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Impact of Different Nitrogen forms on Soil Fertility and Cowpea Productivity under Conditions Saline Soil

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MHAA and EMN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MHAA, EMN and AAAEK managed the analyses of the study. Authors EMN and AAAEK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The field experiment was carried out at the south-west portion of Shall El-Tina region, north Siena Governorate Egypt. The study investigated evaluations of different nitrogen fertilizers sources (ammonium nitrate, ammonium sulphate and urea fertilizers) and rates (100%, 75% and 50% recommended dose of all different nitrogen forms) alone or combined with bio-fertilizer (*Rhizobium radiobacter* sp.) strain on improve nutrients available and contents in cowpea productivity in newly reclaimed saline soil conditions during two summer seasons 2019 and 2020. The studies treatments were disturbed among the experimental plots in split design. The obtained results showed that using ammonium sulphate fertilizer was more effect on EC, pH, and available N, P, K, Fe, Mn and Zn values by increasing application rates compared with other treatments. Moreover, data recorded the applied ammonium sulphate75% with bio-fertilizer was increase of plant height, weight of 100 seeds, weight of pods (g plant^{-1}), seeds yield (ton fed^{-1}) have been affected by inoculation with bio-fertilizer combined with different nitrogen forms and different application rates than other treatments. The highest values of N, P, K, Fe, Mn and Zn concentrations and uptake in seeds were obtained with ammonium sulphate application combined with bio-fertilizer compared

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with other treatments. The increase of chlorophyll, protein contents in cowpea plants with decreasing soil salinity, while the increase of proline content was increasing soil salinity. The application of ammonium sulphate at application rate 75% N combined with bio-fertilizer improves soil properties and cowpea productivity under saline soil conditions.

Keywords: Saline soils; available nutrients; cowpea productivity; and cowpea quality.

1. INTRODUCTION

Salinity and soil nutrient deficiencies are the main factors reducing plant productivity in arid and semi-arid areas. Among the essential elements, nitrogen is usually the most limiting of plant growth in saline or non-saline soils [1]. Improving salt affected soils may be achieved by using different practices such as subsoiling, mole drain, soil amendments, farm manure and bio-fertilizer. These previous practices are important tools for improving crop productivity and soil properties in salt affected soils at the North Delta [2].

In Egypt, cowpea cultivation area according to [3] was about 14830 fed. with production of about 17248 tons with (an average yield of 1.16 ton fed^{-1}). In fact, salinity is one of abiotic stress, which severely limited cowpea productivity. Whereas in Egypt 33% out of total cultivated land is suffering from salinity [4]. Also, the reduction of cowpea characters had may be due to the accumulation of salt at high level in cells which in turn affecting many of biochemical process in plants such as translocation of assimilates towards organ regeneration and photosynthesis of the plant. For pod length, clear variation among genotypes in both normal and stress conditions [5]. Decrease of pod fresh values, seed yield and weight of 100 seeds of Cowpea with increasing soil salinity [6]. The yield significant increase of cowpea yield component with decreased soil salinity stress [7].

Nitrogen as a macronutrient has an eminent role in plant nutrition. Some nitrogen fertilizers such as urea and ammonium nitrate have a high mobility and leaching potential. Excessive use of nitrogen fertilizers in agriculture has resulted in leaching of fertilizers and their derivatives below the root zone, contaminating groundwater. Groundwater pollution caused by leaching of NO_3-N from agricultural systems has caused public concerns for decades [8]. Mineral N fertilizer application may have an effect on soil organic matter and other soil parameters were increase. The addition of N fertilization decreased the average values of soil pH. The decrease of soil pH values resulted in decrease of base saturation in N treatments and this effect was more intensive with higher doses of N fertilization [9].

Bio-fertilizers are environmentally friendly contains microorganisms that enrich the nutrient quality of soils. The major concerns in today's agricultural world are: Mining of nutrients, decreasing fertilizer use efficiency and the pollution and contamination of soils. The beneficial use of microorganisms in order to reduce or replace chemical fertilizers has been studied [10] and [11]. Bio-fertilizers and/or bio-protectors improve plant growth stimulation and crop protection. Beneficial microorganisms in improving plant rooting maybe by production of metabolites related to pathogen control (phytohormones, antimicrobials, antibiotics) and root development growth, and (ii) the difficulty to separate the direct and indirect effects on the specific/total activities as a result of enhanced nutrients availability and growth regulators [12]. Inoculated seeds with biofertilizers significantly influenced the total and available phosphorus, dehydrogenase enzyme activity and alkaline phosphate activity in cowpea [13]. Rhizobia can be used as bio inoculants, particularly for stressed agro-ecosystems. Beside main role of Rhizobia in symbiotic nitrogen fixation for plant uptake it also able to perform different plant growth promoting (PGP) activities such as solubilization of phosphate, Zn and potassium, production of phytohormones, exopolysaccharides (EPS), siderophore and bio control of phytopathogens [14] and [15]. Soil salinity is one of the major abiotic stresses affected negatively soil quality and plant growth PGPR known as highly tolerant to several abiotic and biotic stresses. Rhizobia known by its salt tolerance abilities, and reported as plant growth promotion in saline conditions [16].

