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Effects of Growth Stimulator Microbes on Growth and Ions Concentration of Stevia under Salinity Stress Conditions

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Abstract
Soil salinity and water deficit are major problems for development of agricultural production. In this study, effects of growth promoting microorganisms (inoculation with *Piriformospora indica* and two isolates of *Streptomyces sp*.) on the leaf yield and absorption rate of some elements in leaves and roots of stevia plant was investigated under salinity conditions for two consecutive years (2016-2017). The fresh and dry weights of the leaves were significantly affected by microorganism and salinity. High salinity level led to sodium accumulation in the root and leaf; while the amount of potassium and K^+/Na^+ ratio decreased in both root and leaf. The accumulation of phosphorus in both leaf and root was significantly decreased in 3 ds m^{-1} salinity. Plants exposed to *Piriformospora indica* and *Streptomyces* strains, showed lower sodium content in their leaves and roots. In contrast, an increase in the content of potassium and phosphorus was observed in the growth stimulator microbes-treated plants. However, the K^+/Na^+ ratio in the growth stimulator microbes-treated plants was to some extent lower than its ratio in control plants. In conclusion, *P. indica* and *Streptomyces* strains improved the biomass formation of the stevia plant under salinity conditions by controlling the uptake of potassium, phosphorus and sodium.

Keywords: Bacteria, Endophyte, Mycorrhiza, Salinity, Stevia.

Introduction

Various environmental stressors, including drought, high temperature, salinity, flood, insecticides, and soil pH, limit agricultural production. Soil salinization is one of the most severe problems among them that have a negative impact on productivity and quality of crops by reduction of the cultivated area (Lanza et al., 2019). Around 20% of arable and 33% of irrigated lands in the world are severely affected by salinity stress (Ali et al. 2014; Aaqil Khan et al., 2019). Plants have evolved mechanisms to adapt to salinity or prevent salinity stress, such as the control of water flux, accumulation of osmolytes (K^+, Ca^{2+}, NO^{3-}, and etc.) and maintenance of ion homeostasis (i.e. coupled exchange of K^+/Na^+ for H^+) (Zanetti et al., 2019). As an example of the regions that suffer from salinization, in Iran, around 24 million hectares of the soils are saline or closed to be salinized (Hosseini et al., 2009). Under saline conditions, plants are exposed to three types of stresses including ionic toxicity, desiccation, and nutritional disturbances (Blumwald, 2000, Jose, 2002; Molassiotis et al., 2006). Most of the plant's tolerance mechanisms against salinity

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stresses require energy (Barrett-Lennard et al., 2003). Therefore, in this situation, plant energy is mostly allocated to adaptation (Noaman & El-Haddad, 2000).

Stevia leaves are naturally sweet and being used for hundreds of years in South America (Soejarto et al., 1982; Purkayastha et al., 2016). Since it has a modest effect on blood glucose, it is appropriate as a natural sweet for the people with a controlled carbohydrate diet (Hajar et al., 2014). Symbiosis with beneficial soil microorganisms is a natural mechanism that might offer a quicker, cost-efficient and eco-friendly solution to mitigate salinity stress (Fallahi et al., 2016, Andrés-Barrao et al., 2017; De Zélicourt et al., 2018). At the same time, many endophytic microbes can help plants with the uptake and utilization of soil nutrients, through their bio-simulation of many compounds and enhancement of their availability. These nutrients have been linked to the promotion of plant growth and development, and thus to the increased plant yields (Martínez-Medina et al., 2011; Hosseini and Gharghani 2015, Xia et al., 2019). Many endophytic fungi are also capable of improving plants' resistance to pathogen and insect attacks and their defenses against abiotic stresses, such as those brought on by drought and excessive salt (Heidari et al. 2016, Xia et al., 2019). Phosphate is a major macronutrient needed by plants for their development, health, and productivity, but available phosphate is very low in soil all over the world because most phosphate in the soil is insoluble and unavailable. Therefore, plant growth and development is limited by the phosphate availability in most natural ecosystems (Wu et al., 2019). Plants have evolved to possess two distinct models to enhance absorption of phosphate from soil. The direct model is that plants use their own phosphate transporters in roots and carry out changes in root morphology, biochemistry, and physiology, especially great increase in the numbers of lateral roots (Péret et al., 2014) and root hairs, secretion of organic acids (Gerke, 2015), and formation of cluster and dauciform roots under phosphate deficiency. The indirect model is that plants symbioses with mycorrhizal fungi and other microorganisms. In the symbiosis system, host plants provide the mycorrhizal fungi with carbohydrates, and the latter helps their hosts to absorb nutrients, especially phosphate (Wu et al., 2019). The use of salt tolerant microbes is an alternative method to improve crop productivity. Recently, it has been shown that plant growth promoting endophytic (PGPE) bacteria can help their host plants to cope with various biotic and abiotic stresses (Zhao et al., 2016). Extensive research has been done to assess the potential of endophytes as inoculants to promote plant growth and as bio control agents (Anuar et al., 2015; Tian et al., 2017). Endophytes that have been found to date fall largely into three major functional groups: 1) defending hosts from biotic stress; 2) alleviating the impact of abiotic stress on the host; and 3) supporting the host nutritionally by increasing phosphorus(P) and nitrogen (N) ion contents (Bacon et al., 2015). At present, scientists study most kinds of fungi and bacteria in the plants, but few focuses have been made on actinomycetes (Santoyo et al., 2016; Zhang et al., 2019). One of the effective indices in salt tolerance is the maintenance of cellular turgescence, thereby reducing plant growth due to salinity (Shabala et al., 2000). The unproductive consumption of chemical fertilizers in recent decades and the revealing of their harmful effects, including water and soil pollution, human health problems and other living organisms, changed human attention to the inherent potentialities of creatures especially microorganisms towards sustainable agricultural policy (Zahir et al., 2004). Rhizospheric microorganisms are associated with the development of root systems (e.g. fungal hyphae) by dissolving high-energy elements such as P or K, increase the ability of plants to absorb elements from the soil (Bucio et al., 2007, Meena et al., 2010). The

