns}	0.034^{ns}	3454.48*	128.8**	1.88**
Error	12	0.45	1.42	0.04	942.69	25.33	0.32
CV		11.93	17.89	7.09	20.94	19.86	8.99
Sources of changes	df	Diameter of the stem	Number of branches	Plant height	Length of the largest root	Root volume	Root DW
Treatment	5	0.05^{ns}	3.12*	71.42*	25.7**	27.06**	0.88**
Error	12	0.24	0.77	15.61	2.87	3.13	0.109
CV		12.17	20.95	7.97	11.058	30.37	24.55
Sources of changes	df	Root FW	Leaf DW	Total plant weight	Leaf width		
Treatment	5	40.95**	135.85**	479.8**	0.084^{ns}		
Error	12	0.92	9.33	33.44	0.03		
CV		12.86	6.45	5.09	7.95		

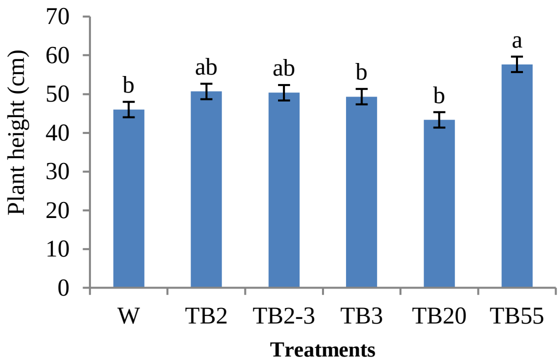

Fig.1. Effect of endophytic fungi on plant height, W: The sample was treated with distilled water as control.

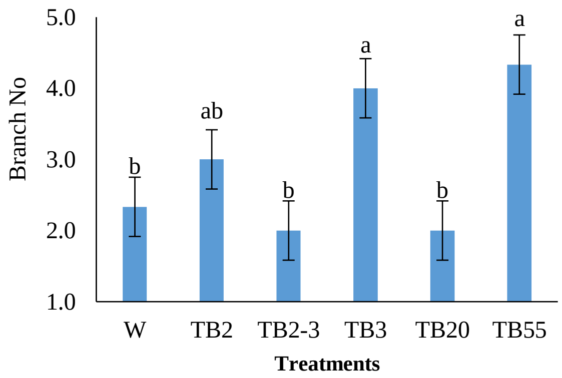

Fig. 2. Effect of endophytic fungi on number of plant branch, W: The sample was treated with distilled water as control.

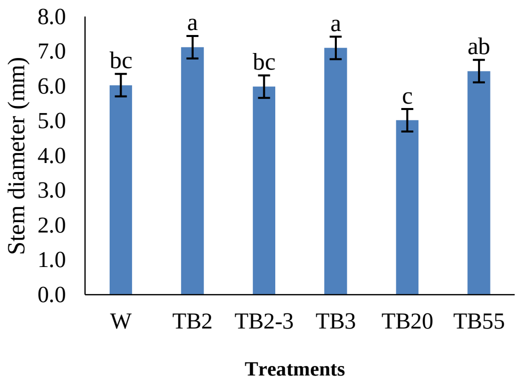

Fig. 3. Effect of endophytic fungi on the main stem diameter, W: The sample was treated with distilled water as control.

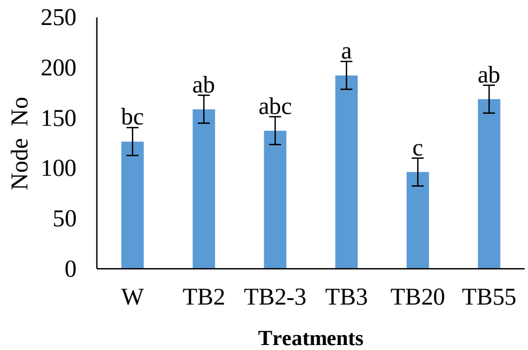

Fig. 4. Effect of endophytic fungi on the number of node, W: The sample was treated with distilled water as control.

