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EVALUATING EFFICACY OF PLANT GROWTH PROMOTING RHIZOBACTERIA AND POTASSIUM FERTILIZER ON SPINACH GROWTH UNDER SALT STRESS

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Abstract

Soil salinity is a major constraint for crop production as it negatively impacts the supply of nutrients and produces ethylene that hampers crops productivity. Several studies have been conducted so far to devise robust strategies for alleviation of soil salinity stress on crops. Inoculation of plant growth promoting bacteria (PGPR) is one of the potential strategy to decrease salinity stress on the plants. The PGPR can produce 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase which decreases stress of ethylene generated by salinity. On the other hand, improving potassium (K) uptake is also an effective strategy to minimize the negative effects of salt stress on crops. Therefore, a pot study was conducted with co-application of two pre-isolated ACC deaminase producing PGPR (i.e. *Bacillus amyloliquefaciens* and *Alcaligenes faecalis*) with half (HRK) and full recommended dose of K (RK) fertilizer in spinach (*Spinacia oleracea*) under salt stress (control = 3.0 and 5.0 dS m^{-1}). Co-application of *B. amyloliquefaciens* + RK significantly enhanced leaf and root fresh and dry weights of spinach. Maximum significant improvement in chlorophyll a (93%), chlorophyll b (50%), total chlorophyll (76%), K concentrations in leaf (70%) and root (60%), and reduction (26%) in leaf electrolyte leakage validated the effectiveness of *B. amyloliquefaciens* and *A. faecalis* with RK to mitigate salt stress. It is concluded that both *B. amyloliquefaciens* and *A. faecalis* with RK can alleviate salt stress in spinach.

Key words: ACC deaminase; Chlorophyll pigments; Electrolyte leakage; Morphological attributes.

Introduction

Plant response to salt stress has been widely investigated by researchers (Lu *et al.,* 2003; Qu *et al.,* 2012; Shahzad *et al.,* 2012; Yasmeen *et al.,* 2013; Yasmeen *et al.,* 2019). It is well documented that 20% of cultivable and half of irrigated area of world is saline (Abogadallah, 2010). Cultivation of crops in saline soils results in development of ionic toxicities, production of reactive oxygen species and osmotic stresses that significantly disturb plants growth (Locy *et al.,* 1996; Flowers & Flowers, 2005; Munns *et al.,* 2006; Zörb *et al.,* 2019). In salt affected soils, Na^+ and Cl^- restrict the development of root and optimum uptake of water by decreasing the water potential in rhizosphere (Rout & Shaw, 2001). Stress induced by salinity accelerates the synthesis of ethylene (Stearns & Glick, 2003; Roychoudhury *et al.,* 2019) that imposes deleterious effects on plants (Van Loon & Fontaine, 1984; Stearns & Glick, 2003). The elevated level of ethylene in plants minimizes the supply of energy and restricts the water uptake due to poor development of root (Taiz & Zeiger, 2010; Ricardo, 2012). Higher rate of transpiration, minimum biological nitrogen fixation, stomatal closure and evoking of physiological responses are also major indicators of elevated ethylene biosynthesis and accumulation (Tamimi & Timko, 2003; Wang *et al.,* 2003; Tanaka *et al.,* 2005; Roychoudhury *et al.,* 2019).

Many scientists have observed that salinity stress stimulates ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) (Shah *et al.,* 1998; Glick *et al.,* 1999; Glick, 2004; Albacete *et al.,* 2008; Roychoudhury *et al.,* 2019). For mitigation of salinity stress, inoculation with ACC deaminase (ACCD) producing plant growth promoting rhizobacteria (PGPR) is frequently recommended (Penrose & Glick, 1997; Glick, 2004; Zahir *et al.,* 2009; Zafar-ul-Hye *et al.,* 2014; Ijaz *et al.,* 2019). The enzyme deaminase breakdown ethylene into α-ketobutyrate and NH_3 (Shah *et al.,* 1998; Glick, 2004; Taiwo *et al.,* 2018), and later one is mostly utilized by PGPR that inhibits the regeneration of ethylene (Shah *et al.,* 1998; Roychoudhury *et al.,* 2019; Ahmed *et al.,* 2020).