This study aim to improve of salt soil productivity by using some mineral nitrogen fertilizer sources alternative bio-fertilizer is a trend optimization to reclaimed soil health, reduce pollution, and increase their cowpea production.

2. MATERIALS AND METHODS

Two field experiments were carried out in south-west portion of Shall El-Tina region, north Siena Governorate, Egypt, during two successive summer seasons 2019 and 2020, to study the evaluation of different application rats for ammonium nitrate (33% N), ammonium sulphate (20% N) and Urea (46% N). The physical and chemical properties of soil before planting were determined according to Page et al [17] and [18]. The obtained data were recorded in Table 1.

In both seasons, each experiment was carried out in split plot design three replicates. Main plot was the rates of N fertilizers, while the sources of nitrogen fertilizers were sub main plot. The area of each experimental plot was 5 × 10 m which divide two division, first division was bio-fertilizer (*Rhizobium radiobacter*) strain (salt tolerant PGPR) deposited in the Gen bank under number of HQ395610 Egypt by Bio-fertilizer Production Unit, Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.

The experimental treatments were as follows:

- Control 100% mineral fertilizers recommended dose (without Bio).
- Ammonium nitrate (AN) 75% + bio-fertilizer. (20 L /400 L water /fed)
- Ammonium nitrate (AN) 50% + bio-fertilizer. .(20 L /400 L water /fed)
- Ammonium sulfate (AS) 75% + bio-fertilizer. .(20 L /400 L water /fed)
- Ammonium sulfate (AS) 50% + bio-fertilizer. .(20 L /400 L water /fed)
- Urea 75% + bio-fertilizer. .(20 L /400 L water /fed)
- Urea 50% + bio-fertilizer. .(20 L /400 L water /fed)

Seeds of cowpea Kafr El-Shakh 1 variety *(Vigna unguiclata L.)* were supplied from Veg. Res. Dept. Hort. Res. Agric. Res. Center. Sowing was April 25 on carried out 2019 and 29 April 2020. Seeds of cowpea were thoroughly mixed with the inoculants in the shade, then sown immediately and covered with soil in order to minimize Rhizobia exposure to the sun. More bio-fertilization was added 3 periods at 30, 45 and 65 days after planting through liquid sprays on soil at a rate of 20 L mixed with 400L water fed^{-1}. Also, the soil fertilizer by potassium sulphat (48 % K_2O) was applied at rate 75 kg fed^{-1} on three periods 31, 45 and 65 days from planting.

Ammonium nitrate (33% N) with bio-fertilizer; ammonium sulphate (20% N) with bio-fertilizer and urea (46% N) with bio-fertilizer were applied on three times 21, 40 and 55 days from planting at rates 75% and 50% recommended dose. Harvest was done on September, 20thand 25th for the two successive seasons of 2019 and 2020, respectively. After harvest, samples of the surface soil layer (0-30cm) from each plot were taken. Samples were analyzed for EC (in soil paste extract), pH (in 1:2.5 soils: water suspension) available N, P, K, Fe, Mn, Zn and Cu as described by [19] and [17].

Plant samples of three replicates were taken (1m×1m) after harvest. Samples of each experiment plot were prepared for parameters and some physiochemical determination. Each fresh plant sample was separated into shoot and pods. Number of pods per plant was counted. Both shoot and pods were air-dried and

Table 1. Physical and chemical properties in initial soil

Corse sand (%)	**Fine sand (%)**	**Silt (%)**	**Clay (%)**	**Texture**	**O.M (%)**	**$CaCO_3$ (%)**		
2.29	26.90	32.10	38.71	Clay loam	0.89	4.74		
F.C.	W.P.	A.W.		B.D (g cm^{-3})		T.P (%)		
28.39	10.56	17.83		1.48		45.00		
Chemical properties								
pH	EC	**Cations (meq l^{-1})**				**Anions (meq l^{-1})**		
(1:2:5)	(dS m^{-1})	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	HCO^{-}_{3}	Cl^{-}	SO^{-2}_{4}
8.39	8.23	21.51	14.46	43.03	1.29	2.29	77.52	4.49
Macronutrients (mg kg^{-1})			**Micronutrients (mg kg^{-1})**					
N	P	K	Fe	Mn	Zn	Cu		
38.59	5.83	158	2.29	1.52	0.63	1.02		

