

Plant Growth Promoting Rhizobacteria and Mycorrhizae for Alleviation of Salinity Stress to *Khaya senegalensis* Seedlings

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Abstract: Salinity stress is an important environmental problem that adversely affects crop production by reducing plant growth. The impacts of rhizobacterial strains to alleviate salinity stress on the vegetative growth and chemical constituents of *Khaya senegalensis* seedlings were assessed using different levels of saline water. Results showed that survival percentage of seedlings was 69.45% under 8000 ppm salinity when inoculated with mycorrhizae. All growth parameters (i.e), plant height, number of leaves, fresh and dry weights were decreased by increasing salinity level. Chlorophyll content, sodium, potassium, chloride, nitrogen and phosphorus were also investigated. Under 4000 ppm, inoculation of seedlings had a positive effect on potassium and phosphorus contents.

Key words: Salinity • PGPR • Mycorrhiza • *Khaya senegalensis*

INTRODUCTION

The African mahogany (*Khaya senegalensis* L.), an exotic species of the Meliaceae family, stands out for its excellent wood quality, high prices in domestic and international markets, wood appreciated for carpentry, woodworking, shipbuilding and production of decorative veneers [1]. *K. senegalensis* is a fast growing trees in Egypt, planted in large area at different plantations. Serapium plantation is an example of successful plantation and the growth rate of this species is 102.5 (m³ /ha) [2].

Salinity (from soil or irrigation water) is a major factor reducing crop productivity and a major cause of the abandonment of lands and aquifers for agricultural purposes especially in the semi-arid areas of the world [3].

Yang *et al.* [4] coined the term "Induced Systemic Tolerance" for PGPR-elicited tolerance in plants against abiotic stress. Various reports have been published that elucidate the effect of PGPR in relieving abiotic stress in different crop plants [5]. Therefore, the rhizobacterial population can improve plant survival under different abiotic stresses, such as drought and salinity, through several mechanisms [6]. Increasing plant hormone synthesis, such as indole acetic acid (IAA) and gibberellins, is the main trigger of the activity of specific enzymes that promote plant growth [7]. The aim of the

present work was to demonstrate the adverse effect of salinity on plant growth and to report the potentiality of rhizobacterial strains to mitigate this effect on the *K. senegalensis* seedlings growth.

MATERIALS AND METHODS

The present experiment was conducted in Woody Trees Research Department - Horticulture Research Institute - Agriculture Research Center - Giza, Egypt during 2015 to 2017.

Plant Materials: Seeds of *Khaya senegalensis* were collected from nursery of Woody Trees Research Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. The seeds were soaked in water for 24 hrs and then treated with PGPR and mycorrhiza before sowing. All seedlings were transferred to culture bags (12 X 20 cm) each one contains 1.5 kg of soil (sand + peat moss + silt in proportion of 1:1:1)

Plant Growth Promoting Rhizobacteria (PGPR): Two bacterial isolates *Kocuria varians* and *Enterobacter cloacae* which are high indole acetic acid (IAA) and polysaccharides producers were isolated from

the rhizospheric soil of *Taxodium disticum* by serial dilution plate technique [8]. PGPR were used at concentration of 1×10^5 CFU for promoting seedlings growth under salinity conditions.

Mycorrhizae Inoculation Treatment: The arbuscular mycorrhizal fungi *Glomus sp.* AMF-1 and *Gigaspora sp.* AMF-2 were kindly supplied by Microbiological Research Center (MRCEN), Ain Shams Univ., Cairo, Egypt.

Inoculation of Seedlings: Seedlings were inoculated with PGPR and mycorrhizae three times: before transplanting (by deeping), when the age of seedlings was one month and before salinity treatments with one month.

Salinity Treatments: Saline solution contains sodium chloride (NaCl), calcium chloride (CaCl_2) and (MgSO_4) in ration of 2: 1:1. Saline solution was prepared at three different concentrations (2000, 4000 and 8000ppm). One year's old seedlings were used for salinity treatments.

Seedlings were irrigated with 200 ml of saline solutions every week intervals for six months and then irrigated with tap water one time for washing. After that it was washed with tap water for one time every three times of irrigation with saline solutions.