Besides the use of PGPR, most of the researchers also suggested to improve the uptake of potassium (K) to reverse the negative effects of salinity on plants (Yahya, 1998; Tzortzakis, 2010). Presence of K ions in large amount increases the selectivity of K over Na that significantly reduces the intake of Na in plants (Volkov *et al.,* 2004). Better uptake of K ultimately increased the K/Na ratio in mesophyll cells which play an imperative role in regulation of proper enzymatic functioning in crops under salt stress (Volkov *et al.,* 2004). However, despite all modern reclamation and mitigation techniques for salinity stress, the natural causes and agricultural practices are continuously contributing towards the development of saline soils (Munns & Tester, 2008). The need of the time is to explore the approaches that are associated with the solution of salinity stress for better production of crops (Chinnusamy *et al.,* 2005; Ashraf & Akram, 2009).

Spinach (*Spinacia oleracea*) is considered the richest source of beta carotene, calcium (Ca), vitamin C, iron (Fe), phosphorus (P) and potassium (K) (Dicoteau, 2000; Massa *et al.,* 2018). It also plays an imperative role for the provision of supplement *p*-coumaric acid derivatives that show a significant antioxidant activity and glucuronic acid derivatives of flavonoids (Bergman *et al.,* 2001; Edenharder *et al.,* 2001; Pandjaitan *et al.,* 2005; Caparrotta *et al.,* 2019). A lot of work has been so far done on inoculation of ACCD producing PGPR and K

individually for mitigation of salt stress in different crops. However, limited literature is available about co-application of ACCD producing PGPR and K for alleviation of salt stress. As spinach is moderately salt-sensitive crop (Zeng & Shannon, 1998; Maftoun *et al*., 2004) therefore, the current study was conducted to examine the effect of co-application of half and full recommended doze of K fertilizer with ACCD producing PGPR on growth attributes and pigments synthesis under salt stress. It is hypothesized that co-application of ACCD producing PGPR with full recommended K fertilizer could be an effective and better approach for mitigation of salt stress than half dose of potassium fertilizer.

Materials and Methods

Inoculation: Two pre-isolated PGPR strains *Bacillus amyloliquefaciens* and *Alcaligenes faecalis* were used in this study. For preparation of inoculum, Dworkin and Foster (DF) media without agar was used having (in mg/L) 4000 KH_2PO_4, 6000 Na_2HPO_4, 250 $MgSO_4.7H_2O$,1 $FeSO_4.7H_2O$,0.01 H_3BO_3,0.01 $MnSO_4$,0.07 $ZnSO_4$, 0.05 $CuSO_4$, 0.01 MoO_3, 10000 glucose, 2000 gluconic acid, 2000 citric acid and 658 ACC (Dworkin & Foster, 1958). On rotatory shaker, the inoculum containing *B. amyloliquefaciens* and *A. faecalis* were incubated for 48 hours at 25 ± 2ºC.

Healthy spinach seeds of variety "Desi Palak" were manually screened out to eliminate the factor of reduction in germination by damaged seeds. For sterilization, seeds were dipped in 0.1% $HgCl_2$ for 2 min. Traces of 0.1% $HgCl_2$were washed 3 times with sterilized water (autoclaved at 120ºC for 20 min) as described by Sadiq & Ali (2013). Ten sterilized seeds were placed in an autoclaved beaker having 1ml PGPR inoculum. Finally, seeds were top dressed with sterilized (autoclaved at 120ºC for 20 min) peat and clay.

Pot study: Bulk sample of soil was collected from plough layer (15cm) at the research area of Department of Soil Science, Bahauddin Zakariya University and analyzed for texture by Hydrometer method (Gee & Bauder, 1986), soil organic matter by Walkley (1935), soil total nitrogen by Richards (1954), available P by Olsen & Sommers (1982), extractable K by Nadeem *et al*., (2013). The pre-experiment characteristics of the soil are provided in Table 1.

Table 1. Pre-experiment soil characteristics (prior to artificial development of salinity).