Variation in the morphological characters of stevia plant affected by fungal treatments

The fungal treatments did not show any significant differences in the variation of the stem diameter of the plants (Table 1). In contrast, the branch diameter at the probability level of 1 % was affected by the type of endophytic fungi. The largest diameter was observed in plants inoculated with TB2 and TB3, and the smallest one was observed affected by the TB20 fungus (Fig. 3). *Piriformospora indica,* an endophytic fungus is known to promote growth and increasing resistance to stress in many vegetables (Sharma et al., 2015). In tomato, the morphological traits and photosynthetic pigments of plant were improved as contaminated with this fungus (Ghorbani et al., 2016). The results (Table 1) showed that, fungal treatment made significant difference in the number of nodes of stevia at 1% probability level, and also significantly increased the number of leaves per plant. Contrast to the TB20 endophyte-treated plants (Fig. 4), the maximum number of nodes was found in treated plants with TB3 fungus (36 and 19.3% in TB2 and TB20, respectively). Unlike TB3, plants treated with TB20 had a lower number of leaves (Fig. 5) than other fungi (96.2 and 192.3% inTB3 and TB2, respectively). The results of other scientists indicated that the growth of peppermint and thyme inoculated with endophytic fungi *Vermifera sebacina* and *Piriformospora indica* were significantly increased (Dolatabadi et al., 2011). The number of nodules in peppermint and number of branches in thyme were significantly increased as affected by the mentioned endophytes. It was also reported that arbuscular mycorrhizal fungi have a significant effect on the vegetative parameters of peppermint (Mahmoudzadeh et al., 2015). Improving the absorption of mineral elements in host plants by mycorrhizal and endophytic fungi often leads to positive growth responses in these plants, which is most commonly found in the vegetative parts. It has been also demonstrated that plant inoculation with endophytic fungus increases the nutrient uptake and resistance to the environmental stresses and diseases, as well as increased vegetative plant function (Porras Soriano & Soriano-Martin, 2009).

According to the results of analysis of variance (Table 1), total plant weights, fresh and dry weight of leaves were significantly affected by endophytes in the probability level of 1%. Fungi increased the mentioned traits in comparison with the control plants. The highest total plant weight, fresh weight and dry weight of leaves (12.3, 100 and 53.75 mg respectively) were obtained in plants treated with TB3, TB2 and TB55 (Figures 6, 7 and 8). The lowest amount of total plant weight was observed in control plants. However, there was no significant difference between the control and the plants treated with TB2-3 and TB20 (Fig. 6). The lowest leaf fresh weight (79.7 mg) and leaf dry weight (39.08 mg) were observed in the control plants (Fig. 7 and 8). Martin & Stutz (2004) showed that, inoculation of pepper with two species of mycorrhizal fungi was effective on growth parameters of the plant. Growth stimulatory effect occurs through stimulation of growth hormone biosynthesis, especially auxin. In this case, the fungus increases the absorption of water and minerals and, in the long term, increases the vegetative growth and yield of the biomass by increasing the production of roots of the host plant. As mentioned above, increasing shoot dry weight in inoculated plants compared to control plants can be due to increased water and nutrient uptake and better transferring of these materials in plant organs and also increase plant photosynthesis which results in the formation of more photosynthetic materials, a subject approved by other researchers (Vamerali et al., 2003). It seems that the production of growth phytohormones including auxin and cytokinin in mycorrhizal contaminated plants increases the shoot dry weight of plants (Dolatabadi et al., 2011). This also was observed in the present study. results showed that, in comparison with two S*cutellospora gilmorei* and *Glomus mossae* fungi a better growth pattern of neem plant (*Azadirachta indica*) was observed as contaminated with *P. indica* (Singh et al., 2000). In another study, under hydroponic culture conditions of tomato it has been showed that the leaf biomass, fresh and dry weight of tomato fruits of *Priformospora indica* contaminated plants increased 20%, 100% and 20%, respectively (Fakhro et al., 2010). Similar results were also reported in Ghasemnajad and Babaeizadeh (2012) and Rahimi Tanha et al. (2017) studies on artichoke.

It has been showed that, the highest leaf chlorophyll content, fresh and dry weight of the shoot and root of Stevia were obtained in 20% vermicompost treatments in combination with the *Glomus mosseae* mycorrhizal (Zare hoseini et al., 2015). Results showed that the growth parameters and essential oil content of *Mentha arvensis* and *Cymbopogon* spp. as two important essential oil bearing plants were significantly influenced as inoculated with endophytic agents (Gupta et al., 2002). Lenin et al. (2010), in a study of four plants of *Lycopersicume sculentum* L. *Capsicum annuum* L., *Abelmoschus esculentus* L. and *Solanum melongena* L., showed that inoculation of the plant with mycorrhizal fungus *fasciculatum Glomus* increased the amount of biomass in each of them via increasing plant absorption potency. In another study it has been found that, inoculation of roots of dill (*Anethum graveolens*) and Ajwain (*Trachyspermum ammi*) with two species of mycorrhizal fungi caused a significant increase in their shoot dry weight (Kapoor et al., 2002). Based on the results (Table 1), fungal treatments had a significant difference in fresh and dry weight leaf at a probability level of 1%. So that, the root dry and fresh weight of the experimental plants treated with TB2-3 and TB3 were significantly increased by increasing the total biomass production. The highest fresh weight (13.8 mg) and dry weight (2.2 mg) of root was observed in plants inoculated with TB3. In a study it has been showed that, simultaneous inoculation of *Capsicum annum* L. with endophytic fungi and plant grow stimulator bacteria significantly increased the fresh and dry weight as well as the chlorophyll content of leaves (Tanwar et al., 2011).It means that this isolate of fungi might have influence on the root phyto hormones balance. In contrast to the lowest fresh weight per plant (4.35 mg) was observed in plants inoculated with the TB55 fungus (Fig. 9 and 10). Versus to grow promoters, it seems that TB55 isolate acts as an elicitor of secondary metabolite production.