genus *Streptomyces* is an aerobic and gram-positive bacterium belonging to the Streptomyceae family (Ludwig et al., 2012) that grows as branched fibers containing vegetative mycelia (Dworkin & Falkow, 2006; Petrus & Claessen, 2014). Currently, about 600 authentic species of *Streptomyces* have been introduced since their separation from environmental sources is the main focus of research to discover new bioactive compounds to use in the pharmaceutical and agricultural industries (Kämpfer et al., 2014). This group of microorganisms has been found in various ecosystems and plays an important role in improving plant growth, plant protection, organic matter decomposition and production of secondary metabolites. The growth potential of *Streptomyces* in tomato plants (El-Tarabily, 2008), wheat (Sadeghi et al., 2012), rice (Gopalakrishnan et al., 2012) and chickpea (Tokala et al., 2002) have been reported. *Streptomyces* induce plant growth through siderophore (Tokala et al., 2002) or indole - 3-acetic acid (Aldesuquy et al., 1998) production. Streptomyces are also widely used for biological control of fungal pathogens in the soil (Gopalakrishnan et al., 2011, Gopalakrishnan et al. 2014). Most of the fundamental and applied studies of the beneficial effects of plant and bacteria relation were focused on germ-negative bacteria (Balsanelli et al., 2015). Elements in the crystalline structure of minerals are released by organic acids (such as gluconic acid, citric acid, succinic acid and oxalic acid) secreted by microorganisms (Rajput et al., 2013). Jog et al. (2014) reported that the release of phosphate increase by the acidification of the environment via secretion of malic acid and gluconic acid by isolates of *Streptomyces* mhcr0816 and *Streptomyces* mhce0811. The metabolism of organic acids in host plants is unknown (Adeleke et al., 2017). The endophytic fungus of *P. indica* belongs to Basidomycete family of Sebacinaceae and in many respects acts as an *Arbuscular Mycorrhizal* Fungus (AMF) (Pan et al., 2017). It has been shown that actomycorrhizal fungi improve the tolerance to salinity of plants through non-transferring Na^+ and improving nutritional conditions (Chen et al., 2014). Endophyte relationship increases the concentration of N, P and K in plants, while reducing the concentration of Na^+ and Na^+: K^+ ratio (Song et al., 2015). Although creating saline tolerant plants through genetic engineering or eliminating soil salinity by washing the excess salt, is being successful, but is not economically valuable (Cantrell, et al., 2001). Therefore, the present study was designed to use bacteria *Streptomyces sp.* and *Piriformospora indica* fungi to improve nutritional status and increase the tolerance of Stevia plant under salt stress conditions.

Material and methods

Preparation of inoculum of Piriformospora indica

Isolate of *P. indica* was prepared from a collection of laboratory of Sari University of Agricultural Sciences and Natural Resources. Since the root colonization of the plant by inoculum of the *P. indica* requires a sufficient amount of fungal spores, therefore, by preparing a sufficient number of petri dishes containing Kafir (1977) medium, the fungal isolate was cultured on this medium and stored in an incubator at 25 °C for four weeks. Then, using 20% Tween solution, the fungal spores were collected from the culture medium and after centrifugation, fungal cells were washed and redissolved for three times, the number of spores of the fungus was counted using a homocytometer slider and about 5×10^7 spores per mL was adjusted for each petri dishes (Drüge et al., 2007).

Preparation of inoculum of Streptomyces sp.

Yeast-malt extract (ISP2) was used to prepare inoculum from isolates of *Streptomyces* sp., with accession number of kj152148 (S1) and kj152149 (S2). First, the mentioned isolates were inoculated in

ISP2 liquid medium and stored at 28 °C for one week on a shaker (150 rpm). After this time, two mL of the bacterial suspension (with a concentration of 10^7 per mL) were prepared to inoculate the root system of the stevia seedlings.

Plant cultivation and treatments

In order to investigate the effect of *Piriformospora indica* and *Streptomyces* isolates on saline sensitive stevia plant, growth, absorption and transferring of salinity-related elements including Na^+, K^+ and PO_4^{3-} an experiment was conducted in a factorial arrangement based on completely randomized design with 6 replications in two consecutive years in Gorgan University of Agricultural Sciences and Natural Resources (Fig. 1 and 2). The treatments consisted of four levels of plant growth promoting microorganisms (non-inoculation, inoculation of *Piriformospora indica* and two isolates of *Streptomyces sp*.), as well as three levels of salinity (0. 85, 1.5 and 3 dS/m). For doing the experiment, approximately 10 cm of herbaceous cuttings were prepared. After root formation, the roots were immersed in a suspension containing 10^7 per mL spores for 3 to 8 h on the shaker at 75 rpm. The plants were then cultured in plastic pots containing sand, peat and field soil (1:1:2). Irrigation of pots was done daily and equally in each pot from planting date (June) to the time of salt stress application using non-saline water. In order to prevent the accumulation of salt around the root, once a week pots were leached with non-saline water. The plants were then harvested in September and dried in an oven at 39 °C.

Preparation of plant extract

First, one gram of sample was powdered and placed in an electric furnace to ashes. The calcination procedure was initially performed at 200 °C and gradually increased to 550 °C for two h and kept for up to 4 h at this temperature until complete mineralization was performed. The specimens were removed and transferred into desiccator to cool down. Then, to the cooled samples, a quantity of 5 mL of 2-chloride acid was added. After completion of the interactions, samples were placed in a bin mania at 80 °C for 30 min to complete the digestion and to remove the first white vapors. The content of each sample was filtered using filter paper (Whatman 41). To ensure complete transfer, the filter paper was washed several times with distilled water, and then volume of volumetric flask reached to 50 ml using distilled water (Emami, 1997).

Measuring the amount of plant phosphorus

Colorimetric method was used to measure the P of plant's leaves and roots. In this method, ammonium heptamolybdate, ammonium vanadate and nitric acid 65% were used to prepare P solution. Then P concentration of the samples was measured using a spectrophotometer (UNICO 2800 model) at a wavelength of 470 nm (Page et al., 1982).

Measuring the amount of plant sodium and potassium

One mL of the plant extract was diluted in 50 mL balloons using distilled water and the K and Na were measured using JENWAY PEP7 flame photometer (Chapman and Pratt, 1982).

Statistical analysis

The experiment was conducted as factorial in a completely randomized design with 6 replications. Statistical analysis was performed using SAS software. Means were compared via LSD test at a significant level of 0.95% ($P < 0.05$).

Results

Plant leaf yield

According to the results of the first and second experimental years, the effect of microorganisms and salinity on fresh and dry weights of the leaves showed a significant difference (Table 1). Although,

the leaf dry weight was not affected by the interaction of microorganisms and salinity in the first year, the fresh weight of the leaves in both years and leaf dry weight in the second year were significantly affected by the interaction of microorganisms and salinity (Table 1). In both experiments, the salinity stress reduced the fresh weight of the above-ground parts. As it can be seen in Fig 1, although in second year of experiment, the fresh weight of the above-ground parts was reduced by 1.37 and 2.23 folds in medium and high saline conditions, respectively, but this reduction was lower in the plants inoculated with fungi and bacteria (Fig. 1). For example, the reduction of the fresh weight of above-ground parts of the plants inoculated with *P. indica* was only 0.11 and 1.62 folds in medium and high saline conditions. Similar results were observed in dry weight of the above- ground part (Fig. 2).

