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Mitigation adverse effects of salinity stress on wheat plants by co-inoculation of plant growth promoting rhizobacteria, arbuscular mycorrhizal fungi and compost amendment

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***Abstract*---**Salinity has become one of the most important challenges in agriculture. A pot experiment was conducted during 2017/2018 and 2018/2019 seasons at the greenhouse of Fac. Agric., Cairo Univ., Giza, Egypt, on *Triticum aestivum* L., var. *Gemmiza* 10 Plants irrigated with diluted seawater with tap water (control), 4.0, 6.0, 8.0 and 10.0 dS m^{-1} to investigate the utilization of co-inoculation of plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) and the addition of compost individually or in combination asan environmentally sustainable tools to alleviate the effects of salinity on wheat plants at both 75 days after sowing (DAS) (elongation stage) and 150 DAS (maturity stage). Salinity stress caused significant reduction in shoot height, shoot fresh and dry weights, K^+/Na^+ ratio and nitrogen, phosphorous and potassium contents of shoot at 75 DAS, however, Na^+ concentration increased significantly. At maturity yield and its attributes, nitrogen, phosphorous and potassium contents in grain and straw decreased significantly with increasing irrigation water salinity level. Co-inoculation and/or compost amendments increased significantly the growth parameters and yield components compared to untreated plants under all irrigation water salinity levels. Both co-inoculation of PGPR+AMF and compost amendments increased nitrogen,

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phosphorus and potassium contents and K^+/Na^+ ratio; however, Na^+ concentration was decreased. The tripartite combination of co-inoculation with PGPR + AMF and compost showed the highest positive impacts. This study recommends the combined use of co-inoculation PGPR+AMF and applied compost for the salinity stress management strategy of wheat plants.

***Keywords*---**salinity, wheat plant, PGPR, AMF, compost.

Introduction

The fresh-water resources available for agriculture are decreasing in quantity and quality. Globally, the shortage of fresh water resources is a critical challenge to sustainable agriculture. Recently, the use of saline water has been increased as an additional source of irrigation water in areas with a lack of fresh water (Oron et al., 2002). Various studies have reported that irrigation with saline water is acceptable for crops with moderate salinity tolerance (Hassanli and Ebrahimian, 2017). The constant use of saline water for irrigation resulted in long-term environmental problems, such as soil salinization (Ramadoss et al., 2013). Globally, according to the FAO, 20% of the irrigated and 2% of dry lands have been affected by salinity (Salwan et al., 2019), which is expected to reach up to50% by the year 2050 (Hossain, 2019). Among cereal crops, about 70% yield loss has been reported including wheat, rice, maize, and barley due to soil salinity and sodicity (Hussain et al., 2019). A critical challenge is to manage poor quality water for sustainable agricultural production system.

Wheat (*Tritium aestivum* L.) is a major global food crop, with annual production of about 765 million metric tons (FAO 2019). It is consider one of the most important trade commodities (Irshad et al., 2021). Recently, wheat production has been negatively affected by global climatic changes and a shortage of water resources, which has compromised the nutritional security of the increasing population (EL Sabagh et al., 2021). The Egyptian government is exerting great efforts to increase agricultural land through establishing national projects to reclaim and cultivate new lands. Salinity of soil and irrigation water is among the biggest challenges facing cultivation in the new lands.

Microorganisms associated with plants contribute to their growth promotion (Chu et al., 2019) and salinity resistance (Mokrani et al., 2020). PGPR is consider an effective biological and environmentally sustainable method to alleviate the adverse impacts of salinity and enhance the growth and yield of plants (Abd El- Ghany and Attia, 2020), by a several mechanisms that cause physiological, biochemical and molecular changes. Which including changes in expression of defense-related proteins, exopolysaccharides (EPS) and indole acetic acid (IAA) synthesis, activation of antioxidant machinery, accumulation of osmolytes, maintaining the Na^+ kinetics and enhancing the levels of phytohormones and nutrient content in plants (Arora et al., 2020). Arbuscular mycorrhizal fungi (AMF) show a symbiotic relationship with 72% of land plant species (Brundrett and Tedersoo, 2018), which help in efficient uptake of nutrients and water by the plants and also provide protection to the plants against pathogens and various abiotic stresses (Eroglu et al., 2020). In wheat plants, AMF has been reported to mitigate the adverse impacts of salinity by various mechanisms, such as improved water relations, stomatal conductance (Zhu et al., 2018), nutrient uptake (Mardukhi et al., 2015), photosynthetic efficiency (Talaat and Shawky, 2014) and production of antioxidants (Chang et al., 2018).

Under both normal and stress conditions, the dual inoculation of PGPR and AMF promotes crop yield and nutrient content relative to a single inoculation. (Ben-Laouane et al., 2019). Positive interactions between PGPR and AMF resulted in a synergistic effect, which enhances each other's growth and, thus, it promotes plant growth (Pan et al., 2020). It is an efficient and cost-effective recipe for improving plant tolerance against salinity stress, which can be an extremely useful approach for sustainable agriculture (Sagar et al., 2021). The metabolites i.e., organic acids, volatile compounds (ethylene), and nonvolatile compounds which produce by some AMF species that attract specific bacteria (Younesi & Moradi, 2014). As well as, some of the bacteria are known as mycorrhiza-helperbacteria (MHB) which enhance colonization of AMF.

The utilization of compost for the restoration of salt-affected soils was reported by many previous studies (Mbarki et al., 2020). Compost can mitigate salinity stress in plants by enhancing soil fertility (Raklami et al., 2019) when it mineralized into the soil, thereby providing a sustained release of available nutrients to plants (Ou Zin et al., 2020), promoting nutrient availability and plant growth (Trivedi et al., 2017) and act as conditioners to enhance soil physicochemical and biological properties thereby enhancing water holding capacity (WHC), and nutrient retention and availability to the plants (Ullah et al., 2021). Organic amendments could enhance soil properties by accelerating leaching of sodium and other salts and reducing exchangeable sodium percentage (ESP). In addition to, organic amendments improve soil biological and enzyme activities and enhance soil microorganism (Ding et al., 2020). Many previous studies reported the combined synergistic effect of compost, and PGPR in alleviate salinity and drought stress of wheat plants (Kanwal et al., 2017; Yaseen et al., 2020). Consequently, the objectives of this study were determination of wheat growth and yield under different irrigation water salinity, and to evaluate the potential role of either co-inoculation of PGPR and AMF, compost application and tripartite combination of co-inoculation with PGPR + AMF and compost in alleviating the harmful effects of salt stress on growth and production of wheat plants.