Growth Parameters of Seedlings: At the end of the experiment, seedlings were measured for: survival percentage, plant height, number of leaves, stem diameter and fresh and dry weights of aerial and root parts.

Determination of Dry Weight: Seedlings were dried in a ventilated oven at 70°C for 24 hours, then at 105°C for three hours then the dry weight was determined [9].

Chemical Analysis of Plants: Seedlings were subjected to analysis of chlorophyll, total soluble indoles, proline and minerals of N, P, K, Na and Cl.

Determination of Chlorophyll Content: Chlorophyll A and B were determined quantitatively as mg/g fresh weight (F.W.), according to the procedure achieved by Saric *et al.* [10]. The color density was spectrophotometrically measured using spectrophotometer (Jasco V630, Japan).

Determination of Total Indoles: The total indoles was determined in the methanolic extract using P-dimethyl amino benzaldehyde test "Erlich's reagent" according to Larsen *et al.* [11] and modified by Selim *et al.* [12].

Determination of Proline Content: Proline content was determined according to the methods of Petters *et al.* [13] and it was expressed as mg/gram fresh weight.

Elements Content: Total soluble Nitrogen (N) was determined according to Pregl [14], Phosphorus (P) according to Piper [15]. Potassium (K) and sodium (Na) were determined by using the flame photometer CORNING M 410. While, chloride (Cl) was determine by titration method with silver nitrate according to Brown and Jackson [16].

Statistical Analysis: The layout of the experiment was designed in factorial experiment, the main plot factor was salinity and subplot was inoculation treatments with three replicates each replicate contained three seedlings. The experiment was arranged in completely randomized design where, the LSD was calculated for comparison among means according to Steel and Torrie [17].

RESULTS AND DISCUSSION

Growth Parameters: As shown in Table (1) with consideration the mean effect of salinity, survival significantly decreased with increasing salinity level. As for the mean values of survival percentages due to each treatment (Mean inoculation) regardless the salinity level, it could be noticed that there is no significant difference in survival range among microbial treatments. With concern of interaction, the highest value of survival percentage (100%) was recorded for seedlings irrigated with tap water and inoculated with PGPR or mycorrhizae and the combined inoculation. Seedlings irrigated with 2000 ppm saline water had no significant difference among different inoculation treatments. For the salinity level 4000, treatments with mycorrhizae, *Kocuria varians* and *Enterobacter cloacae* had a significant value compared to control. Under 8000 ppm treatment, using Mycorrhizae allow the seedlings to be survived at 69.5%. Generally survival percentage was decreased with increase salinity level.

There is no significant difference between plant heights as a result of salt stress. Whereas, it was found a significant difference in plant height due to microbial inoculation by which *Kocuria varians* shows the highest significant mean 23.75cm among microbial inocula.

Inoculation plants with *Kocuria varians* under 2000 ppm water salinity recorded the highest value of plant height (25.67cm). The plant height increased by 148.34%, 145.03% and 117.4% under 4000ppm and 94.4%, 97.2%

Table 1: Effect of water salinity and inoculation with PGPR and /or mycorrhizae on growth parameters of *Khaya senegalensis* seedlings.