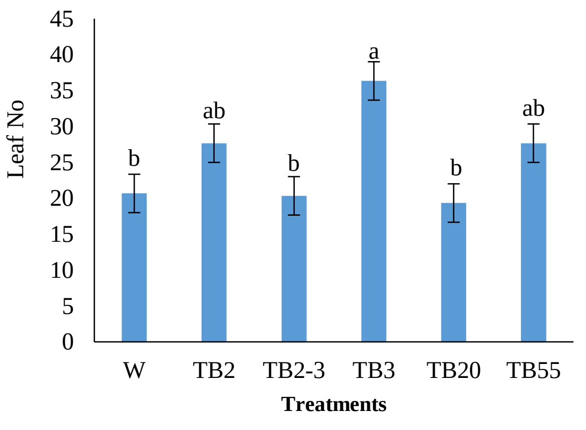

Fig. 5. Effect of endophytic fungi on the number of leaves, W: The sample was treated with distilled water as control.

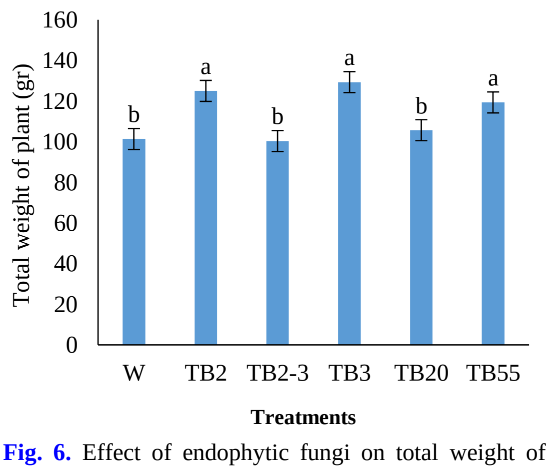

Fig. 6. Effect of endophytic fungi on total weight of plant, W: The sample was treated with distilled water as control.

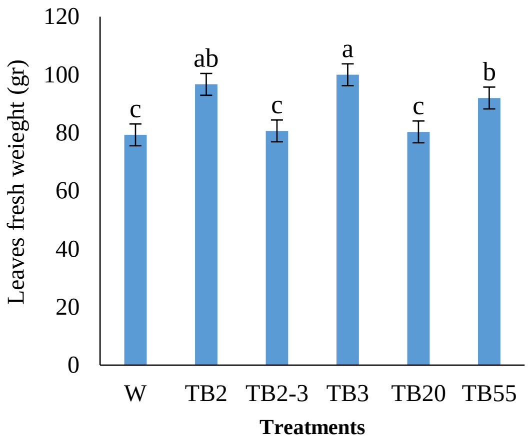

Fig. 7. Effect of endophytic fungi on fresh weight of leaves, W: The sample was treated with distilled water as control.

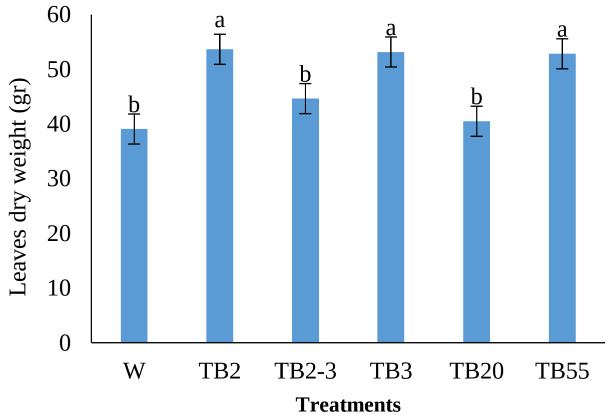

Fig. 8. Effect of endophytic fungi on the dry weight of leaves, W: The sample was treated with distilled water as control.