Materials and Methods

Soil Sampling and Analysis

A pot experiment was carried out at the green house of Fac. Agric., Cairo Univ., Giza, Egypt, during 2017/2018 and 2018/2019 winter seasons. Surface soil sample was collected from Nubaria District AL-Behaira Governorate, Egypt (latitude: 31°05'02" N; longitude: 29°50'50 E; mean altitude: 16 m above sea level). Soil samples were analyzed for their some physical and chemical properties (Table 1), before starting the experiment, according to the methods described by Chapman and Pratt, 1978.

Table 1. Some physico-chemical properties analysis of soil used

Particle size distribution				Chemical properties				Soluble cations (meq l^{-1})			
Sand (%)	Silt (%)	Clay (%)	Soil texture	pH (1:2.5)	EC (dSm^{-1})	$CaCO_3$ %	O.M. %	Ca^{++}	Mg^{++}	K^+	Na^+
70.80	25.60	3.60	Sandy loam	8.23	1.70	3.00	0.20	2.10	1.70	4.90	8.00

Macronutrients (ppm)			Available micronutrients (ppm)				Soluble anions(meq l^{-1})		
Total N	AvailableP	Available K	Fe	Mn	Zn	Cu	HCO_3^-	Cl^-	SO_4^{--}
103.20	4.70	78.60	7.60	0.35	0.12	0.31	1.40	13.50	1.80

Bacterial and Fungal Material

Bacterial inocula, preparation and inoculation techniques

Highly efficient strains of salt-adapted plant growth promoting rhizobacteria (PGPR) *Azotobacter chroococcum, Azospirillum brasilense*, phosphate solubilizing bacteria (*Bacillus megaterium* var. *phosphaticum*) , potassium solubilizing bacteria (*B. cereus*) and growth stimulator bacteria (*Pseudomonas fluorescens*) which isolated previously from rhizoplane and rhizospheric soil fraction salt-affected soil were obtained from cultural collocation of Agric. Microbiology Dep. National Research Centre, Egypt. The PGPR were grown in nutrient broth for 48 hours at 30°C in a rotary shaking incubator. The density of each bacterial culture in the broth was counted using a haemocytometer. Liquid broth cultures initially containing $8x10^{10}$, $7x10^{9}$, $8x10^{10}$, $7x10^{10}$ and $8x10^{10}$ viable cell/ml, respectively. In PGPR treatments, 10 ml of either tested microorganisms suspension were added to the soil in each pot just after sowing.

Mycorrhizal inocula and inoculation techniques

Mycorrhizal inocula was including roots, hyphae, spores and growth media from a pot culture of onion plants colonization with *Glomus mosseae* NRC31 and *G. fasciculatum* NRC15 originally isolated from Egyptian soils and multiply on peat: vermicolite: perlit (Badr El-Din et al., 1999). The inoculum material consists of 295 spores g^{-1} oven dry bases plus the colonization roots pieces. Mycorrhizal inoculation was performed by sowing the seed above a thin layer of the mycorrhizal inoculum material at the time of sowing at rate of 10 g/pot.

Compost Application

Compost was applied during soil preparation at a rate of 10 ton fed^{-1}. The source of compost was obtained from Biogreen Company and some chemical properties of the compost are shown in Table (2).

Table 2. Some characteristics of the compost used

pH (1:10)	EC (1:10) (dSm^{-1})	O.M. %	O.C.	Ash (%)	C:N Ratio	
7.23	2.04	32.20	18.10	68.80	16:1	
Available micronutrients (mg kg^{-1})				Total macronutrients %		
Fe	Mn	Zn	Cu	N	P	K
81.75	34.56	25.43	8.55	1.26	0.42	1.20

Experimental Design and Treatments

The experiment was conducted in a randomized complete block design, each treatment consisted of six replicates, three replicates were collected at growth stage (elongation stage at 75 DAS) and the others replications at maturity stage (150 DAS). The experiment including two factors i.e., factor A, treatments of salinity which irrigate by five salinity levels: tap water as a control EC_{IW} = 0.43 dS m^{-1} (S0), diluted sea water with 4 dS m^{-1} (S1), 6 dS m^{-1} (S2), 8 dS m^{-1} (S3) and 10 dS m^{-1} (S4). Factor B: four bio- and organic amendments which including, control (T1), co-inoculation of PGPR and AMF (T2), compost (T3) and tripartite combination of co-inoculation with PGPR+AMF and compost (T4). Some chemical properties of the used irrigation water were illustrated in (Table 3), according to the methods described by Chapman and Pratt, 1978.

Table 3. Some chemical characteristics of the used irrigation water

Irrigation water	pH	EC (dSm^{-1})	Cations (meq l^{-1})				Anions (meq l^{-1})			
			Ca^{++}	Mg^{++}	K^{+}	Na^{+}	HCO_3^-	CO_3^-	SO_4^{--}	Cl^-
Tap water	7.21	0.43	1.10	0.45	0.31	2.44	0.21	--	1.23	2.85
Sea water	7.82	50.76	40.15	18.50	2.34	446.55	5.41	--	78.92	425.51

Experimental Procedure

A plastic pot with a capacity of 10 kg was filled of soil used. In addition to nitrogen fertilizers, phosphate and potassium fertilizers were included in this study in accordance with the fertilizer amounts recommended by the ministry of agriculture. Irrigation was conducted every 1-2 day throughout the growing season. Saline irrigation water will be prepared with diluted sea water to give salinity levels of (control (tap water), 4.0, 6.0, 8.0 and 10.0 dS m^{-1}). Ten wheat grains (*Triticum aestivum* L., var. *Gemmiza* 10) were sown in each pot, ten DAS, seedlings were thinned to 6 seedlings per pot and irrigated with equal volumes of tap water until 30 DAS. Starting from this date plants were irrigated with either tap water or differently diluted seawater. At 75 DAS, three replications were randomly selected from each treatment to study some growth parameters and nutrient concentrations in shoot. The others replications were used at maturity stage.