Soil characteristics	Units	Values
Sand	%	50
Silt	%	25
Clay	%	25
Texture	Sandy clay loam	
pH*s*	-	8.19
EC*e*	dS m^{-1}	3.00
Organic matter	%	0.40
Organic nitrogen	%	0.02
Extractable phosphorus	mg kg^{-1}	6.63
Extractable potassium	mg kg^{-1}	175

Each pot was filled with8 kg soil. Nitrogen22 kg and $P_2O_5$15.6 kg ha^{-1}were applied as urea and diammonium phosphate. Potassium was applied as per treatment plan. To maintain the half dose of recommended potassium fertilizer (HRK), 13.75 kg ha^{-1} and for recommended potassium fertilizer (RK), 27.5 kg ha^{-1} K_2O was applied as murate of potash (ZTBL, 2018).

Treatments plan and experimental design: Initially, soil electrical conductivity (EC) was 3.0 dS m^{-1} (control) which was increased to 5.0 dS m^{-1} by using NaCl to induce salt stress. At these two EC levels, following treatments were applied: (1) Half rate of recommended potassium fertilizer (HRK), (2) *B. amyloliquefaciens*+ HRK, (3) *A. faecalis* + HRK, (4) Recommended potassium fertilizer (RK), (5) *B. amyloliquefaciens* + RK and (6) *A. faecalis* + RK. Treatments were arranged in two-factorial completely randomized design in four replicates.

Initially, 10 seeds pot^{-1} were sown manually. After seven days of germination, 5 healthy seedlings were maintained. To fulfil the water requirement, the plants were irrigated frequently according to their demand. Plants were harvested after 65 days of sowing for examining the morphological and biochemical growth attributes. Leaves and roots fresh weight was assessed soon after harvesting. For determination of dry weight of leaves and root, samples were oven dried at 65ºC for 24 hours.

Leaf and root analysis: Chlorophyll a, b and total in fresh leaves were determined by methodology of Arnon (1949) and Zafar-ul-Hye *et al*., (2019). Fresh leaves were grinded in 80% acetone and then filtered through a filter (Whatman filter paper no. 42) for measuring chlorophyll at an absorbance of 663 and 645 nm wavelength on a spectrophotometer. Final values were calculated by equations:

$$\text{Cholorophyll a (mg g}^{-1}\text{ f.wt.)} = \frac{12.7\,(OD\ 663) - 2.69\,(OD\ 645)\,V}{1000\,(W)}$$

$$\text{Cholorophyll b (mg g}^{-1}\text{ f.wt.)} = \frac{22.9\,(OD\ 645) - 4.68\,(OD\ 663)\,V}{1000\,(W)}$$

$$\text{Total Cholorophyll (mg g}^{-1}\text{ f.wt.)} = \frac{22.9\,(OD\ 645) - 4.68\,(OD\ 663)\,V}{1000\,(W)}$$

where V is for final volume made and W is g of fresh leaves sample.

Electrolyte leakage (EL)was assessed according to Lutts *et al*., (1996). Dust on fresh leaves was removed with deionized water and then equal discs of diameter 1 cm were cut by using the steel cylinder. In test tubes of 20ml, one gram of uniform size leaves discs were immersed and incubated at 25ºC for 24h. After that, EC of incubated leaves (EC1) was measured on EC meter. The test tubes were heated for 20 min at 100ºC in a water bath and again EC was measured (EC2). The EL was assessed by using the equation:

$$\text{Electrolyte Leakage (EL)} = EC1/EC2 \times 100$$

Harvested plant samples were digested with the di-acid mixture of HNO_3 and $HClO_4$ in 2:1 ratio as described by Chapman and Pratt (1961). After digestion, Na and K were analyzed on the flame photometer as described by Nadeem *et al*., (2013).

Statistical analysis: Data was analyzed using standard statistical procedures of Steel *et al*., (1997). Treatments were compared by ANOVA followed by using Tukey's test at $p \leq 0.05$. Computer based program Statistix 8.1 was used for statistical computations.

