Env. Biodiv. Soil Security **Vol. 4** pp. 225-238 (2020)

Environment, Biodiversity & Soil Security (EBSS)

http://jenvbs.journals.ekb.eg//

Impact of Bio-Inoculation with *Bradyrhizobium spp.* on Yield and Seed Quality of Forage Cowpea (*Vigna unguiculata* L.) under Soil Salinity Conditions

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A STUDY was carried out to assess the effect of single and/or combined inoculation with *Bradyrhizobium* spp. isolates on seed yield and quality of forage cowpea (*Vigna unguiculata* cv. Baladi) under salinity-affected soil conditions. The experiment was conducted in a split plot design for two growing seasons, 2018 and 2019, with two main plot treatments of irrigation water (normal and saline) and four sub-plot inoculation treatments. Results revealed that sub-main plot treatments differed significantly for number of branches, number of pods, pod length, number of seeds pod^{-1}, pod weight plot^{-1} (Kg) and seed yield (Kg fed^{-1}) of forage cowpea plants as affected by the type of irrigation water in the two growing seasons. Also, combination treatment (*Bradyrhizobium* SARS-Rh3 and *Bradyrhizobium* SARS-Rh5) significantly minimized the negative effects of saline irrigation water treatment for seed content of elements (N, P, K, Zn, Mn, Fe and Cu). In the same context, inoculation treatments followed the descending order of SARS-Rh3 + SARS-Rh5>SARS-Rh5>SARS-Rh3 > control for total carbohydrate and protein % of cowpea seeds during 2018 and 2019 seasons. For assessment of seed quality viz., seed germination (%), seedling length (cm), seedling weight (g), vigour index I and vigour index II were higher in 2019 than 2018 season. Similar finding was observed in economic evaluation for SARS-Rh3+SARS-Rh5 treatment which enhanced profitability of cowpea during the two growing seasons.

Key words: Cowpea, Salinity-affected soil, *Bradyrhizobium* spp., Seed mineral content, Seed quality

Introduction

Cowpea (*Vigna unguiculata* L.) is one of the most important legume crops which widely cultivated in semi-arid tropical regions reached 14.5 million hectares, with a total grain production of 6.2 million tons, worldwide (Kebede and Bekeko, 2020). On the other hand, cowpea can be used as food, forage and green manure as well as a cheap source of protein and a rich source of folic acid (Timko and Singh 2008 and Witthoft et al. 2016). Moreover, cowpea can contribute to improving soil fertility and sustainability of cropping systems (Bell et al. 2017 and Abd-elgwad 2019).

Recently, about one-third of the world's soils is not in use which due to salinity stress. This problem results in the degradation of 10 million hectares annually (Giri and Mukerji, 2004), and this is reflected in reduced plant growth and crop yield by reducing the water potential in the rhizosphere zone, nutrient imbalance and phytotoxicity of ions (Tester and Davenport, 2003 and El-Ramady et al. 2019). In this regard, it is necessary to apply different biotechnological approaches to reduce the harmful effect of this problem through genetic techniques, breeding crops for salinity tolerance and biological products

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Received 18/08/2020; Accepted 6/09/2020
DOI: 10.21608/jenvbs.2020.39713.1102

(El-Ramady et al. 2018; Leal et al. 2020 and Farid et al. 2020). Among of which, biological products play an important role to ameliorate salt stress by N_2 fixation, phytohormone, antibiotic, ammonia and siderophore production as well as phosphate solubilization (Kumar et al. 2019; Khan et al. 2020). Moreover, associated with plant roots leading to increase germination, leaf area, chlorophyll, protein, nutrients and yield (Habib et al. 2016 and El-Nahrawy and Omara 2017), and upon this association with roots, beneficial microorganisms divided to: (1) extracellular where the interaction occurs between the cells and the root cortex (rhizoplane), *e.g. Bacillus* spp., *Pseudomonas* spp., *Azospirillum* spp., *Azotobacter* spp., etc., (2) intracellular where the interaction occurs inside the root resulting root nodules e.g. *Rhizobium* spp., *Bradyrhizobium* spp., *Allorhizobium* spp., *Azorhizobium* spp., *Sinorhizobium* spp., *Mesorhizobium* spp., etc. (Bhattacharyya and Jha 2012 and El-Akhdar et al. 2019), and in both cases, these microorganisms have several mechanisms that allow to grow and survival in saline environments such as: (1)specific composition of membrane; (2) adaptation of proteins and enzymes; (3) regulation of intracellular ionic concentration; (4) increasing the energetic capacity; (5) accumulation of compatible solutes *e.g.* sucrose, glycosyl and glyceroletc. and (6) production of extracellular polysaccharides (Ruppel et al. 2013 and Sandhya et al. 2010).

Therefore, several studies have demonstrated these beneficial microorganisms on alleviating salinity stress in different crops.Ullah and Bano (2015) showed that maize plants inoculated with *Bacillus* sp. and *Arthrobacter pascens* enhanced shoot, root length and shoot, root fresh as well as dry mass under induced salinity stress. These results due to accumulation of osmolytes, like sugar, proline and the elevation of antioxidant enzymes activity. Also, inoculation of *Camelina sativa* (camelina) plants with halotolerant *Pseudomonas putida* UW4 and *P. migulae* 8R6 led to increase seed production by approximately 30–50% under moderately saline conditions which due to production of ACC deaminase (Heydarian et al. 2016). Dakora and Belane (2019) showed that different genotypes of forage cowpea can accumulate significantly high values of protein and trace elements (Fe, Mn and Zn) in leaves and seeds, when inoculated with N_2-fixers microorganisms.