Data Recording

After 75 days from sowing growth characteristics (shoot height in cm, and the fresh and dry weights of the shoots (g plant^{-1})) were recorded. Shoot nutrient concentrations (N, P, K and Na) were determined following the methods described by Jackson (1973) and then K^+/Na^+ ratio was calculated. In order to determine the mean values of the yield at harvest time and its related parameters, (i.e., weight of 1000 seeds (g), grain and straw yield (ton ha^{-1}), and biological yield (ton ha^{-1}), as well as nutritive value of the yielded as nitrogen, phosphorus, and potassium content of grain and straw (Kg ha^{-1}). Harvest index (HI) was calculated as the ratio between grain and biological yields, and expressed as a percentage.

$$\text{Harvest index} = \frac{Grain\ yield\ (ton\ ha^{-1})}{Biological\ yield\ (ton\ ha^{-1})} \text{X } 100$$

Statistical Analyses

Separate RCBD analysis of obtained data for each season was performed. Combined analysis over seasons was conducted as indicated of normality and homogeneity tests. Normality according to the Shapiro–Wilk test (1965). However the homogeneity test based on homogeneity error variances of both seasons for each trait was performed according to Hartley's Fmaxtest (1950). The statistical analysis due to estimate the performance of mean treatments depend on significant of means of square according to ANOVA table at a significance level of ($p=0.05$) had been tested according to Duncan's multiple range test (Duncan 1955), where a different superscript letter means refer to a significant difference among the treatments.

Results

Vegetative Growth Parameters of Wheat Plants

The results presented in Table 4 show that, irrespective of the bio- and organic amendments treatments all growth parameters of the wheat plants at 75 DAS (shoot length, shoot fresh and dry weight) irrigated with different levels of diluted sea water (4.0, 6.0, 8.0 and 10.0 dS m^{-1}) decreased significantly at $p \leq 0.05$ relative to the control plants (irrigated with tap water (S0)). The magnitude of decrease in the shoot length was 4.3%, 9.91%, 21.59% and 37.08%, whereas it was 9.80%, 25.16%, 43.76% and 54.87%, in the shoot fresh weight and 12.00%, 30.32%, 51.48% and 63.78% in the shoot dry weight of wheat plants irrigated with S1, S2, S3 and S4, respectively, relative to the control plants (S0).

The results in Table 4 showed also that, co-inoculation of PGPR and AMF, and application of compost alone or in combination stimulated all the studied growth parameters at $p \leq 0.05$ compared with the control treatment (without addition), irrespective of irrigation water salinity level. The magnitude of increase in shoot length of plants co-inoculated with PGPR plus AMF (T2), addition with compost

(T3) and tripartite combination of co-inoculation with PGPR + AMF and compost (T4) compared to control plants (T1) was 10.28%, 6.48% and 14.71%, respectively, whereas this increase in shoot fresh weight was 23.86%, 12.19% and 33.28%, the magnitude of increase in shoot dry weight was 34.09%, 16.95 and 56.12, respectively compared to the control plants (untreated), irrespective of irrigation water salinity level.

The data of the interaction between (bio and\or organic) treatments and salinity levels showed that, all growth parameters of the plants co-inoculated with PGPR and AMF and\or application of compost increased significantly at $p \leq 0.05$ relative to their corresponding control plants under normal (S0) and salinity stressed conditions (S1, S2, S3 and S4). The maximum increase for all growth parameters was observed in the wheat plants treated with compost and co-inoculated with PGPR and AMF in combination, compared to those tested alone under all irrigation water salinity levels. The highest values of shoot length, shoot fresh and dry weight (61.65 cm, 34.25 and 11.76 mg pot^{-1}, respectively) were recorded with wheat plants irrigated with tap water (S0) and co-inoculation with PGPR and AMF with compost amendment in combination. However, the lowest values for the same parameters (32.74 cm, 10.82 and 2.53 mg pot^{-1}, respectively) were recorded with wheat plants irrigated with the highest irrigation water salinity level (10.0 dS m^{-1}) and untreated with any bio or organic amendment.

Table 4. Mean of wheat shoot height (cm), shoot fresh and dry weight (mg pot^{-1}) at 75 DAS irrigated with 5 different salinity levels as factor (A) by using 4 bio- and organic amendments as factor (B) and the effects of interaction among them (AB) over 2 seasons

Factor A	**Factor B**														
	Shoot height(cm)					Shoot fresh weight (mg pot^{-1})					Shoot dry weight (mg pot^{-1})				
	T1	T2	T3	T4	Mean A	T1	T2	T3	T4	Mean A	T1	T2	T3	T4	Mean A
S0	55.09	59.34	57.61	61.65	58.42	27.48	31.58	29.09	34.25	30.60	8.14	10.28	9.16	11.76	9.83
S1	53.10	56.67	55.45	58.42	55.91	24.58	28.01	26.64	31.17	27.60	7.10	9.10	8.19	10.21	8.65
S2	48.84	53.40	51.96	56.34	52.63	19.45	24.35	22.31	25.51	22.90	5.48	7.16	6.12	8.63	6.85
S3	41.79	47.26	45.13	49.06	45.81	13.24	18.75	16.33	20.50	17.21	3.31	5.05	4.12	6.60	4.77
S4	32.74	38.67	35.52	40.12	36.76	10.82	15.65	12.84	15.93	13.81	2.53	4.00	3.47	4.25	3.56
Mean B	46.31	51.07	49.13	53.12		19.11	23.67	21.44	25.47		5.31	7.12	6.21	8.29	
$LSD_{0.05}$ A		0.32				*$LSD_{0.05}$ A*		0.53			*$LSD_{0.05}$ A*		0.14		
$LSD_{0.05}$ B		0.28				*$LSD_{0.05}$ B*		0.47			*$LSD_{0.05}$ B*		0.12		
$LSD_{0.05}$ AB		0.63				*$LSD_{0.05}$ AB*		1.05			*$LSD_{0.05}$ AB*		0.28		