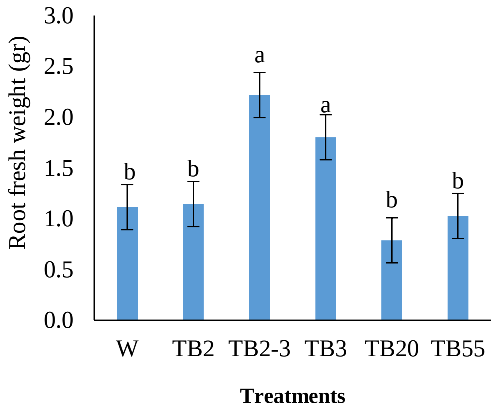

Fig. 9. Effect of endophytic fungi on the fresh weight of roots, W: The sample was treated with distilled water control.

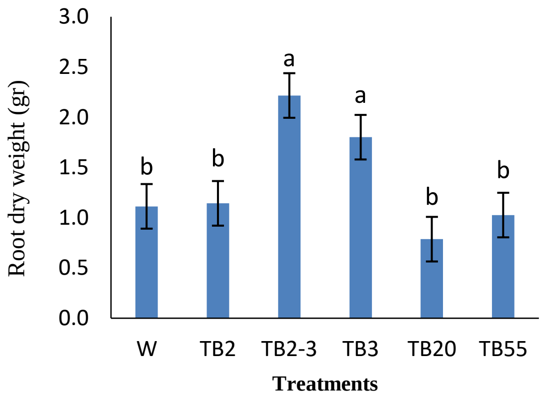

Fig. 10. Effect of endophytic fungi on root dry weight, W: The sample was treated with distilled water control.

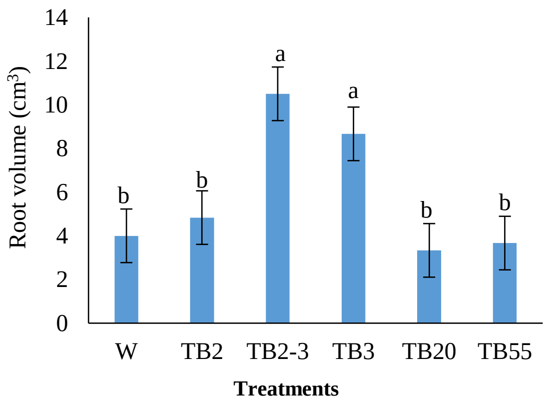

Fig. 11. Effect of endophytic fungi on changes in root volume of stevia, W: The sample was treated with distilled water control.

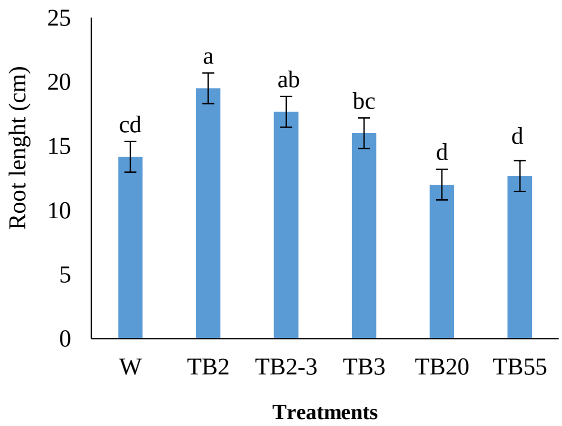

Fig. 12. Effect of endophytic fungi on root length of stevia, W: The sample was treated with distilled water control.

Root development affected by endophyte

Both parameters of root volume and root length were significantly varied at the probability level of 1% (Table 1). The highest root volume (10.5 cm^3) was observed in plants contaminated with TB2-3 fungus without significant difference with TB3. Other treatments had no significant effect than control (Fig. 11). Although the root length of plant increased as treated with TB2, TB2-3 and TB3, the plants treated with TB20 and TB55 even had shorter roots than the control plants.

The highest root length (19.5 cm) was found in plants treated with TB2 (Fig. 12). In the study on *Brassica oleracea* L., spinach (*Spinacia oleracea*) and mustard (*Brassica juncea*), it was shown that *P. indica* contributes to the growth of the over and uder-ground parts of plant, as well as the flowering and early ripening of these plants (Kumari et al., 2003). The mechanism of this increase is likely to be that a part of the endophyte is introduced into the rooted system and by reducing the concentration of the indole acidic acid and increasing the amount of cytokines and gibberllic acid, expand the root system and increase the absorption of water and nutrients (Azcon-Aguilar & Barea, 1996). It is believed that, extracellular mycelia release organic acids such as malic acid and make soluble the insoluble phosphates in rhizosphere and increases the phosphorus absorption of the plant which these interactions indirectly affect morphological traits of plants.