The present study was to evaluate the impact of single and/or dual inoculation with *Bradyrhizobium* spp. isolates on seed yield and seed quality of forage cowpea under salinity-affected soil conditions.

Materials and Methods

Field experiment

Field experiments were undertaken during the summer seasons of 2018 and 2019 in the experimental Research Farm of Sakha Agric. Res. Station, Kafr El-Sheikh, Egypt. The location stands at 31° 05' 20.43" N and 30° 56' 9.29" E at an elevation of 6 m above mean sea level.The present investigation aimed to study the effect of single and/or dual inoculation with *Bradyrhizobium* spp. isolates on seed yield and seed quality of forage cowpea under salinity-affected soil conditions.These bacteria (halo-tolerant *Bradyrhizobium* spp. isolates SARS-Rh3 and SARS-Rh5) were previously selected by (Omara and El-Gaafarey, 2018).

The experiment was conducted as split plot with three replicates during the both seasons. Irrigation treatments were considered as main-plots and inoculation treatments as sub-plots. Main-plots were irrigation water type (normal and well water at a depth of 20 m.), but sub-plots were T1: control (uninoculated), T2: inoculated with SARS-Rh3 isolate, T3: inoculated with SARS-Rh5 isolate, and T4: dual inoculation with SARS-Rh3 and SARS-Rh5 isolates (1:1). The physicochemical and biological properties of soil used and irrigation water are shown in Table 1.

Forage cowpea seeds (*Vigna unguiculata* cv. Baladi) originated in Forage Research Department, Field Crops Research Institute, Agricultural Research Centre, Giza, Egypt, were sown at the rate of 3 seeds/ hole with 15 cm space and a seeding rate 30 kg Fed^{-1} on June10th during 2018 and June 15th during 2019. Each plot (12 m^2) consisted of 5 ridges, 4 m length and 60 cm apart and separated by 1.5 m unplanted distances. During soil tillage, phosphorus (15.5% P_2O_5) at a rate of 150 Kg fed^{-1} and potassium (48% K_2O) at a rate of 50 Kg fed^{-1} were broadcasted. Ammonium nitrate (33.5% N) as a source of nitrogen fertilizer was added at a rate of 60 Kg fed^{-1} in one dose (1/3) before the first irrigation for inoculated treatments and two equal doses before the first and second irrigation for control treatments. All fertilizers treatments was applied according to the recommendation of Ministry of Agriculture and Land Reclamation. The inoculation treatments were prepared and applied according to Omara and El-Gaafarey (2018).

TABLE 1. Some physicochemical and biological properties of soil and irrigation water used

Soil properties

				Cations (meq L^{-1})				Anions (meq L^{-1})			
Season	**O.M (%)**	**EC (dS m^{-1})**	**pH**	**Na^+**	**K^+**	**Mg^{++}**	**Ca^{++}**	**Cl^-**	**CO_3^-**	**HCo_3^-**	**So_4^{--}**
2018	1.15	9.24	8.15	15.6	0.40	1.30	5.10	17.06	0.0	4.21	1.13
2019	1.19	9.29	8.18	16.0	0.44	1.33	5.18	17.18	0.0	4.20	1.57

Water irrigation properties

Normal irrigation Water

Season	**pH**	**EC (dS m^{-1})**	**SAR**	meq L^{-1}				mg L^{-1}			
				Na^+	**Cl^-**	**SO_4^{--}**	**NH_4^+**	**COD**	**BOD**	**SS**	**DS**
2018	7.28	0.59	1.47	2.01	3.65	0.18	1.75	10.87	6.12	181	354
2019	7.22	0.54	1.44	1.98	3.58	0.19	1.84	10.97	6.46	189	369

Saline irrigation Water

Season	**pH**	**EC (dS m^{-1})**	**SAR**	meq L^{-1}				mg L^{-1}			
				Na^+	**Cl^-**	**SO_4^{--}**	**NH_4^+**	**COD**	**BOD**	**SS**	**DS**
2018	8.34	2.43	7.34	16.2	10.9	7.83	2.22	0.00	0.00	17	2710
2019	8.47	2.48	7.41	16.7	11.1	7.90	2.30	0.00	0.00	19	2796

COD: Chemical Oxygen Demand; **BOD**: Biological Oxygen Demand; **SS**: Suspended solids; **DS**: Dissolved solids.

Measurements and analyses

Yield and yield attributing characteristics

At maturity stage, number of branches plant^{-1}, number of pods plant^{-1}, pod length (cm), number of seeds pod^{-1}, pod weight plot^{-1} (Kg) and seed yield (Kg fed^{-1}) were determined.

Seed mineral content

According to Black et al. (1965), nitrogen, phosphorus and potassium content (%) were determined. On the other hand, micronutrients Zn, Mn, Fe and Cu content (mg kg^{-1}), were determined using atomic adsorption spectrophotometer (Perkin Elmer 3300) according to (Cottenie et al. 1982). From dry finely ground cowpea seeds, total carbohydrate contents were extracted according to (Herbert et al. 1971) and estimated colourimetrically by (Montogomery 1961). Also, protein content was calculated as total N × 6.25 (Allen, 1953).

Assessment of seed quality

For seed quality assessment germination test was conducted by rinsed ten seeds of cowpea from each treatment in 70% (v/v) ethanol and surface sterilized with sodium hypochlorite (5% w/v) for 3 min then rinsed the seeds 3 times with sterile distilled water. In 9-cm Petri dish, seeds were distributed on sterile filter papers containing 10 mL saline solution (0.85% NaCl) with 5 replicates per each treatment. Seed germination (%), seedling Length (cm) and seedling dry weight (g) were measured after incubation at 25 °C for 10 days (Belimov et al., 2005). Vigour indices were computed as suggested by Abdul Baki and Anderson (1973) as follows:

Vigour Index I = Germination (%) X Seedling length (cm)

Vigour Index II = Germination (%) X Seedling dry weight (g)

Economics of cowpea seed production

On the basis of market prices and inputs used in cultivation, all different costs was calculated according to Egyptian local market price (LE) during 2018 and 2019 seasons. Total seasonal return was determined by multiplying the produce from different treatments with the Egyptian local market price of cowpea seed. Also, net seasonal return was calculated by deducting total seasonal return from the costs of cultivation. The benefit cost ratio was calculated by dividing the total seasonal return with the costs of cultivation.