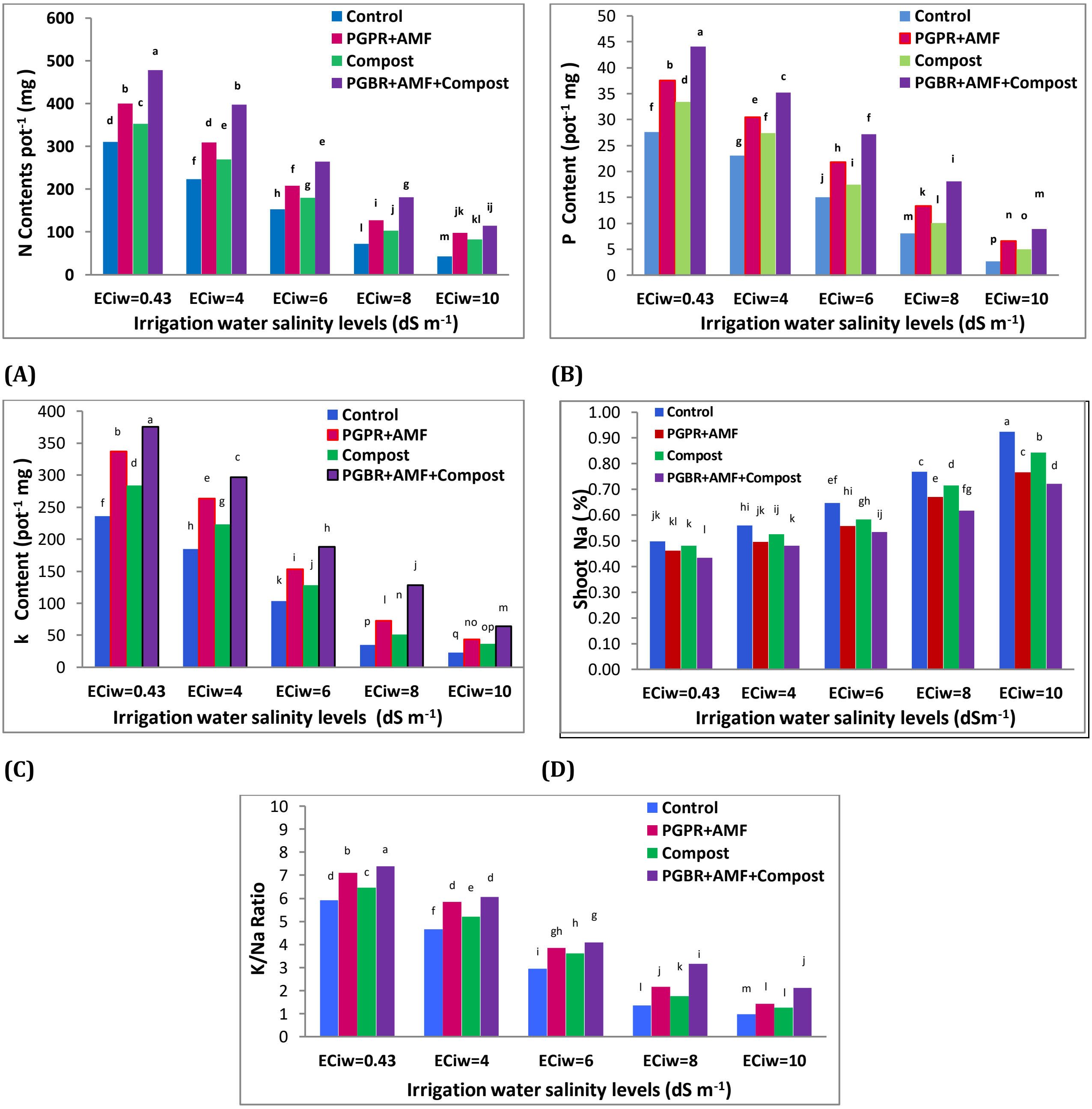
Data are displayed as mean (n = 3). Least Significant Difference (LSD) test at ($p \leq 0.05$). S0= tap water as a control EC_{IW} = 0.43 dS m^{-1}, S1=diluted sea water with 4 dS m^{-1}, S2=6 dS m^{-1}, S3=8 dS m^{-1} and S4=10 dS m^{-1}. T1=control, T2=co-inoculation of PGPR and AMF, T3=compost, T4=tripartite combination of PGPR+AMF+ compost.

Shoot Nutrient Contents of Wheat Plants

Results in Figure 1 showed that, increasing irrigation water salinity level significantly at $p \leq 0.05$ decreased shoot content of nitrogen, phosphorus and potassium at 75 DAS (Fig. 1 a, b, c, respectively). While the concentrations of Na^+ increased significantly *at* $p \leq 0.05$ (Fig. 1d), which reversely affected the K^+/Na^+ ratio compared to wheat plants irrigated with tap water (Fig. 1e). On the other hand, co-inoculation with PGPR and AMF and\or application of compost, were tested to improve nutrient status of wheat plants under normal (S0) and salinity stressed conditions (S1, S2, S3 and S4). The results showed that, nitrogen, phosphorus and potassium contents and the K^+/Na^+ ratio increased significantly at $p \leq 0.05$ in treated plants (T2, T3 and T4), while Na^+ concentration decreased significantly at $p \leq 0.05$ in shoot relative to untreated plants, irrespective of irrigation water salinity level.

The co-inoculated plants with PGPR and AMF, and those treated with compost in combination (T4) achieved the highest nitrogen, phosphorus and potassium contents and K^+/Na^+ ratios followed by the co- inoculated plants of PGPR and AMF (T2), then the plants treated with compost alone (T3) under all irrigation water salinity levels. In contrast, the plants treated with tripartite combination of co-inoculation with PGPR + AMF and compost application produced the lowest Na^+ concentration under all irrigation water salinity levels. Compared with the T1 treatment (untreated plants), the highest nitrogen, phosphorus and potassium contents and K^+/Na^+ ratios for the T4 treatment increased by 78.91%, 74.61%,81.08% and 43.85, respectively, while the Na concentration for T4 treatment decreased by 17.65%, irrespective of irrigation water salinity level. The triple combination of co-inoculation with PGPR + AMF and compost application was clearly superior in uptake the beneficial nutrients (nitrogen, phosphorus and potassium) and excluding the harmful one (Na^+).

The highest values of nitrogen, phosphorus and potassium content and also K^+/Na^+ ratio, were obtained with the plants irrigated with tap water (S0) and co-inoculation of PGPR with AMF and compost amendment together (T4), which were increased significantly at $p \leq 0.05$ compared to all other values of nitrogen, phosphorus and potassium contents and also K^+/Na^+ ratio. However, the highest value of Na% was obtained with the plants irrigated with the highest saline level (10 dS m^{-1}), and untreated (T1) which was increased significantly at $p \leq 0.05$ relative to all other values of Na%, resulting in significantly at $p \leq 0.05$ lower K^+/Na^+ ratio in the shoot. The content of nitrogen, phosphorus and potassium increased significantly at $p \leq 0.05$ in plants irrigated with 4 dSm^{-1} saline water (S1) and co-inoculation of PGPR with AMF and compost amendment in combination (T4) over untreated plants (T1) irrigated with tap water (S0). Similarly, under all other saline irrigation water levels, N, P and K content values obtained with the plants irrigated with both 6 dS m^{-1}, 8 dS m^{-1} and 10 dS m^{-1} and tripartite combination of co-inoculation with PGPR + AMF and compost (T4) increased significantly at $p \leq 0.05$ over values obtained with the untreated plants (control); irrigated with 4 dS m^{-1}, 6 dS m^{-1} and 8 dS m^{-1}, respectively (Figure 1).