The effect of fungal treatments on the amount of chlorophyll and carotenoids

The chlorophyll and carotenoid content of plant leaves were significantly affected by endophytic fungi at 1% probability level (Table 2). Also, total chlorophyll content in inoculated plants decreased with TB55, TB2 and TB3 isolates (Fig. 13). The chlorophyll content of control plants and TB2-3 and TB20 fungal treated plants was same and the effect of endophytes on total chlorophyll content was not significant (Fig. 15). The highest total chlorophyll content was observed in TB2-3 inoculated samples (Fig. 15). The results showed that chlorophyll a was increased under the influence of treatments compared to control. The highest chlorophyll “a” was observed in TB2 treatment. Surprisingly the highest amount of chlorophyll b was observed in control plants (Fig. 13). Increasing the content of chlorophyll (b) and (a) and total chlorophyll in inoculated plants with endophytic fungi could be due to the improvement of the aquatic conditions of the plant (Jenschke et al., 2000). Another reason for increasing chlorophyll content affected by endophyte could be related to the absorption of more mineral elements, the point that also referred by Jenschke et al. (2000). Therefore, the difference in the level of influence of used fungi on the chlorophyll concentration can be attributed to the difference in the concentration of nutrient uptake in host plants. Apart from the TB20 fungus and compared to the control plants the carotenoid concentration was increased as affected by the rest used endophytic fungi (Fig. 14). Carotenoids protect the photosynthetic tissues. Maintaining the structure of carotenoids under stress conditions is related to the ability of the non-enzymatic part of the plant to remove active oxygen species (Bandyopadhyay et al., 1999). Therefore, the effect of endophytes on increasing carotenoid concentration of plants can probably be considered as one of the positive resistance mechanisms activated by these fungi in increasing the tolerance of plants to summer heat. In *Coleus forskohlii*, the interaction of *Piriformospora indica* with plant roots resulted an increasing in the total biomass of the shoot, the content of chlorophyll “b” and “a” and total chlorophyll, plant height, number of branches, branch length, number of leaves and leaf area, and producing more hairy roots which is directly related to the absorption of valuable nutrients (Das et al., 2012). The average of measured traits of tomato seedlings inoculated with *Piriformospora indica*, showed a significant increase in relative humidity and a significant decrease in leaf area chlorophyll a, b and carotenoid compared to the control (Ghorbani et al., 2016).

Table 2. Effect of fungal treatments on measured phytochemical factors

Sources of changes	df	TP	TF	AA	Chl. a	Chl. b	Total chl.	Carotenoids	Total R.H.	Total sugar
Treatment	5	53.03 ns	5.35*	579.8**	0.004**	0.0004**	0.0037**	0.025**	903.5**	8970.12**
Error	12	23.13	1.68	24.62	0.0002	0.00001	0.00008	0.0001	150.35	654.65
C.V. (%)		13.9	1.5	7.18	16.98	8.57	11.76	0.01	20.96	15.03

*, **, ns: significant at 5 and 1% of probability levels, and non-significant.

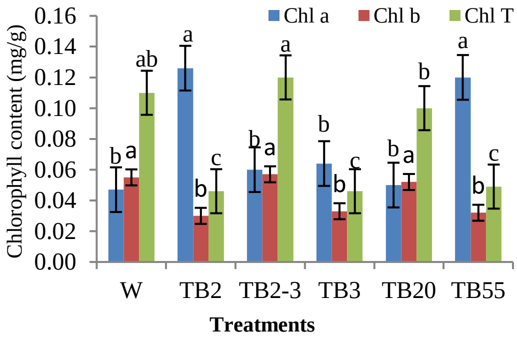

Fig. 13. Effect of endophytic fungi on chlorophyll a, b and total (T), W: The sample was treated with distilled water as control.

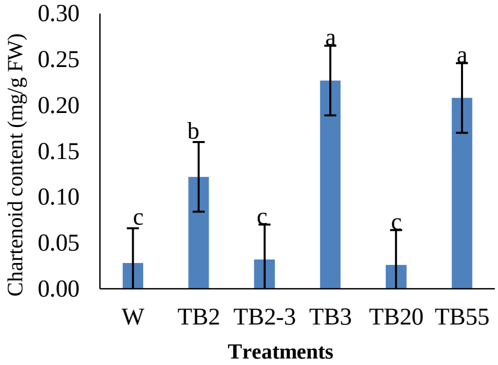

Fig. 14. Effect of endophytic fungi on the amount of carotenoid of stevia leaves, W: The sample was treated with distilled water as control.