Data analyses

Treatment means were compared by using (MSTAT–C, 1986), according to Duncan's multiple range test (Duncan, 1955).

Results and Discussion

Yield and yield attributing characteristics

In general, yield and yield attributing characteristics viz., number of branches plant^{-1}, number of pods plant^{-1}, pod length (cm), number of seeds pod^{-1}, pod weight plot^{-1} (Kg) and seed yield (Kg fed^{-1}) was higher in 2019 than 2018 season (Tables 2 & 3).

Number of branches, number of pods and pod length

Among main plot treatments of irrigation type, analysis of variance shows that application of normal irrigation water (NIW) significantly increased number of branches plant^{-1}, number of pods plant^{-1} and pod length (cm) in both seasons compared to saline irrigation water (SIW),(Table 2). Sub plot of inoculation treatments viz., seed inoculation alone (SARS-Rh3 and SARS-Rh5) and in combination with SARS-Rh3 + SARS-Rh5 differed significantly for the above-mentioned parameters. The highest results was observed at the dual inoculation treatment which was 3.78 and 3.88, for number of branches plant^{-1}, 8.20 and 9.20 for number of pods plant^{-1}, 17.50 and 19.50 cm for pod length compared to control treatment which was 1.88 and 1.98, for number of branches plant^{-1}, 4.73 and 5.73 for number of pods plant^{-1}, 12.91 and 14.88 cm for pod length during 2018 and 2019 seasons, respectively (Table 2).

For the interaction effect between the main plot and sub main plot treatments, data showed that an increase in number of branches plant^{-1}, number of pods plant^{-1} and pod length (cm) were observed with inoculation treatment (SARS-Rh3 + SARS-Rh5) resulted in attaining 5.20, 11.00 and 22.66 cm under normal irrigation water conditions, and 2.36, 5.40 and 12.33 cm under saline irrigation water conditions during 2018 season, respectively. Similar trend was observed during 2019 season (Table 2).

The increases in number of branches, number of pods and pod length as a result of inoculation treatments under different types of irrigation water may be attributed to enhance cell division,

TABLE 2. Effect of inoculation treatments and type of irrigation water on number of branches plant^{-1}, number of pods plant^{-1} and pod length (cm) of forage cowpea plants during 2018 and 2019 seasons

Treatments	N. branches plant^{-1}		N. pods plant^{-1}		Pod length (cm)	
	2018	2019	2018	2019	2018	2019
Irrigation Type						
NIW	4.02 a	4.12 a	8.66 a	9.66 a	20.12 a	22.12 a
SIW	1.77 b	1.87 b	4.20 b	5.20 b	10.41 b	12.41 b
LSD 0.05	0.28	0.26	0.49	0.45	0.65	0.63
Inoculation treatments						
Control	1.88 d	1.98 d	4.73 d	5.73 d	12.91 d	14.88 d
SARS-Rh3	2.68 c	2.78 c	5.93 c	6.93 c	14.66 c	16.66 c
SARS-Rh5	3.25 b	3.35 b	6.86 b	7.86 b	16.00 b	18.00 b
SARS-Rh3 + SARS-Rh5	3.78 a	3.88 a	8.20 a	9.20 a	17.50 a	19.50 a
LSD 0.05	0.40	0.38	0.70	0.67	0.92	0.90
Interaction						
Control NIW	2.63 d	2.70 d	6.33 c	7.29 c	17.50	19.50
SARS-Rh3 NIW	3.70 c	3.81 c	8.33 b	9.30 b	19.33	21.33
SARS-Rh5 NIW	4.56 b	4.66 b	9.00 b	10.03 b	21.00	23.00
SARS-Rh3 + SARS-Rh5 NIW	5.20 a	5.30 a	11.00 a	12.07 a	22.66	24.66
Control SIW	1.13 g	1.22 g	3.13 e	4.10 e	8.33	10.33
SARS-Rh3 SIW	1.66 fg	1.74 fg	3.53 e	4.51 e	10.00	12.00
SARS-Rh5 SIW	1.93 ef	2.03 ef	4.73 d	5.67 d	11.00	13.00
SARS-Rh3 + SARS-Rh5 SIW	2.36 de	2.44 de	5.40 cd	6.37 cd	12.33	14.33
LSD 0.05	0.55	0.52	0.96	0.93	ns	ns

NIW: Normal Irrigation Water, SIW: Saline Irrigation Water. Means in the same column followed by the same letter are not significantly different according to Duncan's test at 0.05 level.

meristematic activity and increasing the vegetative growth viz., leaf initiation, photosynthesis process and chlorophyll concentration. Also, cowpea plants can be increase the size of sap vacuoles under inoculation treatments due to accumulated a lot of water and minerals (Munns, 2002). In the same context, several studies showed that relationship between the symbiosis root-nodule bacteria and various legumes species under salinized soil conditions. Meng et al. (2016), showed that soybean plants grown under salt soils and inoculated by *B. japonicum* had improved morphological and physiological characters. On the contrary, the absence of inoculation treatments, salinity significantly reduced physiological parameters of alfalfa plants (Latrach et al. 2014).