(A)

(B)

(C)

(D)

(E)

Figure 1.The interaction effects of 5 different salinity levels (EC_{iw} = 0.43, 4, 6, 8, 10 dS m^{-1}) as factor (A) and 4 bio and organic amendments (control, co-inocul--ation of PGPR and AMF, compost, tripartite combination of PGPR+ AMF +compost) as factor (B) on shoot nutrient contents [A: Nitrogen (N) (mg pot^{-1}), B:Phosphorus (P) (mg pot^{-1}), C: Potassium (K) (mg pot^{-1}), D: Na% and E: the K^+\$Na^+$ratio] of wheat plants at 75 DAS over 2 seasons. The bars of treatment followed by the same letter are not significantly different according to an LSD test (p ≤ 0.05).

Yield and its Components of Wheat Plants

The results presented in Tables 5 & 6 show that, the mean values of the yield at harvest time and its related parameters, (i.e., weight of 1000 grains (g), grain and straw yield (ton ha^{-1}), and biological yield (ton ha^{-1}), as well as harvest index % (HI%) of wheat plants were decreased significantly at $p \leq 0.05$ by irrigation of different diluted sea water S1, S2, S3 and S4 relative to plant irrigated with tap water (S0). The magnitude of reduction in the weight of 1000 grains was 6.9%, 22.88%, 38.17% and 52.81%, whereas this reduction was 6.6%, 23.58%, 36.42% and 51.88%, in the grain yield, and 5.5%, 18.67%, 29.17 % and 39.67% in the straw yield of wheat plants irrigated with S1, S2, S3 and S4, respectively, relative to the control plants (S0) (Table 5).

The results also showed that, the yield of wheat plants and its related parameters treated with co-inoculation of PGPR with AMF and applied with compost singly or in combination under normal and salinity stressed conditions increased significantly (at $p \leq 0.05$) compared to the control plants. The most prominence treatment was co-inoculation with PGPR and AMF and compost application in combination (T4); it increased all yield attributes more than single treatments. The magnitude of increase in grain yield of plants co-inoculated with PGPR and AMF, addition with compost and tripartite combination of co-inoculation with PGPR + AMF and compost application compared to control plants (untreated) was 15.47%, 11.05% and 20.44%, respectively, whereas this increase in straw yield was 9.50%, 8.48% and 11.52%, and it was 11.94%, 7.55% and 15.59%, respectively, in biological yield compared to the control plants (untreated), irrespective of saline irrigation water level.

Wheat plants irrigated with tap water (S0) and tripartite combination of co-inoculation with PGPR + AMF and compost application (T4) gave the highest significant (at $p \leq 0.05$) increment in the grain yield (ton ha^{-1}), biological yield (ton ha^{-1}), and HI% when compared to all treatments, followed by plants irrigated with tap water (S0) and co-inoculated with PGPR and AMF (T2) and then plants irrigated with tap water (S0) and treated with compost alone (T3). While the lowest values for the same parameters were recorded with wheat plants irrigated with the highest salinity level (10.0 dS m^{-1}) and untreated. The obtained results indicate that the beneficial effects of tripartite combination of co-inoculation with PGPR + AMF and compost application are more pronounced for the grain yield (ton ha^{-1}), biological yield (ton ha^{-1}), and HI% in plants irrigated with the highest salinity level (10 dSm^{-1}) than those irrigated with tap water. The magnitude of increase in the grain yield (ton ha^{-1}), biological yield (ton ha^{-1}), and HI% resulting from tripartite combination (PGPR + AMF and compost) in non-stressed plants was 11.75%, 9.84%, and 1.67%, respectively, while it reached 35.41%, 22.02%, and 12.05%, respectively, in plants irrigated with the highest salinity level (10dSm^{-1}), over the respective controls.

Table 5. Mean of the weight of 1000 grain (g), grain and straw yield (ton ha^{-1}) irrigated with 5 different salinity levels as factor (A) by using 4 bio- and organic amendments as factor (B) and the effects of interaction among them (AB) over 2 seasons

Factor A	Factor B														
	Wt.1000 grain (g)					**Grain Yield (ton ha^{-1})**					**Straw Yield (ton ha^{-1})**				
	T1	T2	T3	T4	Mean A	T1	T2	T3	T4	Mean A	T1	T2	T3	T4	Mean A
S0	39.40	42.37	41.64	44.60	42.00	5.02	5.34	5.26	5.61	5.30	5.76	6.09	5.92	6.23	5.00
S1	36.36	39.92	37.99	42.14	39.10	4.66	5.08	4.87	5.22	4.95	5.36	5.76	5.58	5.98	5.67
S2	29.99	32.84	31.45	35.29	32.39	3.59	4.24	3.96	4.43	4.05	4.58	5.05	4.71	5.19	4.88
S3	23.32	26.74	25.52	28.31	25.97	2.75	3.55	3.44	3.74	3.37	3.92	4.38	4.25	4.45	4.25
S4	17.41	20.35	19.04	22.47	19.82	2.09	2.70	2.58	2.83	2.55	3.37	3.69	3.61	3.83	3.62
Mean B	29.30	32.44	31.13	34.56		3.62	4.18	4.02	4.36		4.60	4.99	4.81	5.13	
LSD $_{0.05}$ *A*	0.606					*LSD* $_{0.05}$ *A*	0.08				*LSD* $_{0.05}$ *A*	0.10			
LSD $_{0.05}$ *B*	0.542					*LSD* $_{0.05}$ *B*	0.07				*LSD* $_{0.05}$ *B*	0.09			
LSD $_{0.05}$ *AB*	ns					*LSD* $_{0.05}$ *AB*	0.16				*LSD* $_{0.05}$ *AB*	ns			

Data are displayed as mean (n = 3). Least Significant Difference (LSD) test at ($p \leq 0.05$). S0= tap water as a control EC_{IW} = 0.43 dS m^{-1}, S1=diluted sea water with 4 dS m^{-1}, S2=6 dS m^{-1}, S3=8 dS m^{-1} and S4=10 dS m^{-1}. T1=control, T2=co- inoculation of PGPR and AMF, T3=compost, T4=tripartite combination of PGPR + AMF + compost, ns = not significant

Table 6. Mean of Biological yield (ton ha^{-1}) and percentage of harvest index (HI%) of wheat plants irrigated with 5 different salinity levels as factor (A) by using 4 bio-and organic amendments as factor (B) and the effects of interaction among them (AB) over 2 seasons