**Notes: Organic Matter (O.M) - Field Capacity (F.C.) – Welting Point (W.P.) – Available Water (A.W) - Bulk Density (B.D) – Total Porosity (T.P)* oven dried at degrees 70C^o for 48 hrs. Dry yield of shoot fed^{-1}) and dry weight of 100 seed (g) were estimated. Ether of oven-dried straw or seeds were ground and kept in plastic bags for chemical analysis. A 0.5 g of each oven dried ground plant sample was digested using H_2SO_4, $HClO_4$mixture according to the method described by [20]. The plant content of N, P, K, Fe, Mn and Zn was determined in plant digestion using the methods described by [20] and [17]. Protein percentage of seeds was calculated by multiplying the nitrogen percentage by the factor 6.25 [21]. Total chlorophyll was estimated in fresh shoot as described by [22]. Proline content was determined according to [23]. Soil sample of each plot was taken after harvest from 0-30cm depth for chemical analyses. The obtained data were statistically analysis using the COSTAT program at the probability levels of 5% was calculated according to [24].

3. RESULTS AND DISCUSSION

3.1 Effect of Different Nitrogen forms Rates Combined with Bio-Fertilizers on Soil Chemical Properties

3.1.1 Soil pH

Soil pH is one important parameter which reflects the overall change in soil chemical properties. Data in Table 2 show that the application of different nitrogen forms combined with bio-fertilizer was positive effect on soil pH. The soil pH values ranged around 8.30 in initial soil and 7.6 after harvest. The lowest pH value 7.6 was recorded with soil application ammonium sulphate at rate 40 kg N/fed combined with or without bio-fertilizer. Concerning those increasing application rates of mineral nitrogen sources combined with bio-fertilizer gave decreased of soil pH values. This result is in agreement by [25] found that the effect of different mineral nitrogen fertilizer combined with bio-fertilizer on Dehydrogenase activity and production of µ moles of H_2 in the rhizosphere of maize root media had a positive effect on increasing the hydrogen moles which react in root zone to form hydrocarbon acid led to decrease soil pH. [26] indicated that the decrease in soil pH could be discussed as follows: calcium ions react with bicarbonate to precipitate calcite ($CaCO_3$) and release protons (H^+) in soil solution which neutralize the hydroxide ions (OH^-) and decrease the soil pH. This result may be due to the decrease in pH values could be attributed to the production of CO_2 and organic acids by soil microorganisms acting and other chemical transformation on the added bio-fertilizer [27]. Reported that the soil pH was decreased with increasing N rate by ammonium sulphate and urea fertilizers. [28] found that the applied of ammonium sulphat on saline soil was decreased of soil pH from (8.5 – 7.8) after 20 week.

3.1.2 Soil salinity (EC dSm^{-1})

As for soil salinity, the obtained data in Table 2 suggested that the application of different nitrogen fertilizer forms caused an appreciated decrease in the EC values. Soil salinity was not significant as affected with nitrogen forms, while the different rates of nitrogen sources on soil salinity was significant with increasing rate of mineral nitrogen. The interaction between nitrogen fertilizers sources and different rates were significant decreased in soil salinity. The soil salinity decrease with increasing rate of mineral nitrogen sources especially soil treated with ammonium sulphate at rate 40 kg N fed^{-1} combined with bio-fertilizer. The relative decreases of mean values EC (dSm^{-1}) was 13.45 and 10.95 % for soil treated with ammonium nitrate combined with bio-fertilizer at application rates 75 and 50% respectively, compared with control. On the other hand, the relative decreases of mean values EC (dSm^{-1}) soil treated with ammonium sulphate different rates were 17.31 and 36.7 % for soil treated with ammonium sulphate at rates 75 and 50% respectively, combined with bio-fertilizer compared with control. The relative decreases of mean values soil salinity (dSm^{-1}) treated with urea fertilizer combined with bio-fertilizer was 22.62 and 19.44 % at rats 75 and 50% respectively, then control.

The efficiencies of nitrogen fertilizers forms in decreasing soil EC arranged as follow: ammonium nitrate > ammonium sulphat > urea. This trend can be due to Rhizobium producing phyto-hormones such as indole acetic acid, cytokines and organic acid which had an effect that decreases salinity stress in the rhizosphere refracted to Na- salt and improve soil structure, increasing aggregate stability and drainable pores enhancing the leaching process of soluble salts [29-30]. Reported that the application of ammonium sulfate caused the decreased in soil salinity as compared to urea.

3.1.3 Available macronutrients in soil

Data in Tables (3 and 4) showed that the values of the available macronutrients N, P and K (mg kg^{-1}) content in soil as affected by different N forms i.e., ammonium nitrate, ammonium sulphate and urea rates combined with bio-fertilizer. Soil available N and P increased as increasing rate combined with bio-fertilizer. The statistically analysis indicated that the effect of different treatments combined with bio-fertilizer on N and P available was not significant, while the K content in soil increase significant with increasing rates of nitrogen forms combined with bio-fertilizer. A similar trend was showed across N rates has been reported by [31].