Salinity (ppm)	0	2000	4000	8000	Mean inoculation
Treatments					
Survival (%)					
Control	91.7 ab	80.6 bc	50.0 d	50.0d	68.6 a
Mycorrhizae	100.0 a	80.6 bc	80.6 bc	69.5 c	82.6 a
<i>Kocuria varians</i>	100.0 a	80.6 bc	69.5 c	50.0 d	75.0 a
<i>Enterobacter cloacae</i>	100.0 a	80.6 bc	80.6 bc	50.0 d	77.8 a
Combination	100.0 a	91.7 ab	50.0 d	38.9 d	70.1 a
Mean salinity	98.3 a	82.8 b	66.1 c	51.7 d	
Plant height (cm)					
Control	16.33 c-e	15.67 de	9.66 f	12.00ef	13.42 c
Mycorrhizae	18.33 b-d	20.00 a-d	24.00ab	23.33 ab	21.42 ab
<i>Kocuria varians</i>	22.00 a-c	25.67 a	23.67 ab	23.67 ab	23.75 a
<i>Enterobacter cloacae</i>	23.33 ab	20.00 a-d	21.00 a-d	23.67 ab	22.00ab
Combination	21.33 a-d	19.00 b-d	20.67 a-d	16.67 c-d	19.42 b
Mean salinity	20.27 a	20.07 a	19.80 a	19.87 a	
Number of leaves/ plant					
Control	8.00 c-e	6.33 c-e	3.67 e	4.33 e	5.58 b
Mycorrhizae	9.67 b-d	8.67 c-e	5.33 de	6.00 c-e	7.42 ab
<i>Kocuria varians</i>	8.67 c-e	10.67 a-c	8.00 c-e	7.67 c-e	8.75 a
<i>Enterobacter cloacae</i>	14.33 ab	7.00 c-e	7.00 c-e	6.00 c-e	8.58 a
Combination	15.33 a	8.67 c-e	7.00 c-e	7.33 c-d	9.58 a
Mean salinity	11.20 a	8.27 b	6.20 b	6.27 b	
Stem diameter (mm)					
Control	4.0 cd	4.2 cd	3.7 d	4.3 b-d	4.0 b
Mycorrhizae	5.0 a-c	5.0 a-c	4.5 b-d	4.8 bc	4.8 a
<i>Kocuria varians</i>	4.7 b-c	5.3 ab	5.0 a-c	6.0 a	5.2 a
<i>Enterobacter cloacae</i>	4.7 b-c	4.7 b-d	4.7b-d	6.0 a	5.0 a
Combination	5.3 ab	5.0 a-c	4.7b-d	4.7 b-d	4.9 a
Mean salinity	4.7 ab	4.8 ab	4.5 b	5.2 a	
Root length (cm)					
Control	23.67 bc	28.33 a-c	24.67 a-c	19.0 c	23.92 c
Mycorrhizae	33.67 a	23.33 bc	32.33 ab	32.0ab	30.33 a
<i>Kocuria varians</i>	32.33 ab	28.00 a-c	27.33 a-c	31.5 ab	29.79 ab
<i>Enterobacter cloacae</i>	27.00 a-c	26.67 a-c	30.00ab	26.0 a-c	27.42 b
Combination	29.67 ab	25.00 a-c	33.33 a	27.5 a-c	28.88 ab
Mean salinity	29.27 a	26.27 a	29.53 a	27.2 a	

and 97.2% under 8000 ppm when seedlings were treated with Mycorrhizae, *Kocuria varians* or *Enterobacter cloacae*, respectively.

There was a negative effect of salinity level on the number of leaves formed per plant. The significant number of leaves was recorded with plants inoculated with *Kocuria varians*, *Enterobacter cloacae* and the combined inoculation. As for interaction effect, treatment with the combined inoculum with control plants resulted in the highest significant leaf number of 15.33 leaves/plant. Whereas, the lowest significant value 4.33 leaves/plant was counted for seedlings under 8000ppm without inoculation. It was noticed that inoculation with *Kocuria varians* lead to an increase in leaf number by 118.58% and 77.07% over un-inoculated seedlings under 4000 and 8000 ppm, respectively.

Stem diameter significantly affected by salinity stress. The highest significant value (5.2 mm) was measured for seedlings irrigated with 8000 ppm regardless the effect of inoculation. The stem diameter was significantly increased due to inoculation with mycorrhizae, *Kocuria varians*, *Enterobacter cloacae* and combination compared to control. Inoculation with *Kocuria varians* and *Enterobacter cloacae* under 8000 ppm resulted in increased stem diameter recorded 6mm, while the stem diameter of plants inoculated with *Kocuria varians* was recorded significant value of 5.3 mm and 5.0 mm compared with non-inoculated seedlings 4.2 mm and 3.7 mm irrigated with 2000 and 4000 ppm of saline water, respectively. It was clear that root length did not significantly affected by the salt stress. The significant root length was recorded with

Table 2: Effect of water salinity and inoculation with PGPR and /or mycorrhizae on plant biomass of *Khaya senegalensis* seedlings.