Factor A	**Factor B**									
	Biological yield (ton ha^{-1})					**HI%**				
	T1	**T2**	**T3**	**T4**	**Mean A**	**T1**	**T2**	**T3**	**T4**	**Mean A**
S0	10.77	11.43	11.17	11.83	11.30	46.57	46.69	47.05	47.35	46.92
S1	10.01	10.84	10.44	11.19	10.62	46.49	46.85	46.57	46.63	46.63
S2	8.17	9.28	8.67	9.61	8.93	44.00	45.60	45.43	46.06	45.27
S3	6.66	7.93	7.69	8.19	7.62	41.23	44.76	44.70	45.64	44.08
S4	5.45	6.45	6.19	6.65	6.18	38.10	41.80	41.63	42.69	41.05
Mean B	8.21	9.19	8.83	9.49		43.28	45.14	45.07	45.67	
LSD $_{0.05}$ *A*	0.14					*LSD* $_{0.05}$ *A*		0.766		
LSD $_{0.05}$ *B*	0.12					*LSD* $_{0.05}$ *B*		0.685		
LSD $_{0.05}$ *AB*	0.27					*LSD* $_{0.05}$ *AB*		1.53		

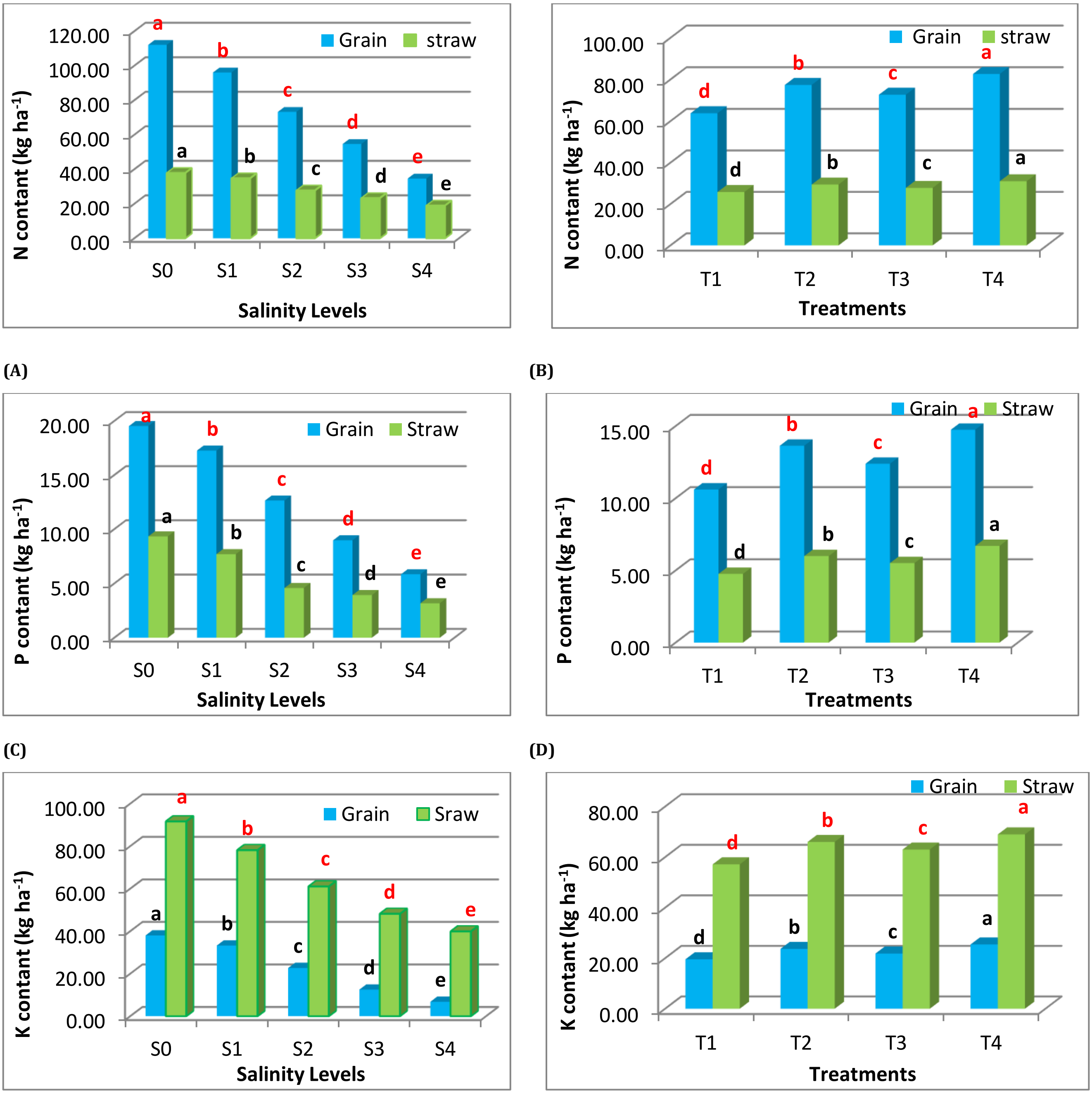
Data are displayed as mean (n = 3). Least Significant Difference (LSD) test at ($p \leq 0.05$). S0= tap water as a control EC_{IW} = 0.43 dS m^{-1}, S1=diluted sea water with 4 dS m^{-1}, S2=6 dS m^{-1}, S3=8 dS m^{-1} and S4=10 dS m^{-1}. T1=control, T2=co- inoculation of PGPR and AMF, T3=compost, T4=tripartite combination of PGPR+ AMF+ compost.

Nutrient Contents in Grain and Straw of Wheat Plants

The effect of saline condition on the content of N, P and K in grain and straw of wheat plants was shown in Figure 2. The results showed that, nitrogen, phosphorus and potassium contents in grain and straw were decreased (at $p \leq 0.05$) sharply under saline condition (plants irrigation of different diluted sea water S1, S2, S3 and S4) compared to the plant irrigated with tap water (S0), irrespective of the bio- and organic amendments treatment. The maximum reduction in N, P, and K contents in grain was 69.12, % 70.08% and 82.17% respectively, while it was 49.92%, 66.02 and 56.47 respectively, in straw of wheat plants irrigated with high salinity irrigation water (10 dSm^{-1}) compared to the plant irrigated with tap water (S0).