Yield components

The results given in Table 3, showed there was significant increases in number of seeds pod^{-1}, pod weight plot^{-1} (Kg) and seed yield (Kg fed^{-1}) of forage cowpea plants as affected by type of irrigation water and inoculation treatments in the two growing seasons.At different inoculation treatments, results showed that highly significant effect of number of seeds recorded 14.66 and 16.06 pod^{-1} for inoculation treatment with SARS-Rh3 + SARS-Rh5 followed by inoculation treatment with SARS-Rh5 recorded 14.00 and 15.40 pod^{-1} followed by inoculation treatment with SARS-Rh3 recorded 12.83 and 14.23 pod^{-1} during 2018 and 2019 seasons, respectively. Similar findings were attained in pod weight plot^{-1} (Kg) and seed yield (Kg fed^{-1}). Also, the positive results was observed in the interaction effect which caused by dual inoculation treatment (*Bradyrhizobium* SARS-Rh3 + *Bradyrhizobium* SARS-Rh5) on number of seeds pod^{-1}, pod weight plot^{-1} (Kg) and seed yield (Kg fed^{-1}) which was 21.66 pod^{-1}, 5.76 Kg plot^{-1} and 1015.00 Kg fed^{-1}in 2018 season and 23.06 pod^{-1},5.85 Kg plot^{-1} and 1037.00 Kg fed^{-1} in 2019 season under normal irrigation water conditions, respectively (Table 3). On the other hand, under saline irrigation water conditions, number of seeds attained 7.66 and 9.06 pod^{-1},pod weight attained 3.23 and 3.31Kg plot^{-1} and seed yield attained 793.33 and 815.33 Kg fed^{-1} during 2018 and 2019 seasons as affected by combination inoculation treatment, respectively (Table 3).

TABLE 3. Effect of inoculation treatments and type of irrigation water on number of seeds pod^{-1}, pod weight plot^{-1} (Kg) and seed yield (Kg fed^{-1}) of forage cowpea plants during 2018 and 2019 seasons

Treatments	N. seeds pod^{-1}		Pod Weight (kg plot^{-1})		Seed yield (Kg fed^{-1})	
	2018	2019	2018	2019	2018	2019
Irrigation Type						
NIW	19.58 a	20.98 a	4.31 a	4.41 a	740.83 a	762.83 a
SIW	6.66 b	8.06 b	2.33 b	2.43 b	562.58 b	584.58 b
LSD 0.05	0.58	0.55	0.19	0.17	40.65	40.01
Inoculation treatments						
Control	11.00 c	12.40 c	2.28 d	2.36 d	477.66 d	499.66 d
SARS-Rh3	12.83 b	14.23 b	2.85 c	2.96 c	554.16 c	576.16 c
SARS-Rh5	14.00 a	15.40 a	3.66 b	3.75 b	670.83 b	692.83 b
SARS-Rh3 + SARS-Rh5	14.66 a	16.06 a	4.50 a	4.59 a	904.16 a	926.16 a
LSD 0.05	0.83	0.80	0.27	0.27	57.49	55.69
Interaction						
Control NIW	16.66 c	18.06 c	3.13 d	3.22 d	595.00 d	617.00 d
SARS-Rh3 NIW	19.33 b	20.73 b	3.63 c	3.71 c	653.33 cd	675.33 cd
SARS-Rh5 NIW	20.66 a	22.06 a	4.73 b	4.80 b	700.00 c	722.00 c
SARS-Rh3 + SARS-Rh5 NIW	21.66 a	23.06 a	5.76 a	5.85 a	1015.00 a	1037.00 a
Control SIW	5.33 f	6.73 f	1.43 g	1.51 g	360.33 f	382.33 f
SARS-Rh3 SIW	6.33 ef	7.73 ef	2.06 f	2.18 f	455.00 e	477.00 e
SARS-Rh5 SIW	7.33 de	8.73 de	2.60 e	2.73 e	641.66 cd	663.66 cd
SARS-Rh3 + SARS-Rh5 SIW	7.66 d	9.06 d	3.23 d	3.31 d	793.33 b	815.33 b
LSD 0.05	1.13	1.08	0.37	0.38	78.24	77.22

NIW: Normal Irrigation Water, SIW: Saline Irrigation Water. Means in the same column followed by the same letter are not significantly different according to Duncan's test at 0.05 level.

Overall, single and dual inoculation treatments led to a significant increase in number of seeds, pod weight and seed yield which due to different hormonal functions in the plant tissues and it is responsible for improvement pollen germination and pollen tube growth. Indeed, these results corroborate those of Senthilkumar and Sivagurunathan (2013) showed higher number of seeds in cowpea plants by dual inoculation of *Rhizobium*, Phosphobacteria and *Azospirillum*. Also, Oliveira et al. (2017) reported that cowpea plants inoculated with *Bradyrhizobium elkanii* and *B. elkanii* + *Rhizophagus irregularis* resulted in seed-yield enhancement by 45% and 42%, under drought stress, respectively. Moreover, Htwe et al. (2019) found that the seed yield of mung bean, cowpea and soybean plants significantly enhancement with the application of biofertilizer produced from *Bradyrhizobium* and *Streptomyces griseoflavus* P4.