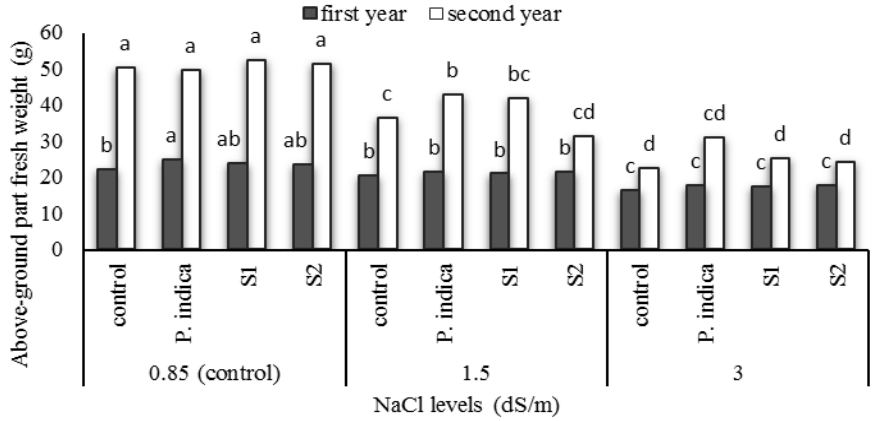


Fig. 1. The interaction effect of salinity levels and growth promoting microorganisms (inoculation with *Piriformospora indica* and two isolates of *Streptomyces sp*.) on fresh weight of the above-ground part of the Stevia plants in two consecutive years. *Streptomyces sp*. with access number kj152148 (S1) and kj152149 (S2). Different letters in the same column indicate significant differences according to LSD's test (P<0.05).

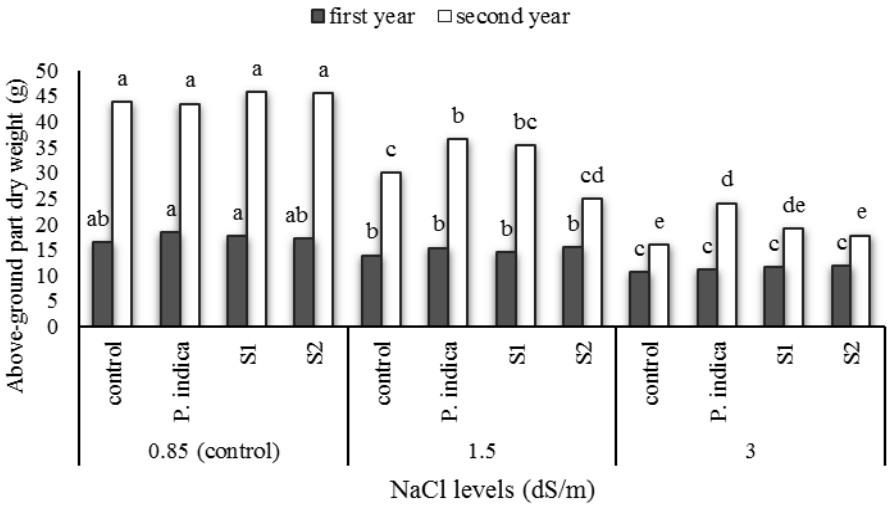


Fig. 2. The interaction effect of salinity levels and growth promoting microorganisms (inoculation with *Piriformospora indica* and two isolates of *Streptomyces sp*.) on dry weight of the above-ground part of the Stevia plants. *Streptomyces sp*. with access number kj152148 (S1) and kj152149 (S2). Different letters in the same column indicate significant differences according to LSD's test (P<0.05).

As mentioned above, under salinity condition, inoculation of fungi and bacteria maintained the fresh and dry weight of the leaf with a relatively stable level and reduced the negative effect of salt stress. There was no significant difference for leaf fresh weight among different treatments for the plants grown under non-saline conditions (0.85 dS/m). In the second year, as salinity increased to 1.5 and 3 dS/m a significant difference was observed among different bio-inoculants. The highest fresh weight was observed in plants treated with *P.indica* (Fig 3). Compare to the control, the amount of increase in inoculated plants was 1.93, 2.05 and 1.67 folds in control, S_1 and S_2 treatment, respectively (Fig 3). However, in the first year such big difference was not observed among treatments under high salinity conditions. As it is presented in Figure 4, in contrast to fresh weight, the control leaf dry weight of first experimental year was relatively higher than that of second year except at high salinity level. Albeit, the leaf dry weight of *P. indica* containing plants was 2.15, 1.9 and 1.6 folds higher than control, S_1 and S_2, respectively. As it can be seen in Figure 5, the special leaf weight (SLW) of plant that grown under medium salinity and treated with the S_1 bacteria, was higher than the other treatments (1.65, 1.50 and 1.54 folds higher than control, fungus and S_2, respectively).

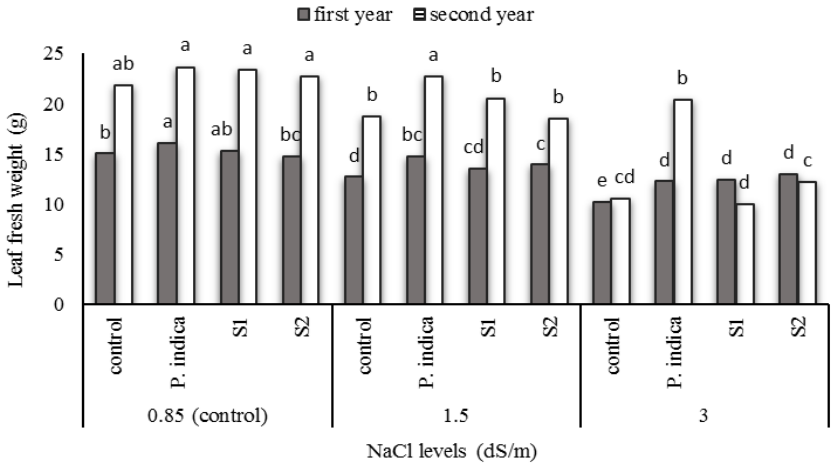


Fig. 3. The interaction effect of salinity levels and growth promoting microorganisms (inoculation with *Piriformospora indica* and two isolates of *Streptomyces sp*.) on leaf fresh weight of the Stevia plants. *Streptomyces sp*. with access number kj152148 (S1) and kj152149 (S2). Different letters in the same column indicate significant differences according to LSD's test (P<0.05).