In general, the results showed also that, co-inoculation of PGPR with AMF and applied with compost singly or in combination under normal and salinity stressed conditions increased significantly (at $p \leq 0.05$) N, P and K uptake in both grain and straw of wheat plants relative to control plants (T1). All bio-and organic treatments single or in combination significantly improved the ability of plants to respond to the negative effects of salinity. The most apparent enhancing was for plants treated with tripartite combination of co-inoculation with PGPR + AMF and compost application (T4) which produced the highest N, P and K content in grain and straw under all saline irrigation water levels (Figure 2).

In addition, there was a significant (at $p \leq 0.05$) interaction of irrigation water salinity levels with treatments for N, P, and K contents in grain and P content in straw. It was observed that further increment for these traits, were detected when co-inoculation of PGPR and AMF were applied alongside with compost (T4) and irrigated with saline water (8 dSm^{-1}), which was on par with the untreated plants (control) irrigated with saline water (6 dSm^{-1}) as compared to their sole applications (Figure 2). Also, sole applications of either co-inoculation PGPR +AMF or compost application produced higher nutrient contents compared to their corresponding untreated (control) plants.



(A)

(B)

(C)

(D)

(E)

(F)

Figure 2. Main effects of salinity levels (A, C and E) and bio- and organic treatments (B, D and F) on nutrient contents [N (Kg ha^{-1}) (A & B), respectively; P (Kg ha^{-1}) (C & D), respectively and K (Kg ha^{-1}) (E& F), respectively] in grain and straw of wheat plants over 2 seasons. The bars of treatment followed by the same letter were not statistically different at $p \leq 0.05$, according to the least significance difference. S0= tap water as a control EC_{IW} = 0.43 dS m^{-1}, S1=diluted sea water with 4 dS m^{-1}, S2=6 dS m^{-1}, S3=8 dS m^{-1} and S4=10 dS m^{-1}. T1=control, T2=co- inoculation of PGPR and AMF, T3=compost, T4=tripartite combination of PGPR + AMF + compost.

Discussion

In arid and semiarid areas, salinity is one of the most important problems affecting agricultural production, which are caused by several factors (Liu, et al., 2020). Salt inhibits plant photosynthesis, protein synthesis and lipid metabolism, therefor salinity reduced crop production. Wheat is the most important cereal crops, particularly for human consumption (Giraldo et al., 2019). Salinity negatively affects the growth and productivity of wheat (EL Sabagh et al., 2021). In this study, wheat plants co-inoculated with PGPR and AMF and treated with compost alone or in combination were evaluated for their effects on the growth, nutrient status, and the yield of wheat plants irrigated with four saline water differ in salinity i.e., 4, 6, 8, 10 dSm^{-1} compared to wheat plants irrigated with non-saline water (tap water). This study reported that, the growth at 75 DAS and the yield components of wheat plants at maturity stage decreased with increasing salinity levels. These results were clearly observed in (tables 4, 5 &6), which agree with the results reported by (EL Sabagh et al., 2021), who observed that, salinity stress adversely affects the growth and development of wheat, which leads to a decrease in grain yield and quality. Salinity leads to osmotic stress and ion toxicity (Hasanuzzaman et al., 2013), which preventing cell division, cell magnification, and extension (Radi et al., 2013), and therefor decreased the growth and yield of crops. Feng et al., (2019) observed that, the grain yield reduced with increasing irrigation water salinity. In this respect, Kumar et al., 2017 observed that, grain yield of wheat plants decreased when salinity of irrigation water increased over 3 dSm^{-1}. They also reported that, this reduction in grain yield may be resulted from the adverse effect of salinity on yield componentswhich also affected by increasing salinity. Results also reported that grain yield was decreased as a result of reducing the weight of 1000 grain (Table 5), which supports the findings of Dikgwatlhe et al., (2008).

The low nutrient ion activity of saline soil due to excessive ratios of Na^+/K^+ and Cl^-/NO_3^- in the soil solution, affect nutrients absorption and therefore plant growth)Bidalia et al., 2019(. The present study, reported that the content of N, P and K^+ and K^+/Na^+ ratio decreased while Na% increased under salinity stress in shoot of wheat plants at 75 DAS (Figure 1). Also at maturity the contents of nitrogen, phosphorus and potassium in grain and straw decreased with increasing irrigation water salinity level (figure 2). Which mean that salinity stressaffecting plant nutrient acquisition and therefore plant growth and yield (Tables 4, 5&6). Wallace and Berry (1981) reported that wheat yield reduction under salinitystress may be caused by the shortage of NO_3^- due to increase the external Cl^-. Day et al., 2021 reported that, under stress conditions phosphorus become unavailable for plants, since phosphate ions precipitate with Ca^{2+}, Mg^{2+}, and Zn^{2+} ions. Moreover, salinity stress causes ion-specific effects, since an increase in the accumulation of Na^+ and Cl^- in the cells, which may be decrease the uptake of K^+ and increase Na^+/K^+ ratio, which is characteristic of Na-induced toxicity (Maathuis and Amtmann, 1999(. This could be due to the competition between the identically-charged ions, Na^+ and K^+, through nutrient absorption at the absorptive sites of plant roots (Bidalia, et al.. 2019), the ionic selectivity of cell membranes (Dikgwatlhem et al., 2008) and photosynthetic activity (Radi, et al., 2013), which resulted in ionic imbalance (Arif et al., 2020).

In the present study, co-inoculation of PGPR and AMF, and compost application alone or in combination (tripartite combination of PGPR, AMF and compost) enhanced the studied growth parameters, i.e., plant height; fresh and dry weights of shoots at 75 DAS (Table 4) and the yield and its related parameters, i.e., weight of 1000 grains (g), grain and straw yield (ton ha^{-1}) (Table 5), and biological yield (ton ha^{-1}), as well as harvest index% (HI%) of both stressed and unstressed wheat plants (Table 6). The results reported that the positive effects of either single or combined treatment are more pronounced for the growth and yield in wheat plants irrigated with saline water than those irrigated with tap water. The best results were obtained when bio-and organic amendments were treated in the tripartite combination (PGPR, AMF and compost), followed by the dual combination (co-inoculation of PGPR+AMF), and then compost application only, whether it is under salt stress or normal conditions. The co-inoculation of PGPRand AMF were tested in this study for their alleviation adverse effects of salinity on wheat plants. Results showed that co-inoculation of PGPR and AMF alleviated salt stress by stimulated growth (Table 4), yield and its component and HI% of wheat plants (Tables 5&6). Nadeem et al. (2014) demonstrated that co-inoculation of AMF and PGPR enhanced the growth of several plants such as tomato, alfalfa, subterranean clover, mung bean, chick pea, and Apple, by the incorporation of various mechanisms that stimulate changes in plant physiology and promote endurance under stress (Hidri et al., 2016).