The interaction between different nitrogen forms and different application rates, it was found that available N, P and K content in soil were significantly increased by applied of different nitrogen forms and different application rates combined with bio-fertilizer. The results indicated that ammonium sulphat applied at 75% rate gave the highest values compared with other treatments. These results are in agreement by [32] indicated that available N, P and K content in soil were significantly increased in soils treated with Bio-fertilizer in combination with chemical fertilizers than soil treated with chemical fertilizers alone.

Table 2. Effect of different nitrogen forms and rates combined with bio-fertilizer on soil pH and EC

Treatments	pH (1:2.5)			Mean	EC paste (dS m^{-1})			Mean
	Rates of N forms				Rates of N forms			
	Control	75%	50%		Control	75%	50%	
AN + Bio	8.30	7.90	7.90	8.03	8.10	7.14	7.30	7.51
AS + Bio	8.20	7.60	7.70	7.83	8.20	6.22	6.00	7.04
Urea + Bio	8.30	8.0	8.10	8.13	7.90	6.99	6.17	7.02
Mean	8.27	7.83	7.90		8.10	6.78	6.49	
L.S.D. at 0.05	Treat. (T) = 0.11Rates (R)= NS T×R = **				Treat. (T) = NSRates (R)= 0.34 T×R = **			

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio – Not significant (NS) - Treatment (T) – Application Rates (R)*

Table 3. Effect of different nitrogen forms and application rates combined with bio-fertilizer on available N and P (mg kg^{-1} soil) in soil

Treatments	N			Mean	P			Mean
	Rates of N forms				Rates of N forms			
	Control	75%	50%		Control	75%	50%	
AN + Bio	40.23	43.96	40.5	41.56	6.14	9.03	8.97	8.05
AS + Bio	41.55	44.50	41.99	42.68	6.12	9.81	8.05	7.99
Urea + Bio	35.12	38.30	36.77	36.73	6.13	6.09	6.00	6.07
Mean	38.97	42.25	39.75		6.13	8.31	7.67	
L.S.D. at 0.05	Treat. (T) = NSRates (R)= 1.02 T×R = ***				Treat. (T) = 1.92Rates (R)=1.13 T×R = ***			

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio – Not significant (NS) - Treatment (T) – Application Rates (R)*

Table 4. Effect of different nitrogen forms and application rates combined with bio-fertilizer on available K (mg kg^{-1} soil) in soil

Treatments	K (mg kg^{-1} soil)			Mean
	Rates of N forms			
	Control	75%	50%	
AN + Bio	165.0	195.0	153.0	171.00
AS + Bio	167.0	205.0	199.0	190.33
Urea + Bio	164.0	198.0	195.0	185.67
Mean	165.33	199.33	182.33	
L.S.D. at 0.05	Treat. (T) = NST×R = ***	Rates (R)= 1.90		

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio – Not significant (NS)- Treatment (T) – Application Rates (R)*

General, the positive effects of the used different nitrogen forms and different rates combined with bio-fertilizer on available N, P and K could be arranged in the following order: ammonium sulphate > ammonium nitrate > urea > initial soil. These results indicate the important role of bio-fertilizer in improving soil nutrients (N, P and K) status due to microorganism's activity in N fixation and by reduction of soil pH. [30] found that the application of mineral nitrogen fertilizer combined with biofertilizer had decreased soil pH and increased available N, P and K.

3.1.4 Available micronutrients in soil

Availability of micronutrients depended on soil pH, date in Tables (5 and 6) show that the variation in available micronutrient contents of soil (Fe, Mn and Zn, mg kg^{-1} soil), results from different nitrogen forms and different rates combined with bio-fertilizer were increase with increasing rate of nitrogen forms especially soil treated with ammonium sulphate combined with bio-fertilizer. On the other hand, effect of different nitrogen forms with bio-fertilizer on Fe, Mn and Zn availably were not significant, while the effect of different rates of nitrogen forms on available Fe, Mn and Zn was significantly increase with increasing application rates compared with control.

Interaction between nitrogen forms combined with bio-fertilizer and different rates, it was found that ammonium sulphat applied at 75% rate gave the highest values for Fe, Mn and Zn available in soil compared was other treatments. Significant increase of micronutrients was affected with interaction between nitrogen forms and different rates alone or combined with bio-fertilizer [33]. Indicated that the soil treated with bio-fertilizer improved soil microbial activity and increase availability of nutrients.

The relative increases of mean values Fe, Mn and Zn available for soil treated with ammonium sulphate were 0.115%, 0.091% and 0.071 % respectively, compared with other treatments [34]. reported that the soil with treated with bio-fertilizer in combination with N-mineral fertilizer caused progressive significant increases in all the studied available micronutrients than without bio-fertilizer.