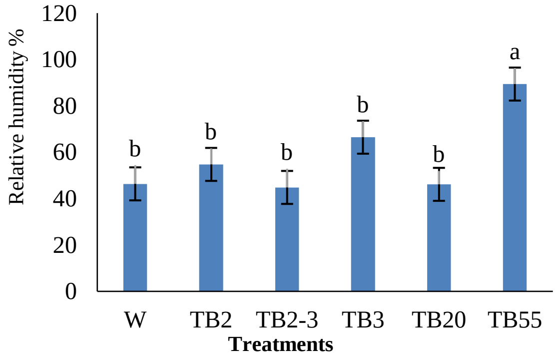

Fig. 15. Effect of endophytic fungi on the relative humidity of stevia leaf. W: The sample was treated with distilled water as control.

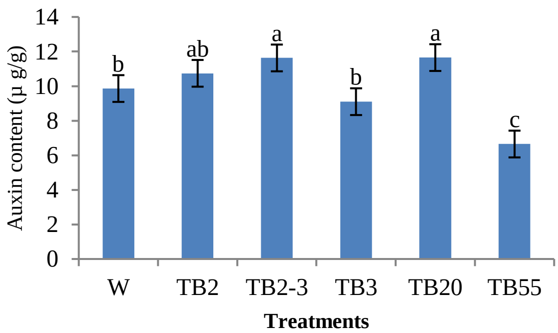

Fig. 16. Effect of endophytic fungi on the amount of stevia root auxin. W: The sample was treated with distilled water as control.

Effect of endophytic fungi on the moisture content of stevia leaves

As can be seen in Figure 15, except the samples treated with TB55, the relative water content of leaf was not significantly varied in different experimental plants. Although there was no significant difference in the relative water content of leaves treated with endophytic fungi compared to control plants, it tended to be more than control. The highest percentage of leaf relative water content was observed in TB55 endophyte treated plants (Fig. 15). It has been argued that mycorrhizal coexistence often leads to a change in the velocity of water flow inside and outside of host plants and can have a significant impact on tissue depletion and leaf physiology (Auge et al., 2001). The mycelium of the fungus in the soil causes water to be absorbed from very fine pores (Bearden, 2001). Probably these fungi, like mycorrhiza, will absorb more water and improve the aqueous relationships of the host plants by changing the root morphology and increasing the root length of the host and increasing the absorption through the fungi rings (Auge et al., 2001).

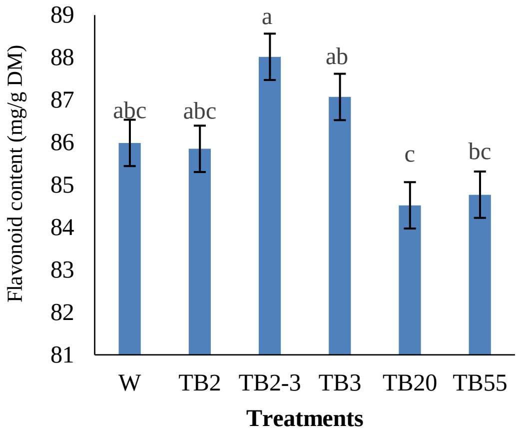

Fig. 17. Effect of endophytic fungi on the amount of leaves stevia flavonoid. W: The sample was treated with distilled water as control.

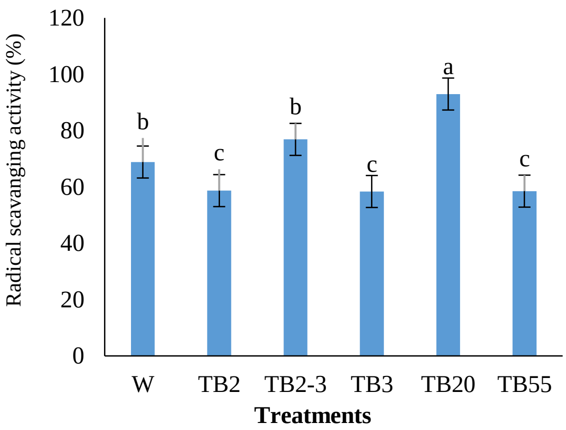

Fig. 18. Effect of endophytic fungi on the radical scavenging activity of stevia leaves. W: The sample was treated with distilled water as control.