Phosphorus content of leaves and roots

In both years, the amount of leaf and root P was significantly affected by single and interaction effects among treatments at the probability level of 1% (Table 2). Compared with control plants, the amount of P in plants inoculated with microorganisms increased at all levels of stress. For example, in second year of the experiment with increasing the salinity level to the concentration of 1.5 dS/m, the amount of P in the inoculated plants was significantly increased (3.9, 2.1 and 4.4 folds in *P. indica*, S1 and S2, respectively). At the highest salinity level (3 dS/m), the reduction in P concentration in fungus inoculated plant was 2.14 folds lower than that of control plants (Fig. 6). Under the low salinity conditions and compared to the control plants, the concentration of P in the leaves of plants inoculated with S_2, increased up to 1.8 folds than that of the control. Relatively similar results were observed in root in both first- and second-year experiment (Fig. 6).

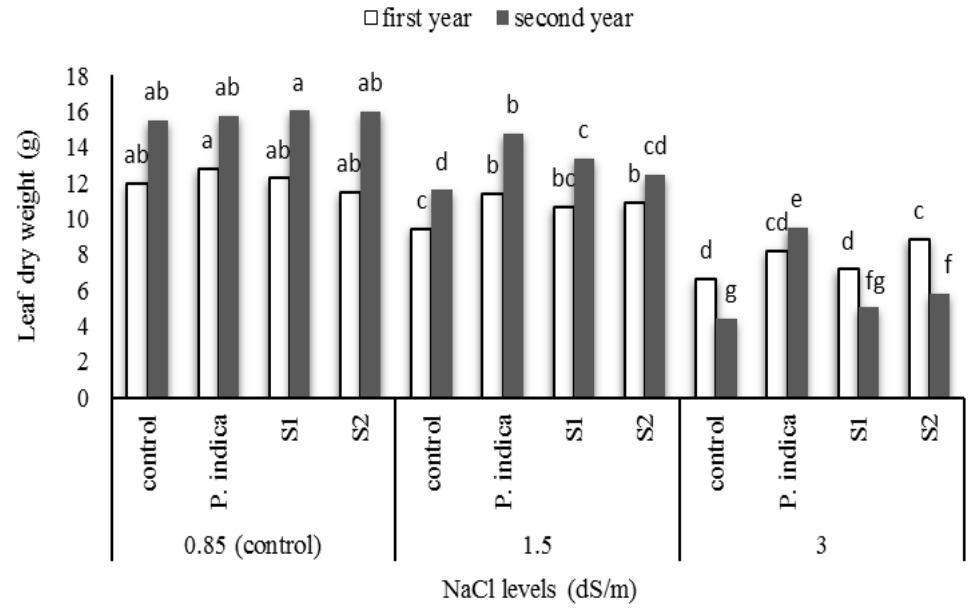


Fig. 4. Mean value of special leaf weight affected by salinity levels and growth promoting microorganisms (inoculation with *Piriformospora indica* and two isolates of *Streptomyces sp*.) in the Stevia plants. *Streptomyces sp*. with access number kj152148 (S1) and kj152149 (S2). Different letters in the same column indicate significant differences according to LSD's test (P<0.05).

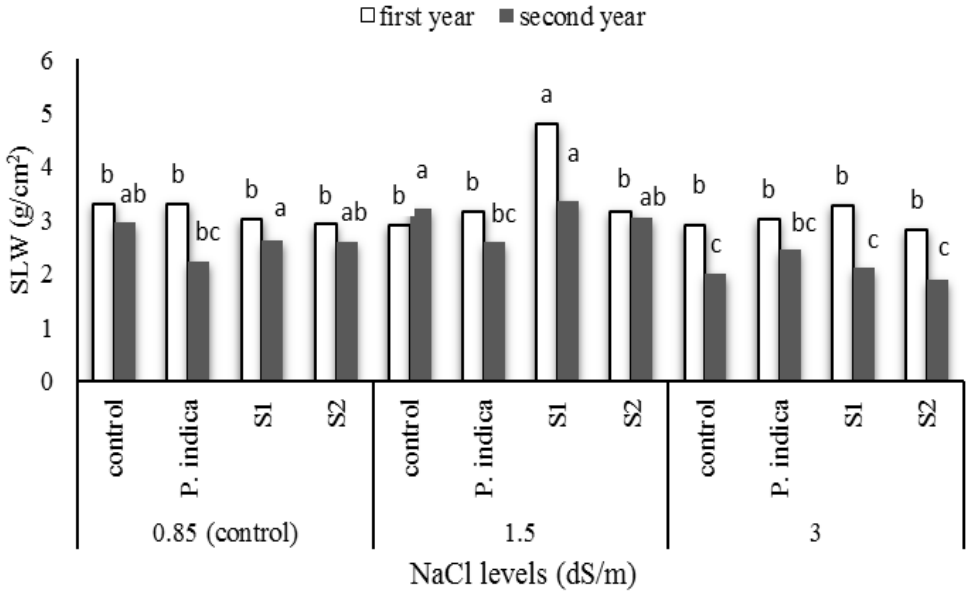


Fig. 5. The interaction effect of salinity levels and growth promoting microorganisms (inoculation with *Piriformospora indica* and two isolates of *Streptomyces sp*.) on leaf dry weight of the Stevia plants. *Streptomyces sp*. with access number kj152148 (S1) and kj152149 (S2). Different letters in the same column indicate significant differences according to LSD's test (P<0.05).