Table 5. Effect of different nitrogen forms and application rates combined with bio-fertilizer on available Fe and Mn (mg kg^{-1}) in soil

Treatments	Fe			Mean	Mn			Mean
	Rates of N forms				Rates of N forms			
	Control	75%	50%		Control	75%	50%	
AN + Bio	2.95	3.89	3.76	3.53	1.92	2.36	2.31	2.20
AS + Bio	2.97	4.93	4.08	3.99	1.95	2.86	2.44	2.42
Urea + Bio	2.94	3.98	3.94	3.62	1.93	2.41	2.34	2.23
Mean	2.95	4.27	3.93		1.93	2.54	2.36	
L.S.D. at 0.05	Treat. (T) = NS Rates (R)=1.03 T×R = ***				Treat. (T) = NS Rates (R)=0.601 T×R = ***			

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio – Not significant (NS) - Treatment (T) – Application Rates (R)*

Table 6. Effect of different nitrogen forms and application rates combined with bio-fertilizer on available Zn (mg kg^{-1}) in soil

Treatments	Zn			Mean
	Rates of N forms			
	Control	75%	50%	
AN + Bio	0.69	0.84	0.81	0.78
AS + Bio	0.68	0.98	0.85	0.84
Urea + Bio	0.67	0.86	0.83	0.79
Mean	0.68	0.89	0.83	
L.S.D. at 0.05	Treat. (T) = NS T×R = ***		Rates (R)= 0.31	

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio – Not significant (NS) - Treatment (T) – Application Rates (R)*

It is worthy to mention that the contents of Fe, Mn and Zn available in soil, in general the positive effects the used different nitrogen sources and rates fertilizers combined and without biofertilizer could be arranged in the following order: ammonium sulphate > urea > ammonium nitrate > initial soil.

3.2 Effect of Different Nitrogen Forms and Application Rates Companied with Bio-fertilizer on Yield Component

The results indicated that plant height, weight of 100 seeds, weight of pods (g plant^{-1}), weight of seeds (g plant^{-1}) and seeds yield (ton fed^{-1}) have been affected by inoculation with bio-fertilizer combined with nitrogen forms under different nitrogen rates Tables 7, 8, 9. However, different nitrogen forms combined with bio-fertilizer showed a significant effect on only weight of pod (g plant^{-1}).

Plant height (cm), weight seed (g plant^{-1}) and weight seeds yield (ton fed^{-1}) were significant increase with increasing different rates of nitrogen fertilizers sources combined with bio-fertilizer, while the 100 seeds (g) was not significant. The interaction between nitrogen forms and different application rates, it was found that different treatments combined with bio-fertilizer were significant increases all parameters of plants, except weight of 100 seeds compared with control.

These results may be due to the ammonium sulphate is an essential element of bio-molecules such as amino acids, proteins, nucleic acids, phytohormones and a number of enzymes and coenzymes and improve of yield component [35]. Reported that the inoculation of cowpea seeds with rhizobia significant increased number pods plant^{-1}, number of seeds pod^{-1}, seed yield plant^{-1} and seed yield fed^{-1} compared with uninoculated seed.

3.2.1 Macronutrients contents in seeds

Data presented in Tables 10, 11, 12 illustrated the macronutrients concentration and uptake by of seeds cowpea under different nitrogen forms combined with bio-fertilizer in saline soil. The data obtained of N, P and K concentration and uptake in seeds show increase with increasing rate of nitrogen fertilizers sources single. The highest values of N, P and K concentrations 4.85, 0.64 and 2.88 (%) and 47.75, 6.26 and 25.18 (kg fed^{-1}) uptake in seeds for seeds treated with bio-fertilizers combined with ammonium sulphate. The results are in agreement with [30] indicated that the application of ammonium sulphate was increase value for P and N content in plant may be due to less saline soil and more nutrition adsorption by plants as compared other N fertilizers.

The effect of application different nitrogen forms on N, P and K concentrations were not significant while, the N uptake was significant. The different rates of nitrogen forms combined with bio-fertilizer were not significant for N concentration in seeds while the N uptake was significant. The P and K concentrations in seeds was significant increases as affected with rates nitrogen fertilizers sources combined with bio-fertilizer, while the P and K uptake in seeds inoculation combined with rates of nitrogen fertilizers sources were significant. The interaction between nitrogen fertilizers forms and rates combined with bio-fertilizer for N, P and K concentrations and uptake in seeds cowpea were significant increases.