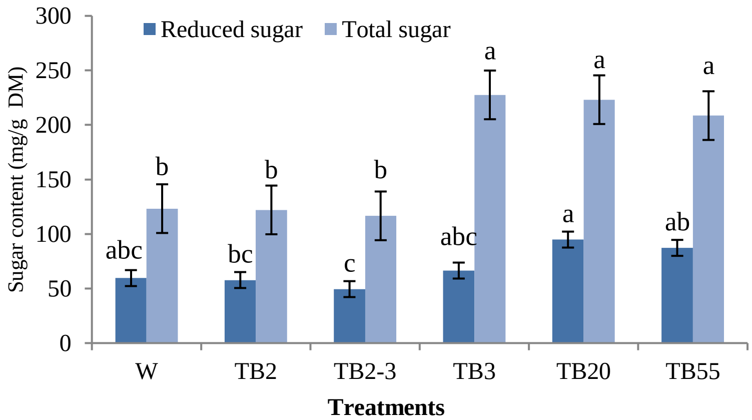

Fig. 19. Effect of endophytic fungi on the concentration of reducing sugar and total leafy stevia. W: The sample was treated with distilled water as control.

Effects of endophytic fungi on the auxin accumulation of the root

Auxin concentration of the root has been shown to be significant at 1% level based on the data analysis of variance (2). The results showed that the inoculation of plants with TB2, TB2-3 and TB20 fungi increased the amount of root auxin compared to the control (Fig. 16). There was no significant difference in TB3 hormone concentration in comparison with the controls. However, contrary to the assumption, the amount of auxin was lower in inoculated samples with TB55 than in control plants. The reason of it is not clear, but it seems that due the role that TB55 plays in secondary metabolite production, as it was found in present experiment, the grow limitation in TB55 contaminated plants could be explained. In a study on apples, using mycorrhizal fungi, the level of internal auxin of the seedling tissues was significantly increased (Parvizi et al., 2014). In studies of Meixtner et al. (2005), increased levels of auxin have been reported as a result of colonization of fungi in corn and soybean plants, respectively. One of the important proposed mechanisms for stimulating plant growth by endophytic microbes is the hormonal balance model (Poupin et al., 2016). However, there is still little information about the molecular messages and the paths of this reciprocal relationship. Studies in Arabidopsis showed that, the expression of a number of genes involved in the biosynthesis of auxin was influenced by *Burkholeria phytofirmans* growth promoters (Poupin et al., 2016). In recent years, researchers have been trying to gain a better understanding of the interactions of microbes and plants, particularly in stimulating growth (Zamioudis et al., 2013). One of the suggested hypotheses in this regard is the "plant stimulation" hypothesis means stimulated by the production of hormones of the microbes or by the hormonal balance model (Lugtenberg & Kamilova, 2009). Increasing the amount of indole acetic acid in the root pointed that the plant is able to produce more roots. Of course, the important thing is the sensitivity of the root tissue to the concentration of auxin. Therefore, as well as the growth probability of the root number of the plant in the presence of growth-promoting endophytes, root growth might be limited. From the obtained data, it can be seen that in some cases there was a correlation between the auxin concentration and root volume. Increasing the amount of auxin in roots of TB20 contaminated plant resulted to the less root volume compared to other used endophytes. However, the plant's response to the endophyte in the case of root development varied based on the type of used fungi.

The effect of endophytic fungi on total phenol, total flavonoid and free radical scavenging activity

According to the analysis variance of data (Table 2), phenol content was not significantly influenced by different fungi. The highest phenol content was observed in plants treated with TB2 (39.57 mg/g DM) However, there was no significant effect between different endophytic fungi (Table 2). The highest flavonoid content (88.017 mg/g dry weight) was observed in plants inoculated with TB2-3 endophyte (Fig. 17). By studying the effect of endophytic fungus (*P. indica*) on the qualitative characteristics of peach, it was showed that despite the root surface contamination of fungi, total sugar and total phenol of leaves of treated plants were higher than the control. However, there was no significant difference in total flavonoid content (Ghasemnajad & Bagheri fard, 2014). The secondary metabolites production of plants is strongly affected by the endophytic fungi via increasing the physiological changes and enzymatic activities (Mathur & Vyas, 1995). Based on Rahmatzadeh et al. (2014) studies on *Catharanthus roseus*, it has been showed that the phenolic content of the over ground part of plants inoculated with *Glomus etunicatum* was significantly higher than that of non-mycorrhizal samples. Many Researches has shown that phenolic compounds are one of the important antioxidant agents in plants, and one of their most important functions is to participate in plant defense mechanisms (Gan et al., 2010). The positive effect of arbuscular mycorrhizal fungi on the activity of enzyme peroxidase and total phenol content in micronized plants was demonstrated during the adaptation period of *Vitis vinifera* L. seedlings (Krishna et al., 2005). Tyrosine and phenylalanine are important precursors for the production of phenolic compounds. Therefore, the high nitrogen uptake potential in colonized plants in producing the amino acids and high activity of the PAL (phenylalanine ammonialyase enzyme), one of the most important enzymes involved in the production of phenolic compounds is expected in the contaminated plants. This enzyme catalyzes the first stage of phenylpropanoid biosynthesis, which splits between primary and secondary metabolism. It also plays an important role in the biosynthesis of flavonoids, lignin, and many other compounds (Toussaint et al., 2004).