Salinity (ppm) Treatments	Shoot fresh weight (g)				
	0	2000	4000	8000	Mean inoculation
Control	5.22 b-g	2.99 e-g	2.68 fg	3.57 d-g	3.61 b
Mycorrhizae	5.43 a-f	5.12 b-g	3.22 d-g	3.35 d-g	4.28 ab
<i>Kocuria varians</i>	7.67 ab	6.03 a-e	4.27c-g	4.31 c-g	5.57 ab
<i>Enterobacter cloacae</i>	8.37 a	7.10 a-c	6.13 a-d	5.60 a-f	6.80 a
Combination	6.92 a-c	2.18 g	4.81 b-g	4.45 c-g	4.58 ab
Mean salinity	6.72 a	4.68 b	4.22 b	4.22 b	
Salinity (ppm) Treatments	Root fresh weight (g)				
	0	2000	4000	8000	Mean inoculation
Control	2.77 b-f	1.83 e-g	1.57 fg	2.15 d-g	2.08 b
Mycorrhizae	2.40 c-g	2.90 b-f	1.98 e-g	2.05 e-g	2.33 b
<i>Kocuria varians</i>	4.17 ab	3.07 a-f	2.42 c-g	2.75 b-g	3.10 ab
<i>Enterobacter cloacae</i>	4.53 a	3.93 a-c	3.37 a-e	2.10 e-g	3.48 a
Combination	3.70 a-d	1.20 g	3.33 a-e	2.10 e-g	2.58 ab
Mean salinity	3.51 a	2.59 b	2.53 b	2.23 b	
Salinity (ppm) Treatments	Shoot dry weight (g)				
	0	2000	4000	8000	Mean inoculation
Control	2.02 a-e	1.08 de	0.95 de	1.12 de	1.29 b
Mycorrhizae	2.03 a-e	2.11 a-e	1.11 de	1.19 c-e	1.61 ab
<i>Kocuria varians</i>	3.36 a	2.32 a-d	1.57 c-e	1.63 b-e	2.22 ab
<i>Enterobacter cloacae</i>	3.40 a	3.05 ab	2.17 a-e	2.3 a-d	2.73 a
Combination	2.65 a-c	0.84 e	1.97 a-e	1.48 c-e	1.73 ab
Mean salinity	2.69 a	1.88 b	1.56 b	1.54 b	
Salinity (ppm) Treatments	Root dry weight (g)				
	0	2000	4000	8000	Mean inoculation
Control	1.55 c-g	0.94 e-g	0.81 fg	0.97 e-g	1.07 b
Mycorrhizae	1.26 d-g	1.53 d-g	1.05 e-g	1.1 e-g	1.24 b
<i>Kocuria varians</i>	2.67 a	1.70 b-f	1.30 d-g	1.58 b-g	1.81 ab
<i>Enterobacter cloacae</i>	2.47 a-c	2.50 ab	1.78 a-e	1.45 d-g	2.05 a
Combination	2.04 a-d	0.68 g	1.77 a-e	1.20 d-g	1.42 ab
Mean salinity	2.00 a	1.47 b	1.34 b	1.26 b	

mycorrhizae, *Kocuria varians*, *Enterobacter cloacae* and combination in comparison with the control. No significant difference was recorded as a result of microbial inoculation under salinity level 2000 and 4000 ppm. Under 8000 ppm mycorrhizae and *Kocuria varians* gave significant values of 32.0 cm and 31.5 cm, respectively.

Data of shoot fresh weight (g/plant), root fresh weight (g/plant), shoot dry weight (g/plant) and root dry weight (g/plant) of *K. senegalensis* seedlings under salinity stress. A negative effect can be observed between salinity levels on shoot fresh weight (Table 2). As for the mean effect of PGPR, a significant shoot fresh weight was recorded with *Enterobacter cloacae*. Under salinity level 2000 and 4000 ppm *Enterobacter cloacae* had a significant value of 7.1g/plant and 6.13g/plant, respectively. Inoculation of seedlings with *Enterobacter cloacae* lead to an increment in shoot fresh weight by 138.25%, 128.73% and 58.19% under salinity levels 2000, 4000 and 8000 ppm, respectively compared with control. Results of current investigations proved that 69.45% of *K. senegalensis* seedlings were survived under 8000 ppm salinity when inoculated with mycorrhizae. All

growth parameters viz, plant heights; number of leaves, fresh and dry weights were decreased by increasing salinity level. Among microbes, PGPR can also modulate phyto hormone levels in plant tissues affecting hormonal balance of host plant [18]. The reduction in stem length, plant height or stem diameter might be due to salinity which decreased each cell division, cell elongation and meristemic activity as indicated by Rug *et al.* [19] and Bolus *et al.* [20].