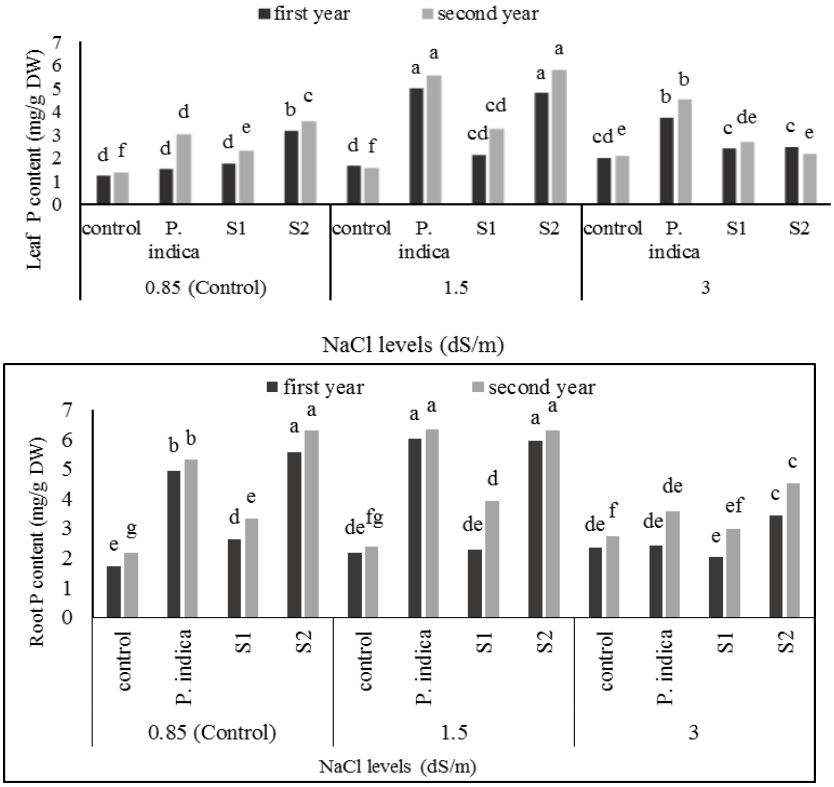


Fig 6. Effect of different levels of NaCl and bio-inoculants (*Piriformospora.indica,* kj152148 (S1) and kj152149 (S2)) on the phosphorus content of *Stevia rebaudiana* Bertoni leaves and roots in two consecutive years. Different letters in the same column indicate significant differences according to LSD's test (P<0.05).

Table 1. Analysis variance for the effects of salinity levels and growth promoting microorganisms (*Piriformospora indica* and two isolates of *Streptomyces sp*.) on some growth characteristics of Stevia plants

Source of variations	Year 1					Year 2				
	Leaf DW	Leaf FW	Over-ground part DW	Over ground part FW	SLW	Leaf DW	Leaf FW	Shoot DW	Shoot DW	SLW
Bio-inoculants (B)	6.84**	9.47**	5.78^{ns}	10.22*	2.001**	25.49**	100.44**	119.65**	124.60*	0.36^{ns}
Salinity (S)	119.57**	66.10**	220.25**	236.14**	1.57**	583.47**	595.93**	3843.94**	3776.39**	5.29**
B × S	2.45^{ns}	2.76**	1.25^{ns}	1.32^{ns}	1.55**	8.47**	33.72**	62.55*	67.11*	0.60**
Error	1.23	0.54	2.69	2.75	0.25	1.16	3.45	25.50	28.84	0.19
CV (%)	2.96	5.41	11.25	8.10	15.72	9.25	9.91	15.80	14.08	16.81

ns, *and **;Non significant, Significant at the 5% and 1% levels probability respectively, DW: Dray weight, FW: fresh weight, SLW: special leaf weight, As there was significant difference between the results of two different experimental years, results of these two years were presented separately

Table 2. Analysis variance for the effects of salinity levels and growth promoting microorganisms (*Piriformospora indica* and two isolates of *Streptomyces sp*.) on phosphorus (P) accumulation and K/Na ratio in roots and leaves of Stevia plants

Source of variations	Year 1				Year 2			
	P		K/Na		P		K/Na	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Bio inoculants (B)	15.29**	36.71**	0.99**	0.14**	24.65**	40.12**	0.83**	0.22**
Salinity (S)	12.61**	15.46**	1.47**	0.28**	14.12**	10.24**	1.38**	0.46**
B × S	5.64**	5.07**	0.09**	0.002^{ns}	5.64**	3.22**	0.06**	0.01**
Error	0.26	0.25	0.0008	0.002	0.16	0.17	0.005	0.0006
CV (%)	19.21	14.52	4.16	10.13	12.75	10.09	10.51	5.09

ns, *and **;Non significant, Significant at the 5% and 1% levels probability respectively, As there was significant difference between the results of two different experimental years, results of these two years were presented separately

Sodium content of leaves and roots

During the first and second experiments, the effect of microorganisms, salinity, and their interactions on the sodium concentrations of root and leaf of Stevia was significant (Table 3). The concentration of leaf and root sodium in both years increased with increasing the salinity levels in all four groups of plants (Fig. 7). In both years, the sodium content increased significantly by the highest salinity level (3 dS/m). While, the sodium level in inoculated plants was lower than its level in the plants that were not inoculated by fungus and bacteria. As shown in Figure 7, the Na^+ content of leaf in the first experiment was significantly influenced by microbial treatments. The Na content of plants treated with both fungus and bacteria was 1.2, 1.4 and 1.1 folds lower than that of control in *P. indica*, S_1 and S_2, respectively. A similar tendency was observed in the root Na^+ content both in the first and second experiments (Fig 7.).

Potassium content of leaves and roots

The potassium content of experimental plants was significantly influenced by treatments in both experiments (Table 3). In contrast to Na^+, the K^+ concentration in the root and leaf of plant was influenced by the microbe inoculations (Fig. 8). As it can be seen in Figure 8, under all salinity levels the K^+ content of both leaf and root of inoculated plants were higher than that of control and it seems that the bacteria were more efficient than that of the fungus. For instance, under the non-saline conditions the K^+ accumulation in control plants was relatively 1.4, 1.3 and 1.1 folds lower than that of plants containing S_2, S_1 and *P. indica*, respectively. As it can be seen in Figure 8, the root K^+ concentration in response to the treatments was relatively similar to the leaf.

K^+/Na^+ ratio of leaves and roots

Regarding to the results of analysis of variance (Table 2), the simple and interaction effects of treatments on the K^+/Na^+ ratio in the second year was significant at 1% level. However, in the first year there was no significant difference for the K^+/Na^+ ratio in the root. Under salt stress conditions, the K^+/Na^+ ratio of leaf and root decreased. However, this reduction in untreated plants was significantly higher than that of treated plants. In the first experiment and under low salinity levels the K^+/Na^+ ratio of control plants was 1.6, 2.8 and 1.8 folds lower than K^+/Na^+ ratio in plants inoculated with *P. indica*, S1 and S2 bacteria, respectively. A similar result was observed in root and the K^+/Na^+ ratio was increased in inoculated plants with microorganisms and with increasing salinity levels, this ratio decreased in leaves and roots compared to the control plants (Fig. 9).

Table 3. Analysis variance for the effects of salinity levels and growth promoting microorganisms (*Piriformospora indica* and two isolates of *Streptomyces sp.*) on K and Na concentrations in roots and leaves of Stevia plants

Source of variations	Year 1				Year 2			
	Na^+		K^+		Na^+		K^+	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Bioinoculants (B)	177.19^{**}	139.53^{**}	127.96^{**}	82.72^{**}	135.25^{**}	48.77^{**}	94.44^{**}	151.70^{**}
Salinity (S)	302.32^{**}	364.09^{**}	142.04^{**}	111.38^{**}	233.18^{**}	677.13^{**}	125.07^{**}	97.08^{**}
B × S	4.41^{**}	15.55^{**}	1.006^{**}	1.08^{ns}	7.31^{**}	13.37^{**}	2.38^{**}	3.49^{**}
Error	1.10	2.11	0.23	2.09	1.52	1.51	0.61	0.52
CV (%)	3.48	3.31	2.62	7.12	4.35	2.86	4.26	3.37

ns, *and **;Non significant, Significant at the 5% and 1% levels probability respectively. As there was significant difference between the results of two different experimental years, results of these two years were presented separately.