Seed mineral content

The inoculation of forage cowpea plants with *Bradyrhizobium* SARS-Rh3 and *Bradyrhizobium* SARS-Rh5 and their mixture under normal irrigation water treatments lead to increases in macro and micro elements over saline irrigation water treatments (main plots) Table 4. In this context, the combination treatment (*Bradyrhizobium* SARS-Rh3 and *Bradyrhizobium* SARS-Rh5) significantly minimized the negative effects of saline irrigation water treatment for seed content of elements (N, P, K, Zn, Mn, Fe and Cu). For sub main plots, N % increased to 2.56 and 2.82 % with the SARS-Rh3 treatment, 2.98 and 3.24 % with the SARS-Rh5 treatment, 3.63 and 3.89 % with the combination treatment for 2018 and 2019 seasons, respectively, compared to control treatment. Furthermore, P % increased to 0.36 and 0.45 % with the SARS-Rh3 treatment, 0.42 and 0.51 % with the SARS-Rh5 treatment, 0.51 and 0.60 % with the combination treatment for 2018 and 2019 seasons, respectively, compared to control treatment. Also, K % increased to 2.49 and 2.61 % with the SARS-Rh3 treatment, 2.66 and 2.78 % with the SARS-Rh5 treatment, 2.93 and 3.05 % with the combination treatment for 2018 and 2019 seasons, respectively, compared to control treatment. Also, the same trend was observed in the interaction effect which the highest N, P and K % were obtained by the combination treatment under normal irrigation water with values of 4.18, 0.70, and 3.82 % in the first season, and 4.44, 0.79 and 3.94 % in the second season, respectively (Table 4). For seed content of microelements (Mn, Fe, Zn and Cu), results showed that highly significant effect was found in the dual inoculation treatment which was 44.50, 456.16, 65.83 and 22.16 mg kg^{-1} in the first growing season, respectively, for sub main plots. However, an increase was found in the interaction effect in the same treatment which recorded 51.00, 603.00, 87.66 and 27.33 mg kg^{-1} under normal irrigation water treatment then decreased in saline irrigation water treatment recoded 37.33, 309.33, 44.00 and 17.00 mg kg^{-1} in the first growing season, respectively. The same trend was findings in 2019 season (Table 4).

In legumes plants, symbiotic effectiveness depends on the specific combination plant and *rhizobium* under the saline conditions (Faghire et al. 2013). So, beneficial microorganisms can keep the soil system rich in all forms of macro- and micronutrients through N_2 fixation, P and K solubilization or mineralization as well as release of plant growth regulators, antibiotics, enzymes, moreover biodegradation of organic matter in the soil (Sinha et al. 2010; Singh et al. 2017; Chaudhary et al. 2020 and Elbaalawy et al. 2020). Therefore, our study supports previous findings that combination of bradyrhizobial strains and *Streptomyces griseoflavus* P4 increased N, P, and K uptakes of soybean and mung bean compared with un-inoculation treatment (El-Nahrawy and Omara 2017 and Htwe et al. (2019). Additionally, Sharif et al. (2019) found that the highest total macro and micronutrients (N, P, K, Zn, Cu, Mn and Fe) in cowpea seeds were achieved in organic treatment (vermicompost) compared to control treatments. Also, mixed application of biofertilizers and organic fertilizers can be enhanced the activity of phosphatase (acid and alkaline) around roots of Syrian cephalaria (*Cephalaria syriaca* L.), which led to increase the availability of N, P, Zn, Cu and Fe (Rahimi et al. 2019).

Total carbohydrate and protein content

Under different types of irrigation water, forage cowpea seeds treated with inoculation treatments had the greatest values of total carbohydrate and protein content over uninoculated control treatment (Fig. 1).

Under normal irrigation water and saline irrigation water conditions, data showed that an increase in total carbohydrate of cowpea seeds was observed with SARS-Rh3 + SARS-Rh5 treatment recorded 95.04 and 62.63 % followed by inoculation treatment with SARS-Rh5 recorded 60.36 and 33.52 % followed by inoculation treatment with SARS-Rh3 recorded 34.22 and 18.42 % more than uninoculated treatment (control), during 2018 season, respectively (Fig. 1a).

TABLE 4. Effect of inoculation treatments and type of irrigation water on some mineral contents of forage cowpea seeds during 2018 and 2019 seasons