Results

Plant growth: There were significant effects of treatments (T) and salinity (S) remained significant but their interaction (T × S) effect was non-significant on root and leaf fresh and dry weight. In case of root fresh weight, *B. amyloliquefaciens*+ RK, *A. faecalis* + RK performed significantly better comparative to the control (HRK) (Table 2). For root dry weight, *B. amyloliquefaciens*+ RK remained significantly better comparative to control. The treatments RK and *A. faecalis* + RK did not differ to each other but performed significantly better as compared to the control for root dry weight. However, application of *B. amyloliquefaciens*+ HRK and *A. faecalis* + HRK did not differ comparative to the control for both root fresh and dry weight. Maximum increase of 130% and 110% in fresh and dry weight of roots was noted as compared to the control in *B. amyloliquefaciens*+ RK. For leaf fresh weight, *B. amyloliquefaciens*+ RK differed significantly comparative to the control (Table 2). Application of *A. faecalis* + RK and RK alone also remained significantly better comparative to *B. amyloliquefaciens*+ HRK, *A. faecalis* + HRK and control for fresh weight of leaf. Maximum increase of 120% in leaf fresh weight was noted as compared to the control in *B. amyloliquefaciens*+ RK. In case of leaf dry weight, *B. amyloliquefaciens*+ RK performed significantly best and all other treatments remained statistically alike to each other.

Chlorophyll and electrolyte leakage: Application of K fertilizer, PGPR and salinity stress had significant effects while their interaction did not significantly affect chlorophyll a, chlorophyll b, total chlorophyll and electrolyte leakage of spinach (Table 3). Statistical analysis showed that *B. amyloliquefaciens*+ HRK and *B. amyloliquefaciens*+ RK performed significantly best as compared to the control for chlorophyll a. Application of *A. faecalis* + RK and RK alone remained statistically alike to each other but performed significantly better as compared to the control for chlorophyll a. However, *A. faecalis* + HRK and the control did not differ to each other for chlorophyll a. Maximum increase of 93% in chlorophyll a content was noted comparative to control where *B. amyloliquefaciens*+ RK was applied. In case of chlorophyll b, HRK and RK did not differ significantly (Table 3). Application of *B. amyloliquefaciens*+ HRK, *A. faecalis* + HRK, *B. amyloliquefaciens*+ RK and *A. faecalis* + RKdid not differ to each other but significantly different as compared to the control. Maximum increase of 50%in chlorophyll b content was noted comparative to control where *A. faecalis* + RK was applied. For total chlorophyll, *B. amyloliquefaciens*+ RK and *A. faecalis* + RK performed significantly best comparative to control (Table 3). The performance of *B. amyloliquefaciens* + HRK and RK was also significantly better comparative to control for total chlorophyll. Maximum increase of 76% was noted comparative to control where *B. amyloliquefaciens*+ RK was applied. For electrolyte leakage, *B. amyloliquefaciens* + HRK, RK, *B. amyloliquefaciens*+ RK and *A. faecalis* + RK remained statistically alike to each other but significantly different comparative to *A. faecalis* + HRK and control. A significant reduction by 26% in electrolyte leakage was noted when compared to the control in the *B. amyloliquefaciens*+ RK treatment.

Table 2. Effect of treatments on root and leaf fresh and dry weight of spinach plant^{-1}grown under normal (3.0 dS m^{-1}) and salinity stressed conditions (5.0 dS m^{-1}).