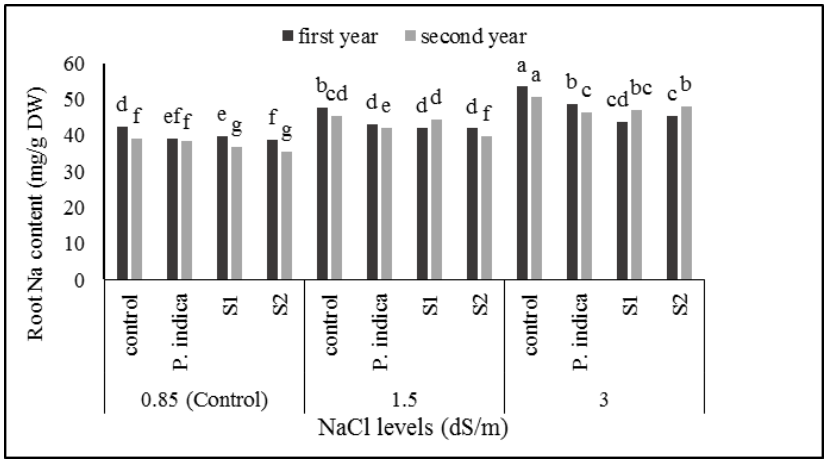
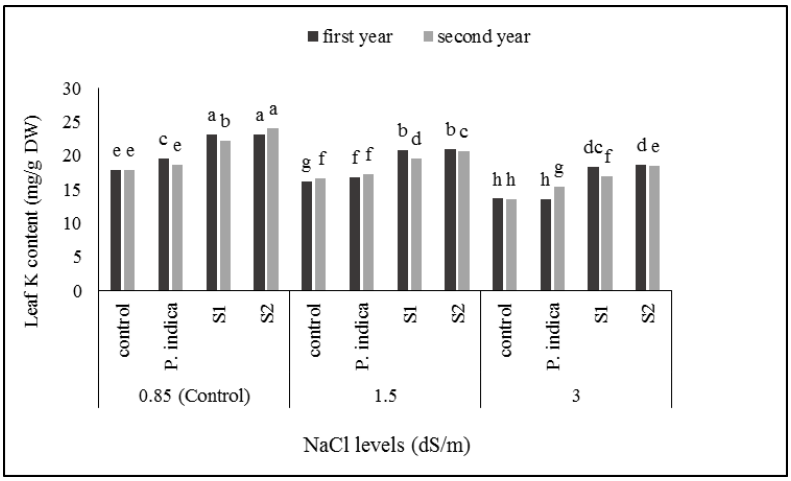


Fig.7. Effect of different levels of NaCl (control=0.85, S1=1.5 and S2=3 dSm^{-1}) and bio-inoculants (*P.indica*, S1= kj152148 and S2=kj152149) on sodium (Na) content of *Stevia rebaudiana* Bertoni leaves and roots in two consecutive years.
Different letters in the same column indicate significant differences according to LSD's test (P<0.05).



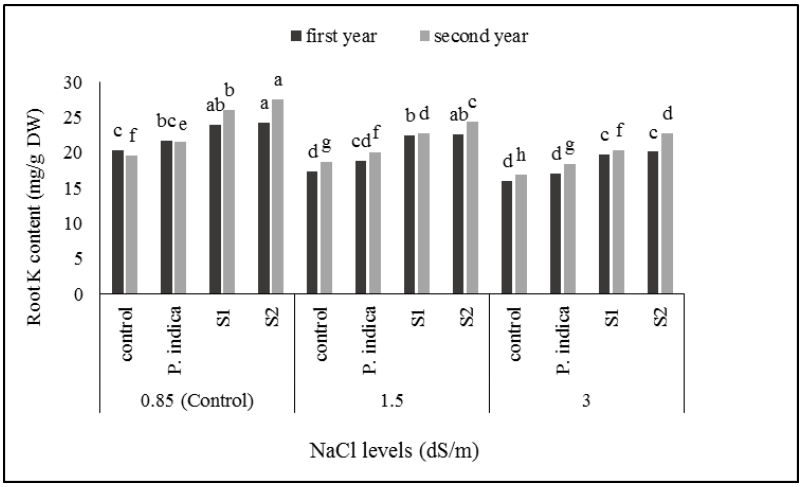


Fig.8. Effect of different levels of NaCl (0.85, 1.5 and 3 dSm^{-1}) and bio- inoculants (*P.indica*, S1= kj152148 and S2=kj152149) on potassium (K) content of *Stevia rebaudiana* Bertoni leaves and roots in two consecutive years. Different letters in the same column indicate significant differences according to LSD's test (P<0.05).