Also, under salinity conditions, the reduction in leaves number/plant might cause a disturbance in natural hormones leading to unbalanced growth of the plants. Bernstien *et al.* [21] found that, the decrease in root length due to salinity treatments might be attributed to the inhibition of water absorption, specific ions concentration in the saline media. The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of *Acacia gerrardii* under salt stress [22].

Chemical Constituents: As shown in Table (3) the data revealed that both salinity treatments and inoculation had no significant effect on chlorophyll a content. As for the



Photo 1: Effect of water salinity and inoculation with PGPR and/or mycorrhizae on *Khaya senegalensis* growth (groups from left to right: tap water, 2000, 4000 and 8000 ppm; inside each group from left to right: control, Mycorrhizae, *K. varians*, *Ent. cloacae* and combined inoculation)

Table 3: Effect of water salinity and inoculation with PGPR and/or mycorrhizae on chlorophyll and proline contents of *K. senegalensis* seedlings.

Salinity (ppm)	Chlorophyll a (mg/g)				
	0	2000	4000	8000	Mean inoculation
Control	0.36 ab	0.34 ab	0.37 ab	0.37 ab	0.36 a
Mycorrhizae	0.34 ab	0.36 ab	0.36 ab	0.30 ab	0.34 a
<i>Kocuria varians</i>	0.42 ab	0.20 b	0.22 ab	0.23 ab	0.27 a
<i>Enterobacter cloacae</i>	0.35 ab	0.46 a	0.33 ab	0.29 ab	0.36 a
Combination	0.28 ab	0.38 ab	0.23 ab	0.44 ab	0.33 a
Mean salinity	0.35 a	0.35 a	0.30 a	0.32 a	
Salinity (ppm)	Chlorophyll b (mg/g)				
	0	2000	4000	8000	Mean inoculation
Control	0.33 a-d	0.26 a-d	0.22 b-d	0.23 b-d	0.26 a
Mycorrhizae	0.21 b-d	0.30 a-d	0.27 a-d	0.17 cd	0.24 a
<i>Kocuria varians</i>	0.39 a-c	0.27 a-d	0.15 d	0.14 d	0.24 a
<i>Enterobacter cloacae</i>	0.48 a	0.27 a-d	0.19 b-d	0.16 d	0.27 a
Combination	0.41 ab	0.24 b-d	0.13 d	0.29 a-d	0.27 a
Mean salinity	0.36 a	0.27 ab	0.19 b	0.20 b	
Salinity (ppm)	Proline (mg/g)				
	0	2000	4000	8000	Mean inoculation
Control	3.15 a	3.18 a	3.11 a	3.12 a	3.14 a
Mycorrhizae	1.71 f-h	3.23 a	3.31 a	2.12 c-e	2.59 b
<i>Kocuria varians</i>	1.97 d-g	1.55 h	2.36 b-d	2.03 d-f	1.98 d
<i>Enterobacter cloacae</i>	1.94 e-h	2.48 bc	2.49 bc	1.81 e-h	2.18 c
Combination	2.62 b	2.62 b	2.47 bc	1.60 gh	2.33 c
Mean salinity	2.28 c	2.61 b	2.75 a	2.14 d	
Salinity (ppm)	IAA (mg/100g f.w)				
	0	2000	4000	8000	Mean inoculation
Control	60.06 gh	72.02 c-g	86.47 bc	80.42 b-f	74.74 ab
Mycorrhizae	84.35 bc	93.59 b	77.58 b-f	58.09 g-i	78.40 a
<i>Kocuria varians</i>	111.16 a	67.32 d-g	60.63 gh	42.65 i	70.44 b
<i>Enterobacter cloacae</i>	82.33 b-e	77.50 b-f	78.84 b-f	50.33 hi	72.25 b
Combination	67.21 d-g	65.12 f-h	83.02 b-d	66.57 e-h	70.48 b
Mean salinity	81.02 a	75.11 b	77.31 ab	59.61 c	

interaction effect the treatment with *Enterobacter cloacae* combined with saline water at 2000 ppm recorded the highest value (0.46mg/g).