Treatments	Root fresh weight (g plant^{-1})			Root dry weight (g plant^{-1})		
	Soil EC*e* (dS m^{-1})					
	IE (T × S)		ME (T)	IE (T × S)		ME (T)
	3	5		3	5	
HRK	0.59 ± 0.06	0.33 ± 0.05	0.46^{D}	0.14± 0.013	0.05± 0.012	0.09^{C}
B. amyloliquefaciens + HRK	0.87 ± 0.06	0.46 ± 0.03	0.67^{C}	0.18 ± 0.011	0.09 ± 0.012	0.13^{BC}
A. faecalis + HRK	0.96 ± 0.07	0.42 ± 0.05	0.69^{C}	0.14 ± 0.016	0.07 ± 0.011	0.11^{BC}
RK	0.93 ± 0.09	0.74 ± 0.03	0.83^{BC}	0.17 ± 0.011	0.10 ± 0.018	0.14^{B}
B. amyloliquefaciens + RK	1.20 ± 0.09	0.93 ± 0.06	1.06^{A}	0.21 ± 0.010	0.18 ± 0.013	0.20^{A}
A. faecalis+ RK	1.11 ± 0.09	0.72 ± 0.04	0.91^{AB}	0.17 ± 0.015	0.12 ± 0.023	0.14^{B}
ME (S)	0.94^{A}	0.60^{B}		0.17^{A}	0.10^{B}	
	Leaf fresh weight (g plant^{-1})			Leaf dry weight (g plant^{-1})		
HRK	1.34 ± 0.08	0.74 ± 0.04	1.04^{D}	0.12 ± 0.05	0.08 ± 0.10	0.10^{B}
B. amyloliquefaciens + HRK	1.77 ± 0.19	0.72 ± 0.15	1.24^{CD}	0.18 ± 0.06	0.08 ± 0.12	0.13^{B}
A. faecalis + HRK	1.94 ± 0.14	0.73 ± 0.09	1.34^{CD}	0.18 ± 0.11	0.09 ± 0.05	0.14^{B}
RK	1.85 ± 0.13	1.00 ± 0.04	1.43^{C}	0.18 ± 0.01	0.09 ± 0.08	0.13^{B}
B. amyloliquefaciens + RK	2.76 ± 0.15	1.84 ± 0.08	2.30^{A}	0.25 ± 0.11	0.17 ± 0.07	0.21^{A}
A. faecalis + RK	2.49 ± 0.15	1.33 ± 0.07	1.91^{B}	0.17 ± 0.10	0.21 ± 0.06	0.17^{B}
ME (S)	2.02^{A}	1.06^{B}		0.18^{A}	0.12^{B}	

Means ± standard error; Means sharing different letters are significantly different ($p \leq 0.05$); No letter means non-significant effect; ME (T) = Main effect of treatments; ME (S) = Main effect of salinity; IE = Interactive effect; RK = Full recommended dose of potassium; HRK = Half recommended dose of potassium

Table 3. Effect of treatments on chlorophyll a, chlorophyll b, total chlorophyll and electrolyte leakage in spinach grown under normal (3.0 dS m^{-1}) and salinity stressed conditions (5.0 dS m^{-1}).

Treatments	Chlorophyll a (mg g^{-1})			Chlorophyll b (mg g^{-1})			Total Chlorophyll (mg g^{-1})			Electrolyte Leakage (%)		
	Soil EC*e* (dS m^{-1})											
	IE (T × S)		ME (T)	IE (T × S)		ME (T)	IE (T × S)		ME (T)	IE (T × S)		ME (T)
	3	5		3	5		3	5		3	5	
HRK	0.51±0.05	0.29±0.02	0.40^{D}	0.34±0.02	0.18±0.02	0.26^{C}	0.85±0.04	0.47±0.03	0.66^{D}	43±1.9	63±1.5	43^{A}
B. amyloliquefaciens + HRK	0.72±0.06	0.54±0.08	0.63^{A-C}	0.41±0.02	0.29±0.03	0.35^{AB}	1.12±0.07	0.83±0.09	0.98^{BC}	39±1.6	49±1.7	38^{B}
A. faecalis + HRK	0.54±0.06	0.44±0.02	0.49^{CD}	0.39±0.02	0.28±0.03	0.33^{AB}	0.93±0.05	0.72±0.02	0.82^{CD}	42±1.7	57±3.0	41^{A}
RK	0.68±0.03	0.51±0.04	0.60^{BC}	0.36±0.03	0.27±0.02	0.31^{BC}	1.04±0.04	0.78±0.06	0.91^{C}	36±2.1	51±1.3	36^{BC}
B. amyloliquefaciens + RK	0.85±0.05	0.70±0.02	0.77^{A}	0.43±0.02	0.34±0.04	0.38^{AB}	1.27±0.05	1.04±0.05	1.16^{A}	32±1.3	43±1.5	32^{C}
A. faecalis + RK	0.79±0.07	0.66±0.04	0.72^{B}	0.42±0.01	0.36±0.02	0.39^{A}	1.21±0.07	1.01±0.05	1.11^{AB}	35±2.4	47±2.3	35^{BC}
ME (S)	0.68^{A}	0.52^{B}		0.39^{A}	0.29^{B}		1.07^{A}	0.81^{B}		43^{B}	63^{A}	