Table 7. Effect of different nitrogen forms and rates combined with bio-fertilizer on plant height and 100 seeds weight

Treatments	**Plant height (cm)**			**Mean**	**100 seeds weight (g)**			**Mean**
	Rates of N forms				**Rates of N forms**			
	Control	**75%**	**50%**		**Control**	**75%**	**50%**	
AN + Bio	75.9	92.85	83.78	84.18	13.80	15.34	14.95	14.70
AS + Bio	78.56	88.70	98.88	88.71	15.85	16.38	15.75	15.99
Urea + Bio	77.85	94.8	84.99	85.88	14.83	15.49	14.99	14.44
Mean	77.44	92.12	89.22		14.83	15.74	14.56	
L.S.D. at 0.05	Treat. (T) =1.02 Rates (R)=1.67 T×R = ***				Treat. (T) =0.51 Rates (R)=0.33 T×R = **			

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio-- Treatment (T) – Application Rates (R)*

Table 8. Effect of different nitrogen forms and application rates combined with bio-fertilizer on Pods weight and seeds weight (g plant)

Treatments	Pods weight g plant^{-1}			Mean	Seeds weight g plant^{-1}			Mean
	Rates of N forms				Rates of N forms			
	Control	75%	50%		Control	75%	50%	
AN + Bio	16.65	18.90	22.87	19.47	13.2	17.36	15.9	15.82
AS + Bio	17.23	23.10	22.40	20.91	13.44	17.3	18.69	16.48
Urea + Bio	16.98	19.22	23.00	19.73	12.75	17.75	16.22	15.57
Mean	16.95	20.41	22.76		12.46	17.14	17.27	
L.S.D. at 0.05	Treat. (T) = 0.91Rates (R)= 1.41 T×R = ***				Treat. (T) = 0.52 Rates (R)=1.13 T×R = ***			

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio. - Treatment (T) – Application Rates (R)*

Table 9. Effect of different nitrogen forms and application rates combined with bio-fertilizer on seeds yield

Treatments	Seed yield (kg fed^{-1})			Mean
	Rates of N forms			
	Control	75%	50%	
AN + Bio	720.10	810.74	793.00	774.61
AS + Bio	755.00	985.00	880.00	873.33
Urea + Bio	773.00	840.00	797.00	803.33
Mean	749.37	878.58	823.33	
L.S.D. at 0.05	Treat. (T) = 22.11T×R = ***	Rates (R)= 16.90		

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio- Treatment (T) – Application Rates (R)*

Table 10. Effect of different nitrogen forms and application rates combined with bio-fertilizer on N concentration and uptake in seeds yield

Treatments	N. Concentration (%)			Mean	N. Uptake (kg/fed)			Mean
	Rates of N forms				Rates of N forms			
	Control	75%	50%		Control	75%	50%	
AN + Bio	3.34	4.78	4.25	4.12	24.02	43.49	37.08	34.86
AS + Bio	3.36	4.85	4.67	4.30	25.37	47.75	42.94	38.69
Urea + Bio	3.42	4.80	4.52	4.25	26.44	45.12	40.57	37.38
Mean	3.37	4.81	4.48		25.27	45.46	40.20	
L.S.D. at 0.05	Treat. (T) = NS Rates (R)= NS T×R = ***				Treat. (T) = 0.92 Rates (R)=2.13 T×R = ***			

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio – Not significant (NS) - Treatment (T) – Application Rates (R)*

Table 11. Effect of different nitrogen forms and application rates combined with bio-fertilizer on P concentration and uptake in seeds yield

Treatments	P. Concentration			Mean	P. Uptake (kg/fed)			Mean
	Rates of N forms				Rates of N forms			
	Control	75%	50%		Control	75%	50%	
AN + Bio	0.35	0.54	0.50	0.47	2.51	4.92	4.40	3.95
AS + Bio	0.38	0.64	0.56	0.53	2.90	6.26	5.18	4.79
Urea + Bio	0.41	0.60	0.58	0.53	3.16	5.64	5.17	4.66
Mean	0.38	0.59	0.55		2.86	5.60	4.92	
L.S.D. at 0.05	Treat. (T) = NS Rates (R)= 0.21 T×R = ***				Treat. (T) =0.62Rates (R)=0.11T×R = ***			

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio – Not significant (NS) - Treatment (T) – Application Rates (R)*

[16] found that the increases in N, P and K content might be due to the interaction effect between rhizobial, which consequently increased the uptake of nutrients in cowpea plant. It is evident from the distribution patterns of N, P and K concentration and uptake by seeds cowpea that it could be arranged according to their contents in the following orders: Ammonium sulphate > Urea > calcium nitrate for seeds treated with 30 kg N fed^{-1} combined with bio-fertilizer.

This increase of N, P and K contents in seeds of Cowpea may be due to applied of different nitrogen fertilizers sources and bio-fertilizer that seems important for Rhizobium radiobacter strain as a salt tolerant to fix relatively more from soil, which resulted in increased N, P and K uptake by root [36]. found that phytohormones produce bacteria which cause pronounced increases for plant root elongation and then uptake of more nutrients via the root system, and hence utilization of N as a result bio-inoculation [37]. Who suggested, that inoculation with N_2-fixer bacteria increased uptake of N, P and K by pea plants.