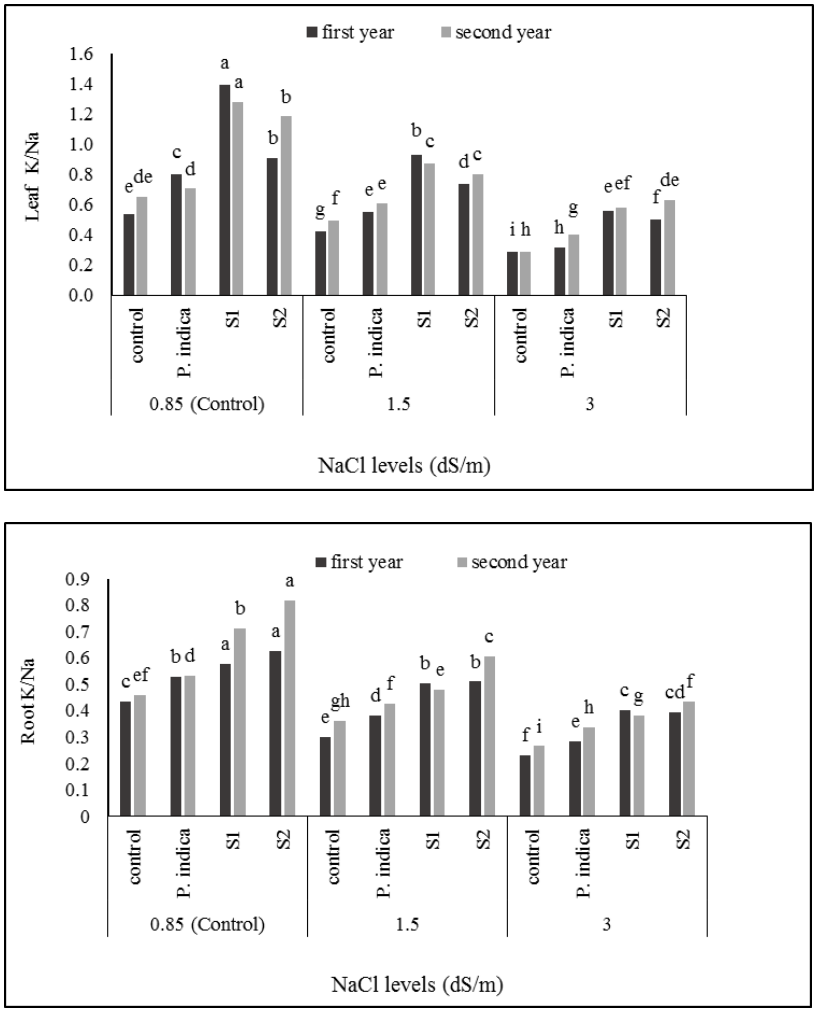


Fig.9. Effect of different levels of NaCl (0.85, 1.5 and 3 dSm^{-1}) and bio- inoculants (*P.indica*, S1= kj152148 and S2=kj152149) on K^+/Na^+ ratio of *Stevia rebaudiana* Bertoni leaves and roots in two consecutive years. Different letters in the same column indicate significant differences according to LSD's test ($P<0.05$).

Discussion

The leaves of Stevia are sweet and its economic value lies therein. The main reason for the interaction effects of salinity and bio-inoculants was to co-increase the biomass of experimental plant and their antioxidant capacity in the form of boosting enzymes such as catalase and peroxidase. These results are in agreement with earlier investigations that explored the ability of *P. indica* to stimulate growth of colonized plants under severe salt stress conditions (Jogawat et al., 2013; Ghorbani et al., 2018). Endophytic and mycorrhizal fungi differ considerably in species diversity, transmission model, and colonization patterns (Jin et al., 2019). It has recently been shown that endophytes have beneficial effects on plant growth, especially under stressful conditions (Qin et al., 2017) Endophytes plays an important role in plant exposed to salt stresses. Egamberdieva et al. (2017) showed *Mesorhizobium Ciceri* and *Bacillus subtilis* could rescue chickpea from salt stress by decreasing H_2O_2 level and accumulation of proline. Portugal et al. (2006) showed that compared to the control plants, Stevia treated with *Glomus intraradices* resulted in a significant increase in total biomass and dry weight. Also, Mandal et al. (2013), Vafadar et al. (2014) and Tavarini et al. (2018) confirmed the positive effect of inoculation of AMF fungus (*Arbuscular*

mycorrhiza) on the Stevia leaf yield. Reducing the amount of biomass under salinity stress is due to the reduction of the relative water content of the tissues and the reduction of chlorophyll content in the leaves. Reducing the relative water content of the leaves means reducing the plant's water status, which can lead to the stomatal closure and the lack of carbon dioxide intake for photosynthesis. In this study, plant growth parameters such as plant height, leaf fresh and dry weights were decreased under salinity stress. The *P. indica* fungi also increased the weight of plant biomass by increasing the relative water content of the leaves due to the accumulation of organic osmoytes and increase of chlorophyll content of the leaf. Regarding the positive effect of different strains of *Streptomyces* bacteria on the biomass production of the aerial parts of the Stevia plant, it was determined that the bacteria improved the plant's water status and increased the chlorophyll content of the leaves, thus increasing the weight of the plant biomass compared to the control plants. Reductions in plant biomass under salt stress have been reported in some other studies. For instance, Hajienia et al. (2012) reported that the salinity of irrigation water could significantly reduce fresh and dry weights of wheat germplasms. Opposite to that, the growth parameters improved under the same conditions when the plants treated with endophytic microorganisms (fungi and bacteria). In recent reports on *Coleus forskohlii* and *C. asciatica* it has been shown that inoculation of the *P. indica* improves the biomass of plants (Das et al., 2012; Satheesan et al., 2012). Bacilio et al. (2004) reported that application of *Azospirillum lipoferum* reduced the negative effects of salinity in wheat. They showed an increase in root and leaf dry weights as well as plant height in inoculated wheat. They deduced that increasing water absorption potency could be one of the reasons for increase in the plant yield due to application of *Azospirillum lipoferum*. Unlike the second year, the special weight of the leaves on the first-year plants was influenced by the single effect of microorganisms. In both years, the single effect of salinity and the interaction between microorganisms and salinity on the leaf weight was significant at the probability level of 1% (Table 1). Based on the comparison of the mean for the first year, except the bacterial treatment, which increased the leaf weight at a salinity level of 1.5 dS/m, in other treatments, no significant differences were found for the weight of the leaf. However, in the second year, this increase was observed at a salinity level of 1.5 dS/m in all treatments. However, there was no significant difference among salinity levels in the plants treated with fungi (Figure1). In both years the highest amount of special leaf weight was obtained in plants treated with *Streptomyces* sp under 1.5 dS/m salinity level (78.4 and 35.3 g/cm^2, in the first and second years, respectively).

Hosseini et al. (2015) found that inoculating Stevia plants with *Glomus mossae* induce more P accumulation than non-inoculated plants. Increased uptake of P from the medium and P translocation to the host and a sharp increase in P content in the shoot were mediated by *Serendipita indica* (Wu et al., 2019). Earanna (2007) also reported a significant difference between inoculated plants of *Glomus fasciculatum* and growth promoters and inoculum plants. Tavarini et al. (2018) demonstrated that, significant increase in P absorption of stems, roots and leaves of Stevia plants inoculated with mycorrhizal arbuscular fungi than non-inoculated plants. In general, plants colonized by the arbuscular mycorrhiza and *P. indica* have a better growth due to higher absorption efficiency by increasing the root surface (Gutierrez-Oliva et al., 2009; Rodriguez-Romero et al. 2011). In the case of P, it has been reported that the fungus can stimulate plant growth regardless of the concentration of mineral P in fertilizer (Franken, 2012). Mycorrhiza

fungi are divided into two categories. Some of them enter the plant system and decrease the acidity of the aquatic acid and increase the amount of cytokinin. Through this mechanism they increase the water absorption results in expansion of the root system. The second groups are outside the root system. These microbes release P-soluble organic acids, such as malic acid, increase P absorption by the plant as a result increase the dry matter content of the plant. P as one of the essential macro-elements for plant is vital for dry matter accumulation. P plays an important role in cell division by regulating phyto-hormones. Furthermore, P plays an important role in the transferring of photosynthetic materials and energy managing in the plant (Khalvati et al., 2005).