It was shown that there were no significant differences in chlorophyll b due to inoculation treatments. Also there were no significant differences in chlorophyll b content under salinity levels of 2000, 4000 and 8000 ppm. For interaction, effect treatment with *Enterobacter cloacae* under control (tap water) condition recorded the highest value (0.48mg/g). According to Barnawal *et al.* [23] PGPR strains, *Arthrobacter protophormiae* (SA3) and *Dietzia natronolimnaea* (STR1), can facilitate salt stress tolerance in wheat crop and these PGPR strains enhance photosynthetic efficiency under salt stress condition. Also, Rojas Tapias *et al.* [24] mentioned that chlorophyll content in leaves of maize increased and PGPR inoculation enhanced plant stress Responses.

Proline Content: Regardless of inoculation treatments, an increasing in plant proline content can be observed with increasing salt stress. Inoculation reduced proline content in both salt-stressed plants and those irrigated with non-salinized water. Inoculation with *Kocuria varians* recorded a significantly lowest value of proline in comparison with control. A significantly low value of proline 1.55 mg/g was recorded in plants inoculated with *Kocuria varians* under salinity level 2000 ppm compared with control 3.19 mg/g. Under salinity level 4000 ppm *Kocuria varians* significantly gave the lowest value of proline 2.37 g/100g compared with control 3.11 mg/g. Under salinity level 8000 ppm combination treatment significantly gave the lowest value 1.60 mg/g compared with control 3.12 mg/ g. Data also stated that proline contents were increased by 1.35% and 6.3% when seedlings were inoculated with mycorrhizae under 2000 and 4000, respectively. Treatments with combination (PGPR+ mycorrhizae) had a significant effect on proline and Na in *Khaya senegalensis* seedlings. These results agreed with Khan and Zaidi [25] showed that the higher proline content (67.8 mg /plant) in wheat was observed with the co-inoculation of *Azotobacter chroococcum* with *Bacillus sp.* and *Glomus fasciculatum*.

IAA Content: The obtained results indicated a decrease in IAA with increasing salinity levels (Table 3). No significant differences could be detected among the IAA content by inoculation treatments, but there is a significant difference between treatment with mycorrhizae and other microbial treatments. Under salinity level 2000 ppm, mycorrhizae had a significant effect which gave the

highest value of IAA (93.59 mg/100g) compared with control (60.06 mg/100g). Under salinity level 4000 ppm *Kocuria varians* gave the lowest value (60.63 mg/100g) compared with 86.47g/100g for control. Under salinity level 8000 ppm plants inoculated with mycorrhizae, *Kocuria varians* and *Enterobacter cloacae* significantly gave the lowest IAA values 58.09, 42.65 and 50.33 mg/100g, respectively in comparison with control (80.42g/100g).

Soil bacteria modulate plant hormone status by releasing exogenous hormones, metabolites and enzymes that may contribute to increased salt tolerance [6]. Together with the plant's endogenous IAA, an auxin signaling pathway is triggered and results in stimulation of cell growth and proliferation. IAA produced by PGPR is one of the most common and widely studied bacterial signaling molecules in plant-microbe interactions. The function of exogenous IAA is dependent on the endogenous IAA levels in plants. At optimal IAA concentration, acquisition of bacterial IAA may result in neutral, promotion or inhibition of plant growth [26]. Moreover, Barnawal *et al.* [23] suggested that PGPR strains increase indole-3-acetic acid (IAA) content of wheat under salt stress condition.