3.2.2 Micronutrients concentration and uptake in seeds cowpea

Data presented in Tables 13, 14, 15 showed the effect of different nitrogen forms and different rates alone or combined with bio-fertilizer on micronutrients concentrations and uptake i. e. Fe, Mn and Zn in seeds cowpea plants were increases with increasing rate of nitrogen fertilizer sources. The highest values of Fe, Mn and Zn concentrations and uptake in seeds treated with ammonium sulphate at rate 75% N than other treatments. The significant increase of Fe, Mn concentrations and Zn uptake in seeds as affected by nitrogen fertilizers sources combined with bio-fertilizer, while the Fe uptake in seeds without bio-fertilizer was significant.

The Mn uptake in seeds treated with bio-fertilizer combined with nitrogen sources was significant. The Fe and Zn concentrations in seeds were significant increase as affected with different nitrogen fertilizers rates alone or combined with bio-fertilizer, while Mn concentrations in seeds without bio-fertilizer was not significant.

Table 12. Effect of different nitrogen forms and application rates combined with bio-fertilizer on K concentration and uptake in seeds yield

Treatments	K. Concentration			Mean	K. Uptake (kg/fed)			Mean
	Rates of N forms				Rates of N forms			
	Control	75%	50%		Control	75%	50%	
AN + Bio	2.23	2.46	2.34	2.34	16.07	22.40	20.42	19.63
AS + Bio	2.26	2.88	2.56	2.90	17.03	25.18	21.86	21.36
Urea + Bio	2.24	2.36	2.47	2.36	17.35	23.23	21.20	20.59
Mean	2.24	2.96	2.50	-	16.81	23.60	21.17	
L.S.D. at 0.05	Treat. (T) = NS Rates (R)= 0.23 T×R = ***				Treat. (T) = 0.65 Rates (R)= 1.65 T×R = ***			

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio – Not significant (NS)- Treatment (T) – Application Rates (R)*

Table 13. Effect of different nitrogen forms and application rates combined with bio-fertilizer on Fe concentration and uptake in seeds of cowpea

Treatments	Fe (mg kg^{-1}) Concentration			Mean	Fe Uptake (g fed^{-1})			Mean
	Rates of N forms				Rates of N forms			
	Control	75%	50%		Control	75%	50%	
AN + Bio	65.30	79.10	77.30	73.90	470.23	720.4	674.83	621.82
AS + Bio	68.20	85.33	82.18	78.57	514.91	840.5	756.06	703.82
Urea + Bio	66.82	84.10	80.22	77.05	516.52	790.54	719.57	675.54
Mean	66.77	82.84	79.90		500.55	783.81	716.82	
L.S.D. at 0.05	Treat. (T) = 1.102 Rates (R)= 2.61 T×R = ***				Treat. (T) =46.21 Rates (R)= 35.11 T×R = ***			

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio.- Treatment (T) – Application Rates (R)*

Table 14. Effect of different nitrogen forms and application rates combined with bio-fertilizer on Mn concentration and uptake in seeds of cowpea

Treatments	Mn (mg/kg) Concentration			Mean	Mn Uptake (g/fed)			Mean
	Rates of N forms				Rates of N forms			
	Control	75%	50%		Control	75%	50%	
AN + Bio	49.83	55.30	53.91	53.01	358.83	503.64	470.63	444.37
AS + Bio	50.51	58.99	55.20	54.90	381.35	518.05	507.84	469.08
Urea + Bio	49.88	57.82	54.30	54.00	385.57	543.51	487.07	472.05
Mean	50.07	57.37	54.47		375.25	521.73	488.51	
L.S.D. at 0.05	Treat (T) =0.55 Rates (R)= 2.11 T×R = ***				Treat. (T) = 9.86 Rates (R)= 44.94 T×R = ***			

Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio. - Treatment (T) – Application Rates (R)

Table 15. Effect of different nitrogen forms and application rates combined with bio-fertilizer on Zn concentration and uptake in seeds of cowpea

Treatments	Zn (mg/kg) Concentration			Mean	Zn Uptake (g/fed)			Mean
	Rates of N forms				Rates of N forms			
	Control	75%	50%		Control	75%	50%	
AN + Bio	32.59	35.87	34.85	34.44	234.68	326.68	304.24	288.53
AS + Bio	33.20	39.21	36.59	36.33	250.66	386.22	336.63	324.50
Urea + Bio	33.00	35.85	34.99	34.61	225.09	336.99	313.86	291.98
Mean	32.93	36.98	35.48		236.81	349.96	318.24	
L.S.D. at 0.05	Treat. (T) =0.66 Rates (R)= 0.92 T×R = ***				Treat. (T) = 1.15 Rates (R)= 9.17 T×R = ***			

Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio. - Treatment (T) – Application Rates (R)

The uptake of Zn in seeds was significant increases with or without bio-fertilizer combined nitrogen fertilizers rates, while the Fe uptake in seeds was significant without bio-fertilizer and Mn uptake in seeds was significant increase with rates of nitrogen fertilizers sources combined with bio-fertilizer.