Nutrition imbalance was another negative effect of NaCl on Stevia plant. One of the strategies that plants use to cope with salt stress is to reduce the accumulation of toxic Na^+ ions in the root and shoot systems. Another way is to counterbalance the entry of Na^+ ions into cells by increasing intracellular K^+ concentrations. Therefore, it is essential to determine the content of Na^+ and K^+ in the roots and shoots under salt stress and control conditions. As already been discussed, Na^+ toxicity in the shoot is responsible for growth reduction (Silva et al., 2015). Extra addition of K^+ increases plant dry weight in both root and shoot, as the ratio $[K^+cyt]/[Na^+cyt]$ increases, because K^+ blocks the uptake of Na^+ into the cytosol the salinity tolerance would increase (Gul et al., 2019). 7% and 2% reduction in non-colonized and colonized plants, respectively, have been reported which led to an overall increase in foliar K^+/Na^+ ratio in colonized plants than non-colonized plants. However, cytosolic K^+ homeostasis is a curial mechanism for salt stress adaptation in a broad range of plant species (Percey et al., 2016). In tomato, *P. indica* colonization increased photosynthetic pigment content, proline and glycine betaine accumulation in inoculated roots than in non-inoculated roots (Ghorbani et al., 2018). It has been showed that, *P. indiac* promoted 14 metabolites and ions conferring tolerance to salt stress in barley grown under 300 mM NaCl (Ghaffari et al., 2016). Yun et al. (2018) reported that the negative effects of NaCl on *Zea mays* seedlings alleviate after *P. indica* inoculation, probably by improving stomatal conductance and lower K^+ efflux from the roots and increase in K^+ content in shoots than in the non-inoculated plants under 200 mM NaCl. Furthermore, rice and wheat, colonized with *P. indica* exhibited better photosynthetic pigment contents and plant growth performance (Zarea et al., 2012; Jogawat et al., 2013).

Therefore, inoculated plants were able to maintain a higher K^+/Na^+ ratio, which is a characteristic of salinity tolerance (Munns and Tester 2008; Xie et al., 2017). Reduction of potassium to sodium ratio under the salinity stress has already been proven in other studies (Ahmad et al., 2012; Kazemeini et al., 2016). The maintenance of a high K^+ level reduces the negative effects of Na^+. However, it is not clear how *P. indica* and *Streptomyces* bacteria affect K^+ transfer and absorption systems in plant. At least, based on the results of this study, it was found that the ability of Stevia plants as a susceptible plant to salinity to cope with salt stress was increased by inoculation with *P. indica* and *Streptomyces* bacteria. It has been reported that plant growth promoting bacteria regulate the growth of plants under salt stress by regulating the absorption of nutrients (increasing the K^+/Na^+ ratio) and maintaining the balance between these elements (Nadeem et al., 2006). In addition, *P. indica* protecting plants against salt stress by altering antioxidant enzyme levels, inducing ROS scavenging systems (Baltruschat et al., 2008) and regulating K^+/Na^+ ratios in the colonized plants (Abdelaziz et al., 2017). Abdelaziz et al.

(2017) indicated that *P. indica* increases K^+/ Na^+ ratios in Arabidopsis by inducing the expression of *HKT1*, *KAT1* and *KAT2* ion channels. Salt stress also causes nutritional imbalance of essential elements, especially K^+, through reducing its uptake by plants (Wakeel, 2013). The exclusion of Na^+ from sensitive tissues is commonly accepted to be related to K^+ contents. Both ions are physicochemically similar and can be transported partly by the same membrane proteins, but their roles and effects in plant cells are completely different. K^+ is an essential macronutrient for plants, which is kept at high concentrations (100–200 mM) to play many cellular and physiological roles, from membrane energization to photosynthesis (Ahmad and Maathuis 2014; Dreyer et al. 2017; Guerrero-Galán et al., 2019). On the other hand, Na^+ is only beneficial in small amounts (in the nano- or micro molar range) and its excess is harmful for plant tissues, except in some particular cases (Guerrero-Galán et al., 2019). High Na^+ concentration in the external medium decreases the K^+/Na^+ ratio in plant tissue partly by an increase of Na^+ uptake at plasma membrane via HKT and non-selective cation channels (NSCCs) (Almeida et al., 2017; Kader and Lindberg, 2005). Furthermore, Na^+ entry into the cell also causes membrane disintegrity and depolarization and leakage of K^+ by activation of K^+ outward rectifying channels (KOR) and NSCCs (Shabala et al., 2015; Gul et al., 2019). Other authors suggest that K^+ efflux of barley and rice under salt stress is caused by membrane disintegration, causing efflux of ions from the cells (Gul et al., 2019). Furthermore, K^+ also ameliorates plant growth during the first phase of salt stress exposure due to its ability for osmotic adjustment. However, maintenance of high intracellular K^+/Na^+ is a critical mechanism for growth and development of plant under salt stress (Sun et al., 2017). Sairam and Tyagi (2004) reported that sodium ion not only reduces K^+ uptake by the root, but also results in enzymatic disorders with high levels of accumulation. Replacement of Na^+ with K^+ can cause damage to the plant, as it will not be able to perform potassium duties. Na^+ concentration in saline environments is more than K^+ and it competes with K^+ adsorption systems, thus preventing K^+ uptake and its concentration in the plants reach to a toxicity threshold. Finally, as the ratio of K^+/Na^+ decreases, it induce a negative effect on plant growth (Zahir et al., 2009). Essa (2002) reported a reduction in K^+ concentration in soybean leaves under salinity stress. Netondo et al. (2004) attributed the reduction of sorghum growth under salinity stress to the reduction of the K^+ concentrations in the roots and leaves. Similar results were also reported by Kao et al. (2006) in Soybean.

Conclusion

Abiotic stresses especially salinity stress, reduces the growth and yield of agricultural products. Therefore, it is important to provide applicable approaches to deal with these stressors. In this regard, the use of bio-fertilizers in optimizing the use of chemical fertilizers is of vital importance for achieving sustainable agriculture. Emphasis on reducing negative effects of environmental stressors is a good alternative for use of chemical fertilizers under stress conditions. The results of this study showed that, microorganisms can induce mechanisms to cope with salinity stress in plant. Reducing the amount of Na^+ in the shoot can have a great effect on the growth of the symbiotic plants. The application of microorganisms could reduce the negative effects of salinity stress by decreasing Na^+ absorption and enhancing plant growth and development, and finally improving the growth characteristics of the plant. Due to the fact that the vegetative and physiological traits of Stevia were improved in the seedlings inoculated with bacteria and fungi, both in control (without stress) and in moderate

and severe stress levels, therefore, growth promoting microorganisms can be used to increase Stevia tolerance to salinity, as well as to increase the biomass of Stevia plants.

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