Elements Content

N, P and K Content: A negative effect of salinity level on nitrogen contents in both inoculated and un-inoculated plants was obtained (Table 4). Plant nitrogen content was significantly affected by inoculation treatment by which the highest value (2.38%) was recorded for seedlings inoculated with *Kocuria varians*. Under salinity level 2000 ppm, plants inoculated with *Kocuria varians* showed a significant high value of N (4.42%) compared with control 1.99%. Under salinity level 4000 ppm *Kocuria varians* had a significantly low value 1.55% when compared to control 3.1%. Under salinity level 8000 ppm *Enterobacter cloacae* had a significant value 2.10% compared with control 1.55%.

Phosphorus content was significantly decreased when seedlings were irrigated with 2000 ppm saline water, while there were no significant differences among other saline levels and tap water irrigation. Microbial inoculation significantly affected P content by which the highest significant value (0.412 %) was recorded for *Enterobacter cloacae* inoculated seedlings and the lowest one was detected for mycorrhizae treated seedlings. A significant difference was recorded in phosphorus content of plants inoculated with *Enterobacter cloacae*. Under salinity level 2000 ppm

Table 4: Effect of water salinity and inoculation with PGPR and/or mycorrhizae on N, P and K (%) of *K. senegalensis* seedlings.

Salinity (ppm) Treatments	N (%)				
	0	2000	4000	8000	Mean inoculation
Control	2.21 c-e	1.99 d-f	3.1 b	1.55 f	2.21 ab
Mycorrhizae	2.43 cd	2.21 c-e	1.77 ef	1.88 ef	2.07 b
<i>Kocuria varians</i>	1.99 d-f	4.42 a	1.55 f	1.55 f	2.38 a
<i>Enterobacter cloacae</i>	2.21 c-e	2.21 c-e	1.88 ef	2.10 de	2.10 ab
Combination	2.21 c-e	2.65 bc	2.21 c-e	1.55 f	2.15 ab
Mean salinity	2.21 b	2.70 a	2.10 b	1.73 c	
Salinity (ppm) Treatments	P (%)				
	0	2000	4000	8000	Mean inoculation
Control	0.43 bc	0.37 cd	0.15 h	0.32 d-f	0.317 bc
Mycorrhizae	0.31 d-f	0.16 h	0.27 fg	0.23 g	0.24 c
<i>Kocuria varians</i>	0.25 e-g	0.32 d-f	0.45 b	0.27 fg	0.322 ab
<i>Enterobacter cloacae</i>	0.32 d-f	0.27 fg	0.52 a	0.54 a	0.412 a
Combination	0.34 de	0.35 de	0.35 de	0.36 cd	0.350 ab
Mean salinity	0.33 a	0.29 b	0.35 a	0.34 a	
Salinity (ppm) Treatments	K (%)				
	0	2000	4000	8000	Mean inoculation
Control	0.83 a	0.78 a-c	0.63 de	0.63 de	0.72 a
Mycorrhizae	0.78 a-c	0.72 a-d	0.81 ab	0.52 e	0.71 a
<i>K. varians</i>	0.72 a-d	0.72 a-d	0.71 a-d	0.6 de	0.69 a
<i>Ent. cloacae</i>	0.81 ab	0.69 b-d	0.78 a-c	0.66 cd	0.73 a
Combination	0.83 a	0.78 a-c	0.66 cd	0.52 e	0.70 a
Mean salinity	0.79 a	0.74 ab	0.72 b	0.59 c	

Table 5: Effect of water salinity and inoculation with PGPR and/or mycorrhizae on Na⁺ (%) and Cl⁻ (%) of *K. senegalensis* seedlings