Treatments	Macro nutrients (%)						Micro nutrients (mg kg^{-1})							
	N		P		K		Mn		Fe		Zn		Cu	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Irrigation Type														
NIW	3.15 a	3.41 a	0.50 a	0.59 a	3.06 a	3.18 a	39.91 a	42.91 a	384.83 a	405.83 a	69.91 a	73.91 a	21.50 a	24.00 a
SIW	2.44 b	2.70 b	0.24 b	0.33 b	1.74 b	1.86 b	29.41 b	32.41 b	229.41 b	250.41 b	35.16 b	39.16 b	13.16 b	15.66 b
LSD 0.05	0.05	0.04	0.02	0.01	0.04	0.04	1.54	1.50	20.77	19.88	1.93	1.90	0.86	0.81
Inoculation treatments														
Control	2.02 d	2.28 d	0.19 d	0.28 d	1.52 d	1.64 d	21.50 d	24.50 d	117.50 d	138.50 d	33.00 d	37.00 d	9.66 c	12.16 d
SARS-Rh3	2.56 c	2.82 c	0.36 c	0.45 c	2.49 c	2.61 c	33.33 c	36.33 c	282.83 c	303.83 c	57.83 c	61.83 c	18.83 b	21.33 c
SARS-Rh5	2.98 b	3.24 b	0.42 b	0.51 b	2.66 b	2.78 b	39.33 b	42.33 b	372.00 b	393.00 b	53.50 b	57.50 b	18.66 b	21.16 b
SARS-Rh3 + SARS-Rh5	3.63 a	3.89 a	0.51 a	0.60 a	2.93 a	3.05 a	44.50 a	47.50 a	456.16 a	477.16 a	65.83 a	69.83 a	22.16 a	24.66 a
LSD 0.05	0.08	0.06	0.03	0.03	0.06	0.04	2.18	2.20	29.38	28.59	2.73	2.68	1.22	1.19
Interaction														
Control NIW	2.14 f	2.40 f	0.32 d	0.32 d	1.83 e	1.95 e	33.00 d	26.00 f	138.66 f	159.66 f	41.66 de	45.66 de	11.66 e	14.16 e
SARS-Rh3 NIW	2.87 d	3.13 d	0.51 c	0.60 c	3.21 c	3.33 c	39.00 c	42.00 c	343.00 c	364.00 c	77.66 b	81.66 b	24.00 b	26.50 b
SARS-Rh5 NIW	3.43 b	3.69 b	0.57 b	0.66 b	3.38 b	3.50 b	46.00 b	49.00 b	454.66 b	475.66 b	72.66 c	76.66 c	23.00 b	25.50 b
SARS-Rh3 + SARS-Rh5 NIW	4.18 a	4.44 a	0.70 a	0.79 a	3.82 a	3.94 a	51.00 a	54.66 a	603.00 a	624.00 a	87.66 a	91.66 a	27.33 a	29.83 a
Control SIW	1.9 g	2.16 g	0.15 g	0.24 g	1.22 f	1.34 f	20.00 f	23.00 g	96.33 g	117.33 g	24.33 g	28.33 g	7.66 f	10.16 f
SARS-Rh3 SIW	2.25 f	2.51 f	0.22 f	0.31 f	1.76 e	1.88 e	27.00 e	30.66 e	222.66 e	243.66 e	38.00 ef	42.00 ef	13.66 d	16.16 d
SARS-Rh5 SIW	2.53 e	2.79 e	0.28 e	0.37 e	1.95 d	2.07 d	32.66 d	35.66 d	289.33 d	310.33 d	34.33 f	38.33 f	14.33 d	16.83 d
SARS-Rh3 + SARS-Rh5 SIW	3.09 c	3.35 c	0.32 d	0.42 d	2.03 d	2.17 d	37.33 c	40.33 c	309.33 cd	330.33 cd	44.00 d	48.00 d	17.00 c	19.50 c
LSD 0.05	0.11	0.10	0.04	0.03	0.09	0.07	2.97	2.94	39.99	39.00	3.72	3.70	1.67	1.66

NIW: Normal Irrigation Water, SIW: Saline Irrigation Water. Means in the same column followed by the same letter are not significantly different according to Duncan's test at 0.05 level.

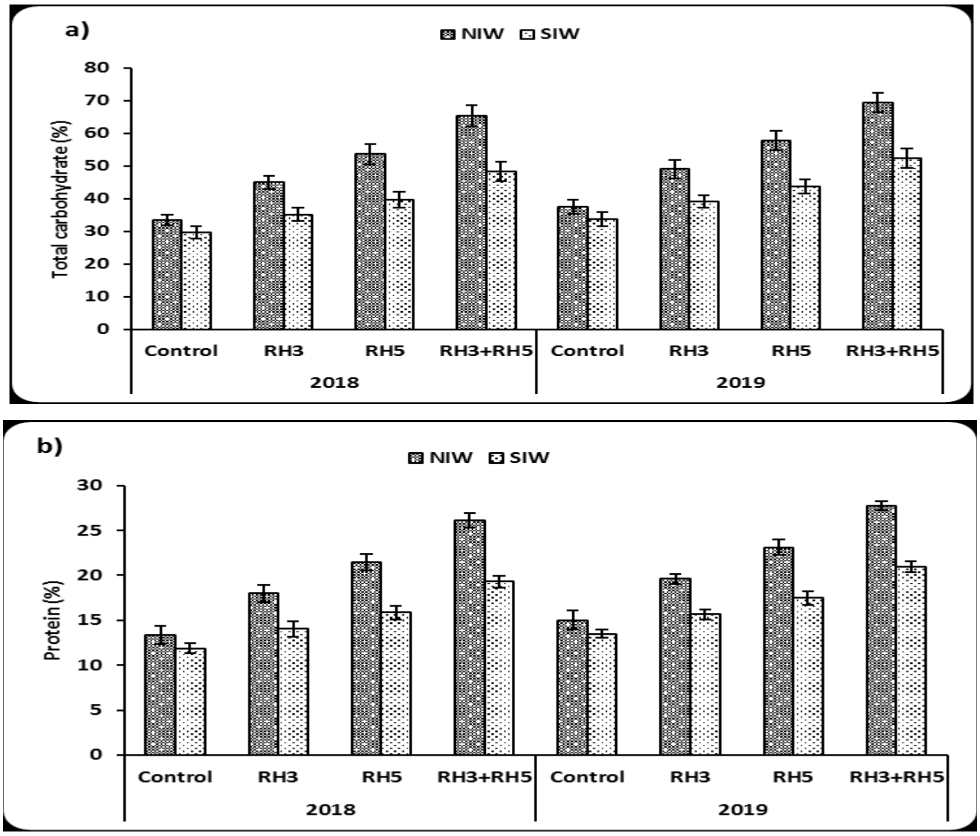

Fig. 1. Effect of inoculation treatments and type of irrigation water on total carbohydrate content (%) and protein content (%) of forage cowpea seeds during 2018 and 2019 seasons

For protein content (%), data of inoculation treatments followed the descending order of SARS-Rh3 + SARS-Rh5 > SARS-Rh5 > SARS-Rh3 > control, which recorded 26.12 % > 21.47 % > 17.97 % > 13.39 % under normal irrigation water treatments and recorded 19.30 % > 15.85 % > 14.05 % > 11.87 % under saline irrigation water treatments in 2018 season, respectively (Fig.1b). Similar findings was observed in 2019 season for total carbohydrates and protein content.