Means ± standard error; Means sharing different letters are significantly different ($p \leq 0.05$); No letter means non-significant effect; ME (T) = Main Effect of treatments; ME (S) = Main Effect of salinity; IE = Interactive Effect; RK = Full recommended dose of potassium; HRK = Half recommended dose of potassium

Concentration of Na and K: The Na and K concertation in leaves and root of spinach were significantly influenced by K fertilizer, PGPR and salinity stress whereas non-significant of their interaction was observed for Na and K concertation (Table 4). For Na in leaves and root, *B. amyloliquefaciens*+ RK and *A. faecalis* + RK remained significantly best comparative to control. Application of RK also differs significantly comparative to control for Na in leaves and root. However, *B. amyloliquefaciens* + HRK remained significant for Na concentration in leaves but non-significant for Na concentration in the root comparative to control. A significant reduction of 45% and 82% in leaves and root Na concentration was noted respectively comparative to control where *A. faecalis*+ RK was applied. In case of K concentration in leaves and root, *B. amyloliquefaciens* + RK and *A. faecalis* + RK differ significantly comparative to control. Application of RK performed significantly better for K concentration in leaves and root comparative to control. However, *B. amyloliquefaciens* + HRK and *A. faecalis* + HRK differ significantly for leaf K concentration but remained non-significant for root K concertation comparative to control. Maximum increase in leaf and root K concertation was noted 70% and 60% comparative to control in *A. faecalis* + RK and *B. amyloliquefaciens* + RK respectively.

Discussion

Soil salinity stress markedly decreased the growth of spinach in the present study. The reduction in fresh and dry weight of leaves and roots of spinach might be due to higher biosynthesis of ethylene and disturbance in the minerals uptake that induced ion toxicity. The findings of Xu & Mou (2016) are in agreement with our results. They argued that ionic imbalance under salinity stress induced toxicity of ions that significantly decreased the fresh and dry weight of spinach. It has also documented that under abiotic stress, salinity or drought, a significant increase in ethylene biosynthesis decreases the growth attributes of the crops (Glick *et al.*, 1995; Zafar-ul-Hye *et al.*, 2007; McDonnell *et al.*, 2009; Zahir *et al.*, 2011; Ahmad *et al.*, 2014; Bano, 2018; Danish *et al.*, 2020). Ali *et al*., (2014) and Glick *et al*., (1998) suggested that ACC deaminase plays a key role in mitigation of salinity stress by decreasing the ethylene accumulation in the crops. According to Glick *et al*., (1998), the ACC deaminase enzyme breaks the molecules of rhizospheric ethylene into an intermediate compound called HN_3 and α-ketobutyrate. As a result of ethylene reduction in rhizosphere, the stress generating ethylene in plants root moved outside along the concentration gradient in the rhizosphere due to which ethylene accumulation in plants is decreased. A significant improvement in root and leaves fresh and dry weight in spinach plants inoculated with *B. amyloliquefaciens* and supplied with HRK validated the efficacious functioning of ACC deaminase in the presence of K under salinity stress. It was also noted that salinity stress significantly increased electrolyte leakage where HK treatment was applied (Table 3). However, *B. amyloliquefaciens* and *A. faecalis* along with RK treatment significantly decreased the electrolyte

leakage in spinach plants which might be due to reduction in ethylene accumulation. Senaratna & McKersie (1983) suggested that more permeability of cell membrane due to damage caused by abiotic stress usually resulted in higher electrolyte leakage. The findings of Nadeem *et al*., (2017) justified our argument as they reported a significant reduction in the electrolyte leakage where they applied *Pseudomonas fluorescens* in combination with biochar under abiotic stress. A significant reduction in the chlorophyll a, b and total in plants also indicated the generation of ethylene stress in HRK where no PGPR was applied (Table 3). Matile *et al*., (1997) observed in their study that when ethylene become in contact with the chloroplast then it activates the chlorophyllase (chlase) gene. As a result of chlorophyllase activation the membrane loss its integrity and destruction of chlorophyll started. Higher synthesis of holorophyll a, chlorophyll b and total chlorophyll in current study indicates the imperative role of *B. amyloliquefaciens* and *A. faecalis*along with RK for mitigation of salinity stress by lowering the ethylene. Greater K concentration in leaves and root might also be an allied factor for the mitigation of salinity stress in spinach plants (Table 4). It was also noted that the concentration of K was significantly improved but Na was decreased where RK was applied along with inoculation of *B. amyloliquefaciens* and *A. faecalis*comparative to control. According to Zhao *et al*., (2001), a significant reduction in the chlorophyll contents of cotton was due to the less intake of K in the plants. This indicates that better uptake of K might also be a pronounced allied factor that can also play an important role in mitigation of salinity stress in plants.