Several studies have shown that salt-adapted PGPR (Gupta et al., 2020) and AMF (Li et al., 2020) enhanced plant growth and yield under salt stress through adjust the endogenous hormonal state of the plant. Synthesis plant growth hormones and various regulators that promote plant growth such as exopolysaccharides (EPS), indole acetic acid (IAA), cytokinin, and abscisic acid (ABA) by PGPR (Del Orozco-Mosqueda et al., 2020) and also by AMF (Li et al., 2020). Furthermore, AMF boost significant changes in more abundance of organic solutes by adjustment the composition of carbohydrates and enhancing accumulation of specific osmolytes such as proline, which helping osmotic adjustment (Pons et al., 2020). AMF enhancing the water absorption capacity of plants by the network expansion of extraradical hyphae in the soil, thereby absorbs more water and transport it inside the cells of plant and maintaining the cellular osmoregulation (Al-Arjani et al., 2020).

Results demonstrated that, the dual combination of PGPR and AMF has a great ability to decreased the adverse impacts of salinity on wheat growth and production (tables 4, 5&6) by improving nutrient status of shoot (figure 1) and of grain and straw (figure 2). The efficiency of co-inoculation with PGPR and AMF could be resulted from the ability of PGPR to dissolve phosphates and the efficient absorption of dissolved phosphorus from soil by AMF hyphae (Saia et al., 2020). The mycorrhizal fungi enhanced phosphorus uptake, which promote plant growth under salt stress (Hidri et al., 2019), increase the uptake of K^+ (Chen et al., 2017), and decreased Na^+ root-to-shoot distribution that, lower Na^+/K^+ ratio in plants (Porcel et al., 2016). In addition, ionic equilibrium and enhance nutrients content (Chen et al., 2017). The improving of salinity resistance has been reported in several AMF-plant symbiosis i.e. wheat, alfalfa, maize, and tomato (Hashem et al., 2018). AMF and PGPR association allows better colonization of the plant by AMF through these PGPR, which are well known as Mycorrhiza Helper Bacteria (Younesi and Moradi, 2014).This enhanced colonization leads to better exploration of the soil by the AMF hyphae and therefore, better uptake of nutrients (Smith and Read, 2008). Various studies have reported that AMF and PGPB enhanced the uptake of nutrients, particularly P, K, and Zn (Yu et al., 2012; Ashrafi et al., 2014).

Compost amendment was evaluated in this study if it could be a sustainable strategy to promote the growth and yield of crops under both non-saline and saline irrigation water. The results observed that, compost improve the growth (table 4), yield (tables 5&6) and nutrient status of wheat plants (figures 1&2) under both normal and salt stress conditions relative to the corresponding controls. While, the results also reported that the addition of compost alone achieved the lowest enhancement under normal and stress conditions and the other dual or tripartite combinations was more effectively. That may be due to PGPR which used in this study are highly efficient strains of salt-adapted plant growth promoting rhizobacteria. Therefor tripartite combination of compost and co-inoculation with PGPR and AMF was the most efficient combination for enhancing the growth and yield of wheat plants under both saline and non-saline irrigation water. Various studies have reported the combined synergistic effect of compost, and PGPR in alleviate salinity and drought stress of wheat plants (Kanwal et al., 2017; Yaseen et al., 2020). Similarly, various studies have reported that the dual or triple combination of PGPR, AMF, and compost has a strong ability to mitigate the adverse effects of salinity stress on plant, by enhancing photosynthesis and water status (Bharti et al., 2016: Toubali et al., 2020). They also demonstrated that, in the interactions between plants and AMF, PGPR and compost, benefit each other from this mutualistic association. The synergistic effect of AMF, PGPR and compost may be due to that compost acts as both carbon sources and suitable environment for PGPR and AMF to boost their growth promotion efficiency (Ullah et al., 2021). Consequently, this leads to enhance compost mineralization into the soil by these microorganisms. Therefore, providing a sustained release of available nutrients from compost which the hyphae of AMF increase, the ability of plants to absorb it (Chen et al., 2017).

In this respect, results also showed that plants treated with tripartite combination of co-inoculation with PGPR + AMF and compost application showed a better performance of the ability to mitigate the adverse impacts of salinity, which produced the highest N, P and K content and K^+/Na^+ ratio and the lowest Na^+ concentration in shoot (figure 1) and produced the highest content of nitrogen, phosphorus and potassium in grain and straw (figure 2) relative to untreated plants and other treatments under both stress or non-stress conditions. This enhancement may be resulted from a combination of different mechanisms due to the synergetic interactions among PGPR and AMF (Nadeem et al., 2014; Raklami et al., 2019), while compost are helpful for promoting the activities of both populations (Yang et al., 2018; Yu et al., 2019). El- Hamahmy et al., (2014) observed that, grain and straw yield beside crude protein and total carbohydrates content were increased when used (*Azospirillum lipoferum* + *Azotobacter chroococcum* + *Bacillus polymyxa* + Compost + compost tea) than control and other treatments could be attributed to the role of N_2- fixers and organic matter amendments in improving plant growth through higher uptake of water and nutrients from soil which decreased the negative effects of salt and thereby enhancing plant yield. The keeping of enough K^+ in the tissue of plants under salt stress might be due to the selective K^+ uptake, the cellular compartmentation of K^+ and Na^+, and distribution in the shoots (Munns 2002). Also, Silini et al., (2012) observed that, inoculation with N_2-fixers + compost extracts enhanced potassium accumulation and decreased Na^+ and consequently increased the uptake of nutrients especially, N, P, and K and finally the microorganisms can produce substances that promote growth like exopolysaccharied that could bind some cations including Na^+.

Conclusions

This study assessed the adverse effects of saline irrigation water on the growth and yield of wheat plants. Co-inoculation of PGPR with AMF and compost application was tested individually or in combination for their ability to mitigate the negative impacts of salinity. Results reported that positively interacting PGPR + AMF combination is an efficient and cost-effective bio-stimulant to improve the tolerance of wheat plants to salinity. In addition, the tripartite combination of compost and co-inoculation of PGPR with AMF has shown a stimulating effect, for improving the growth and yield of wheat plants either with saline and non-saline irrigation water. This may represent an evolving sustainability strategy to alleviate salinity stress with the possibility of using alternative irrigation in wheat plants and save fresh water resources. Thus, the need for more research has become necessary to test whether organic and bio- amendments can be used as a general strategy to enhance the salinity resistance of other crops.

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