By salt stress, carbohydrate and protein concentration in cowpea seeds can be affected negatively or positively. In spite of that, our results showed that an increase in carbohydrates and protein concentrations in cowpea seeds treated with bacterial inoculation under both of normal and saline irrigation water compared to control. Therefore, beneficial microorganisms can be fixes N_2, produce phytohormones (IAA and GA) and enhanced the roots to uptake the different nutrients which led to performed and produce carbohydrates and proteins in legumes seeds (Dahmardeh et al. 2009 and Sindhu et al. 2020). These results are similar to cowpea plants (Musa et al. 2011), syrian cephalaria plants (Rahimi et al. 2019), mung bean, cowpea, and soybean (Htwe et al. 2019), and common bean, pea, cowpea and fenugreek plants (El-Batanony et al. 2020)

Assessment of seed quality

In general, mean performance for seed quality parameters viz., seed germination (%), seedling length (cm), seedling weight (g),vigour index I and vigour index II were higher in 2019 than 2018 season (Fig. 2).

Inoculation treatments improved germination % of cowpea seeds, as shown in Fig. 2a. Data showed that single inoculation with *Bradyrhizobium* SARS-Rh3 recorded 88.66 and 81.66% and *Bradyrhizobium*SARS-Rh5 recorded 90.43 and 85.00 %, but the combination treatment (*Bradyrhizobium* SARS-Rh3 + *Bradyrhizobium* SARS-Rh5) recorded 92.66 and 86.33 % compared to control treatment for normal irrigation water and saline irrigation water conditions in 2018 season, respectively. In season 2019, similar trend was observed for cowpea seed germination. On the other hand, the highest results for seedling length was observed

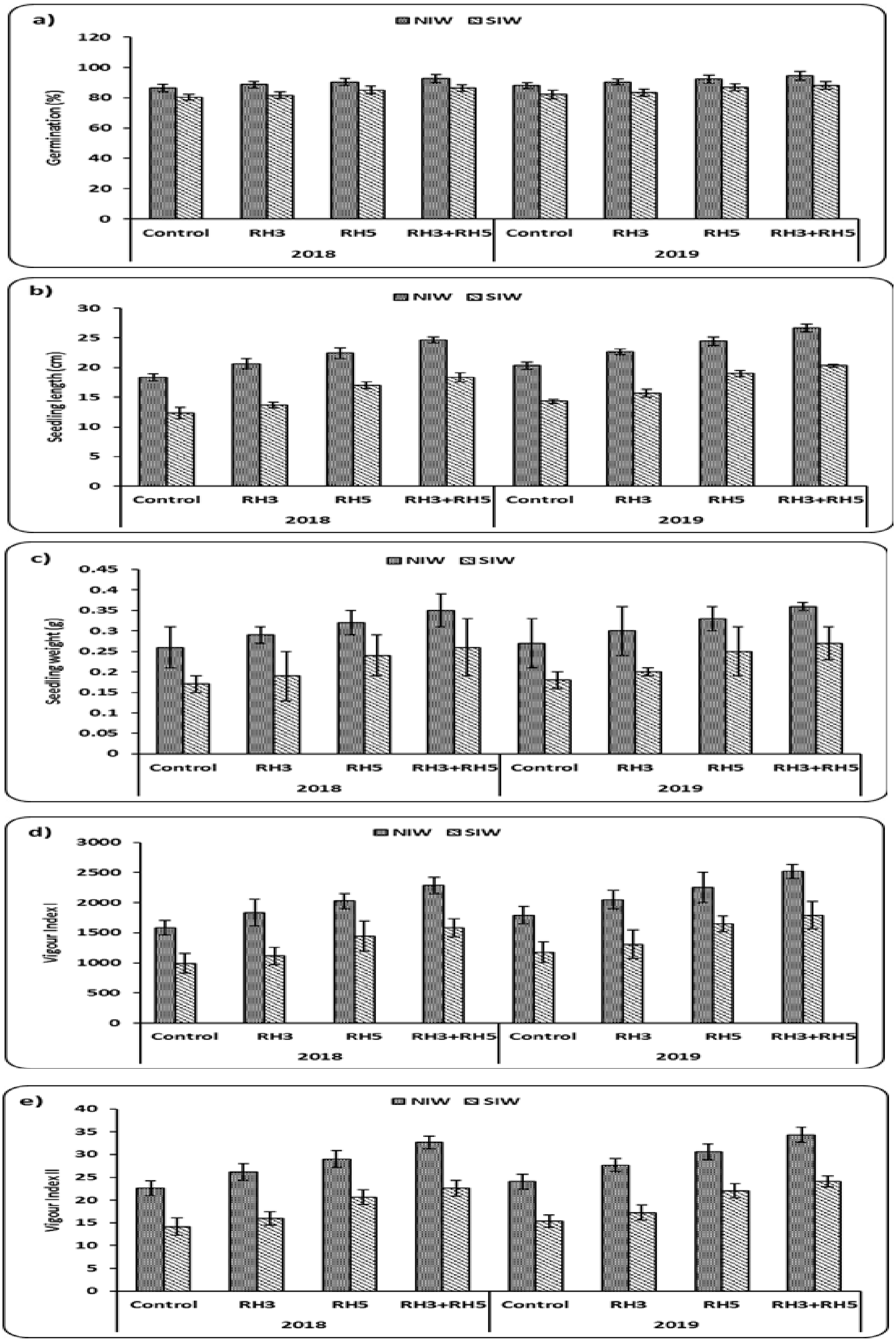

Fig. 2. Effect of inoculation treatments and type of irrigation water on seed quality parameters, a) seed germination (%), b) seedling length (cm), c) seedling weight (g), d) vigour index I and e) vigour index II of forage cowpea seeds during 2018 and 2019 seasons