Salinity (ppm) Treatments	Na (%)				
	0	2000	4000	8000	Mean inoculation
Control	0.89 d-f	0.78 fg	1.06 cd	1.64 a	1.09 a
Mycorrhizae	0.57 hi	0.81 fg	1.09 bc	0.83 fg	0.83 b
<i>Kocuria varians</i>	0.66 gh	0.66 gh	0.66 gh	0.78 fg	0.69 c
<i>Enterobacter cloacae</i>	0.66 gh	0.92 c-f	1.01 c-e	0.92 c-f	0.88 b
Combination	0.75 fg	0.86 ef	1.24 b	0.43 i	0.82 b
Mean salinity	0.706 d	0.806 c	1.012 a	0.92 b	
Salinity (ppm) Treatments	Cl (%)				
	0	2000	4000	8000	Mean inoculation
Control	5.85 e	11.7 a	5.83 e	8.19 c	7.89 a
Mycorrhizae	4.39 f	4.39 f	7.31 d	7.02 d	5.78 bc
<i>Kocuria varians</i>	5.85 e	5.85 e	8.19 c	8.78 b	7.17 ab
<i>Enterobacter cloacae</i>	4.72 f	2.93 g	5.85 e	7.31 d	5.20 c
Combination	8.78 b	8.78 b	8.78 b	8.78 b	8.78 a
Mean salinity	5.92 d	6.73 c	7.19 b	8.02 a	

mycorrhizae inoculated plants had a low significant value 0.16% compared with control 0.37%. Also, under salinity level 4000 ppm mycorrhizae, *Kocuria varians*, *Enterobacter cloacae* and their combination resulted in significantly higher phosphorus values compared with control (0.27, 0.54, 0.45 0.52 and 0.35 %, respectively).

Data also stated that under salinity level 8000 ppm *Enterobacter cloacae* significantly gave the highest value of phosphorus (0.54%) compared with control (0.32%).

There are no significant differences in plant K content due to inoculation. Under salinity level 2000 ppm, no significant differences were recorded in inoculated plants compared with un-inoculated. Whereas, under salinity level of 4000, mycorrhizae and *Enterobacter cloacae* resulted in significantly higher values of 0.81 and 0.78% compared with control 0.63%. When 8000 ppm

irrigation water was used, potassium content significantly decreased with inoculation of seedlings, except that inoculated with *Enterobacter cloacae*.

Na and Cl Contents: Away from inoculation, plants received non salinized water accumulated significantly lower amounts of Na compared with those grown under salt stress (Table 5). It can be shown that there is a positive effect of salinity level on Na content. Salinity level at 4000 and 8000 ppm gave the highest Na contents compared to control. Plants inoculated with *Kocuria varians* had the lowest Na content. Under salinity level 4000 ppm, the combined inoculation significantly decreased Na content to reach 1.24% compared with control 1.06%. At the salinity level 8000 ppm, the combined inoculum gave the lowest Na content of 0.43% compared with control 1.64%.

The overall means of chlorine content of plants grown under salt stress were significantly higher compared with non-salt stressed seedlings. Inoculation with mycorrhizae or *Enterobacter cloacae* significantly decreased Cl content. Under salinity level 4000 ppm mycorrhizae, *Kocuria varians* and combination gave a significant value 7.31%, 8.19% and 8.78% compared with control 5.83%, respectively. Data also stated that under salinity level 8000 ppm *Kocuria varians* and combined inoculation significantly gave the highest values, 8.78% and 8.78%, respectively, compared with 8.19% for control.

Under salt-stress conditions, accumulation of Na in plant tissue inhibits cellular processes like protein synthesis, transport of nutrients and restricts the growth of the plant. Therefore, plants had developed certain strategies to maintain a low level of cytosolic Na by its exclusion and compartmentation under salinity stress [27]. An ability to accumulate K and exclude Na is an important approach for salt tolerance in the plants [28].

Therefore, estimation of the K/ Na ratio has been recommended as one of the most important decisive factors for evaluating the level of salinity tolerance in several plant species [27].

It is assumed that a few PGPR assist host plants in changing the selectivity of ions (Na^+ , K^+ , Ca^{2+}) and maintaining a higher K/ Na ratio, which in turn reduce the salinity-induced damage in various crop plants [29, 30]. Hashem *et al.* [22] reported that the interaction between arbuscular mycorrhizal fungi and endophytic bacteria under salt stress had increase, P absorption and reduced Na. PGPR help maintaining ion homeostasis and high K/Na ratios in shoots by reducing Na and Cl accumulation in leaves, increasing Na exclusion via roots and boosting the activity of high affinity K transporters. Inoculation of *Azotobacter* strains C5 (auxin producing) and C9 in maize plants under salt stress improved K uptake and Na exclusion.

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