The interaction between different rates and different nitrogen forms, it was found that Fe, Mn and Zn concretions and uptake in seeds were affected significant by different treatments. These results are in agreement by [29] suggested that the application of N fertilizers significant increase Fe, Mn and Zn uptake seeds cowpea may be attributed to the role of microorganisms in improving these Fe, Mn and Zn available in soil and seeds cowpea.

3.2.3 Effect of Different Nitrogen forms and Application Rates Combined with Bio-fertilizer on Cowpea Quality

Data in Tables 16, 17 show that the increase of mean value of protein (%), chlorophyll (mg g^{-1} f.w.) and proline (%) content in cowpea plants as affected with different nitrogen forms combined with bio-fertilizer. The highest values of protein percentage, chlorophyll (mg g^{-1} f.w.) and proline (%) content in cowpea plants treated with 75% N fed^{-1} were by application ammonium sulphate combined with bio-fertilizer. The effect of different nitrogen forms combined with bio-fertilizer on protein (%), chlorophyll (mg g^{-1} f.w.) and proline (%) content in cowpea plants were not significant, while the different rates of nitrogen sources alone on protein (%) while, the chlorophyll was significant increase with nitrogen forms combined with bio-fertilizer compared with control, where the best values were by application ammonium sulphat combined with bio-fertilizers. The Proline (%) was significant as affected with different rates of nitrogen fertilization combined with bio-fertilizer.

The interaction between the nitrogen sources fertilizers and rates were significant for protein and proline contents in cowpea plant. [38] found that bio-mineral fertilization was more effective in increasing protein content of peanut plants as

Table 16. Effect of different nitrogen forms and application rates on of Protein and total chlorophyll of cowpea

Treatments	Protein (%)			Mean	Total Chlorophyll (mg g^{-1} f.w.)			Mean
	Rates of N forms				Rates of N forms			
	Control	75%	50%		Control	75%	50%	
AN + Bio	17.38	24.88	22.13	21.46	20.14	23.85	22.39	22.13
AS + Bio	17.50	25.25	24.31	22.35	21.67	25.55	24.88	24.03
Urea + Bio	17.81	25.00	23.56	22.12	21.44	24.89	23.98	23.44
Mean	17.56	25.04	23.33		21.08	24.76	23.75	
L.S.D. at 0.05	Treat. (T) =NS Rates (R)= 1.61 T×R = **				Treat. (T) =0.77 Rates (R)= 1.14 T×R = **			

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio – Not significant (NS) - Free water (f.w.) - Treatment (T) – Application Rates (R)*

Table 17. Effect of different nitrogen forms and application rates on Proline of cowpea

Treatments	Proline (mg g^{-1} f.w.)			Mean
	Rates of N forms			
	Control	75%	50%	
AN + Bio	28.78	55.20	40.88	41.62
AS + Bio	35.20	58.93	45.90	46.68
Urea + Bio	30.88	56.00	42.97	43.28
Mean	31.62	56.71	43.25	
L.S.D. at 0.05	Treatments (T) = 1.04 T×R = ***		Rates (R)= 3.71	

**Notes: Ammonium nitrate (AN) – Ammonium sulphate (AS) – control 100% mineral without bio - Free water (f.w.) - Treatment (T) – Application Rates (R).*

compared with the individual mineral fertilization. The proline increase with decreasing different rates of nitrogen sources this result attributed due may be to the increase of soil salinity. These results are on agreement by [39] revealed that the increases in proline and concentration by increasing salt level. In addition, proline protects membranes and proteins against the adverse effects of high concentration of inorganic ions. It also functions as a hydroxyl radical scavenger. On the other hand, the chlorophyll content in cowpea content increase with increasing nitrogen fertilizers sources rate especially plants treated with ammonium sulphate single or combined with biofertilizer. These results may be decreased of soil salinity. These results are in agreement by [40] indicated that the increase of levels of nitrogen fertilizer application led to the increase of chlorophyll content in plants.

4. CONCLUSION

The obtained results, indicated that the increasing application rate of ammonium sulphate 75% combined with bio-fertilizer lead to increase improve soil fertility and Cowpea productivity under saline soil conditions compared with other treatments.

From the aforementioned, it is recommended to use ammonium sulfate as source of nitrogen in combination with biological fertilizers at rate 75% N lead to increase improve soil fertility and Cowpea productivity under saline soil conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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