at the dual inoculation treatment under normal irrigation water condition which was 24.66 and 26.66 cm, compared to the same treatment under saline irrigation water condition which was 18.33 and 20.30 cm, during 2018 and 2019 seasons, respectively (Fig. 2b). Concerning seedling weight (Fig.2c), the maximum values was recorded at the combination treatment which attained 0.35 and 0.26 g in 2018 season, and 0.36 and 0.27 g in 2019 season for normal and saline irrigation water conditions, compared to control and other studied treatments, respectively. For vigour index I and II, data showed that dual inoculation treatment gave significantly higher vigour index I (2286, 1583) and II (32.65, 22.61) followed by SARS-Rh5 treatment (2029.20, 1445.66) for vigour index I and (28.98, 20.65) for vigour index II than control (1583, 991) for vigour index I and (22.61, 14.15) for vigour index II under normal and saline irrigation water conditions in 2018 season, respectively (Fig.2 d,e).Similar findings was observed in the second season. From above results of seeds quality under saline irrigation water,inoculation treatments can be arranged as follows:SARS-Rh3 + SARS-Rh5>SARS-Rh5>SARS-Rh3 > control.

Similar findings regarding single inoculation by *Bradyrhizobium* SARS-Rh3 and *Bradyrhizobium*SARS-Rh5 or mixed inoculation with their showed improved seed quality and vigour index compared to non-treated plants. This results may be due to the presence higher amount of metabolites which helps in resumption of embryonic growth during germination and due to accumulation of higher quantity of seed constituents like protein and carbohydrates which increase germination and vigour of the seeds. This results are in agreement with Zaidi and Khan (2006) in green gram, Ashrafuzaman et al. (2009) in rice, Kumar and Pandita (2016) in cowpea, Monalisa et al. (2017) in common bean, Kamaraj and Padmavathi (2018) in cowpea.

Economics evaluation

There is a great difference in economics of forage cowpea cultivation due to application of different treatments of biofertilizers (Table 5).

TABLE 5. Values of total seasonal costs (LE Fed^{-1}), total seasonal return (LE Fed^{-1}), net seasonal return (LE Fed^{-1}) and benefit cost ratio for forage cowpea plants during 2018 and 2019 seasons

Treatment	Total seasonal costs(LE Fed^{-1})	Total seasonal return(LE Fed^{-1})	Net seasonal return(LE Fed^{-1})	Benefit Cost Ratio
		2018		
Control NIW	8250	11900	3650	1.44
SARS-Rh3 NIW	7950	13066.6	5116.6	1.64
SARS-Rh5 NIW	7950	14000	6050	1.76
SARS-Rh3 + SARS-Rh5 NIW	7950	20300	12350	2.55
Control SIW	8250	7206.6	-1043.4	0.87
SARS-Rh3 SIW	7950	9100	1150	1.14
SARS-Rh5 SIW	7950	12833.2	4883.2	1.61
SARS-Rh3 + SARS-Rh5 SIW	7950	15866.6	7916.6	1.99
		2019		
Control NIW	8250	12340	4090	1.49
SARS-Rh3 NIW	7950	13506.6	5556.6	1.69
SARS-Rh5 NIW	7950	14440	6490	1.81
SARS-Rh3 + SARS-Rh5 NIW	7950	20740	12790	2.60
Control SIW	8250	7646.6	-603.4	0.92
SARS-Rh3 SIW	7950	9540	1590	1.20
SARS-Rh5 SIW	7950	13273.2	5323.2	1.66
SARS-Rh3 + SARS-Rh5 SIW	7950	16306.6	8356.6	2.05

Total seasonal costs according to the Egyptian local market price (LE); chemical fertilizer (680 LE for uninoculated treatments and 580 LE for inoculation treatments); seeds (30 kg fed^{-1}, 600 LE); machinery costs (1580 LE); labour wages (320 LE); land rent for summer season (5000 LE) and seeds yield (20000 ton^{-1}) .

The total cost of cultivation in cowpea cv. Baladi varied between 8250 LE fed^{-1} for control treatment to 7950 LE fed^{-1}for inoculation treatments under different irrigation water treatments.Combined use of *Bradyrhizobium* SARS-Rh3 + *Bradyrhizobium* SARS-Rh5 treatment recorded the highest total seasonal return 20.300 and 20.740 LE fed^{-1} under normal irrigation water treatment and 15.866.6 and 16.306.6 LE fed^{-1}under saline irrigation water treatment during 2018 and 2019 seasons, respectively. Similar findings was observed in net seasonal return for inoculation treatments which arranged as follows: SARS-Rh3 + SARS-Rh5>SARS-Rh5>SARS-Rh3. However, negative net seasonal return was obtained in control treatment recorded -1043.4 and -603.4 LE fed^{-1} under saline irrigation water treatment during the first and second seasons, respectively. Concerning benefit cost ratio (Table 5), the maximum values was recorded at the combination treatment which attained 2.22 and 1.99 in 2018 season, and 2.60 and 2.05 in 2019 season for normal and saline irrigation water conditions, compared to control and other studied treatments, respectively.

Similar results of enhanced profitability of cowpea were reported by Subbarayappa et al. (2009), Kumar and Pandita (2016) and Kalegore et al. (2018).

Conclusion

From our results, applying dual inoculation with *Bradyrhizobium SARS-Rh3* + *Bradyrhizobium* SARS-Rh5, can alleviate the harmful effects of salt stress by enhancement of yield and yield attributing characteristics, seed mineral content and assessment of seed quality

Acknowledgements

Thanks to all staff members and colleagues in The Bacteriology Research Laboratory and Forage Crops department, Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt for their valuable cooperation which made completion of this work possible.

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