Table 4. Effect of treatments on Na and K concentration (%) in leaves and roots of spinach grown under normal (3.0 dS m^{-1}) and salinity stressed conditions (5.0 dS m^{-1}).

Treatments	Na concentration (%) in leaves			Na concentration (%) in root		
	Soil EC*e* (dS m^{-1})					
	IE (T × S)		ME (T)	IE (T × S)		ME (T)
	3	5		3	5	
HRK	0.69 ± 0.03	0.79 ± 0.03	0.74 ^{A}	0.09 ± 0.003	0.12 ± 0.006	0.10 ^{A}
B. amyloliquefaciens + HRK	0.57 ± 0.02	0.71 ± 0.03	0.64 ^{B}	0.07 ± 0.005	0.09 ± 0.005	0.08 ^{AB}
A. faecalis + HRK	0.56 ± 0.04	0.75 ± 0.03	0.65 ^{AB}	0.09 ± 0.006	0.11 ± 0.008	0.10 ^{BC}
RK	0.48 ± 0.02	0.58 ± 0.03	0.53 ^{C}	0.07 ± 0.006	0.09 ± 0.005	0.08 ^{C}
B. amyloliquefaciens + RK	0.31 ± 0.04	0.42 ± 0.05	0.37 ^{D}	0.04 ± 0.006	0.06 ± 0.004	0.05 ^{D}
A. faecalis + RK	0.37 ± 0.02	0.45 ± 0.03	0.41 ^{D}	0.05 ± 0.009	0.06 ± 0.006	0.05 ^{D}
ME (S)	0.50 ^{B}	0.62 ^{A}		0.07 ^{B}	0.09 ^{A}	
	K concentration (%) in leaves			K concentration (%) in root		
HRK	2.01 ± 0.15	1.46 ± 0.09	1.74 ^{D}	6.03 ± 0.39	3.85 ± 0.29	4.94 ^{D}
B. amyloliquefaciens + HRK	2.72 ± 0.16	2.16 ± 0.08	2.44 ^{BC}	6.79 ± 0.31	4.98 ± 0.43	5.88 ^{CD}
A. faecalis + HRK	2.50 ± 0.11	2.09 ± 0.11	2.29 ^{C}	6.63 ± 0.37	5.10 ± 0.41	5.86 ^{CD}
RK	2.81 ± 0.11	2.06 ± 0.07	2.44 ^{BC}	7.52 ± 0.28	5.68 ± 0.30	6.60 ^{BC}
B. amyloliquefaciens + RK	2.91 ± 0.20	2.56 ± 0.11	2.74 ^{AB}	8.52 ± 0.36	7.25 ± 0.29	7.88 ^{A}
A. faecalis + RK	3.38 ± 0.13	2.52 ± 0.16	2.95 ^{A}	7.98 ± 0.44	6.28 ± 0.47	7.13 ^{AB}
ME (S)	2.72 ^{A}	2.14 ^{B}		7.24 ^{A}	5.52 ^{B}	

Means ± standard error; Means sharing different letters are significantly different ($p \leq 0.05$); No letter means non-significant effect; ME (T) = Main effect of treatments; ME (S) = Main effect of salinity; IE = Interactive effect; RK = Full recommended dose of potassium; HRK = Half recommended dose of potassium

Conclusion

It is concluded that ACC deaminase containing *B. amyloliquefaciens* and *A. faecalis* have the potential to reduce the salt stress in spinach plants when applied with recommended K fertilizer. Both *B. amyloliquefaciens* and *A. faecalis* were effective but *B. amyloliquefaciens* was more efficacious comparative to *A. faecalis* along with recommended K fertilizer to mitigate salinity stress in spinach.

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