In present research, based on the treatments, the level of antioxidant activity was significant at 1% probability (Table 2) and the highest antioxidant activity was observed in plants in which contaminated with TB2-3 and TB20 fungi (Fig. 18). Surprisingly, other used fungi significantly reduced the antioxidant activity of plant compared to the control (Fig. 18). Kumar et al. (2009) reported that inoculation of *P. indica* increased the activity of antioxidant enzymes in corn plants affected by endophytic fungi. They also stated that, *P. indica* increased plant tolerance to *fusarium* pathogenic fungi by increasing the antioxidant capacity of barley. The results of the other studies indicate that in most cases mycorrhizal fungi increase the tolerance of host plants to a wide range of environmental stresses (Gholami & Kochaki, 2001). Therefore, reducing the activity of antioxidant enzymes under stress conditions can be explained by the positive effect of fungi on plant. Endophytic fungi reduce the stress effects by increasing the absorption of the elements (NPK) and thus providing better growth conditions for the plant. In other words, tested endophytes, especially TB20, improve plant growth and decrease the production of reactive oxygen species, reduce oxidative damage and consequently increase the plant tolerance to stress, especially the stress caused by high temperature.

Effects of endophytic fungi on total and reduced glucose of stevia

According to Table 2, the effect of endophytic fungi on total sugars was significant. So, total sugar content of the infected plants was higher than the control (Fig. 19). However, the effect of TB2 and TB2-3 fungi on total sugar was not significantly different than the control (Fig. 19). In the present study the highest amount and yield of sugar in inoculated plants with endophytic fungi indicates that, the fungi improved vegetative growth and sugar accumulation via managing mineral absorption and reducing the environmental stress effect. Ali et al. (2009) reported that, *Pseudomonas* AMK P6 strain enhanced the chlorophyll content, amino acids and sugars in sorghum seedlings. Effect of endophytic fungi in mycorrhizal communities is based on the carbohydrates exchange and the synthesis of polysaccharides. On the other hand, mycorrhizal or endophytic fungi increase the photosynthesis and eventually the formation of carbohydrates in the plant, by increasing the concentration of plant hormones such as cytokinin, which has important role in stomata activity (Ali et al., 2009). Demir (2004), showed that the accumulation of sugars including fructose and alpha glucose significantly increased in pepper plant contaminated with *G. intraradices*. Sandhya et al. (2010) reported that inoculation of corn seeds with *Pseudomonas putida* bacteria significantly increased the levels of soluble sugars, proline and free amino acids under drought stress. These results may be related to the ability of strains to produce diaminase ACC enzymes that reduce ethylene production. In addition, it has been reported that in corn seedling the metabolism of carbohydrates and photosynthesis proteins accumulation of plants which contaminated by *Fluorescent Pseudomonads* was significantly higher than those of non-inoculated plants (Shoresh et al., 2010).

CONCLUSION

In general, the present study revealed that endophytic fungi isolated from yew were effective on stevia vegetative growth. The results indicate a positive effect of endophytic fungi isolated from the yew root on growth parameters and chemical composition of the stevia plant. It was found that TB3 isolates had the most positive effect on morphological factors (number of branches, main stem diameter, number of nodes and leaves per plant, total weight, fresh and dry weight of leaves). However, in some cases there was no significant difference between TB2 and TB55. The increased effect of isolate TB2-3 on fresh and dry weight of root, root volume and total chlorophyll pigment was higher than other used isolates. Regarding the changes in chemical composition, the highest antioxidant activity and carotenoid pigment density were observed in samples treated with TB20 fungal isolates. From the point of view of the concentration of sugar compounds, treatment with TB55, TB20 and TB3 increased the total and reduced sugar contents accumulation, respectively. According to the present work and based obtained results, endophytic isolates used in this study can be divided into two groups of growth promoters (TB3, TB20 and TB55) and metabolic stimulants (TB2 and TB2-3). Also, the findings of this study indicate that stevia, as an economical medicinal herb showed good compatibility with the yew endophytes. If Yew tree endophytes successfully transfer to stevia, it is possible to study the production of taxol in herbaceous plants, thereby opening a new door to easier and cheaper access of taxol.

Acknowledgements

We would like to thank Gorgan University of Agricultural Sciences and Natural Resources for this research financial support. Grant number: 95-354-67.

Conflict of interest

There is no conflict of interest between authors of this research.

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