Review Paper

Impact of different nitrogen sources on improve nutrients in soil, cowpea productivity and quality under newly saline soil conditions

Abstract

Two field experiments were conducted at El-Rod village at Sahl El-Houssinia, El-Sharkia governorate, (32°15' 00" N 30°50' 00" E), Egypt.. The study investigated evaluations of different nitrogen fertilizers sources (calcium nitrate, ammonium sulphate and urea fertilizers) and rates (0, 20, 30 and 40 kg N/fed)alone or combined with bio-fertilizer (using bio-fertilization with *Rhiobium radiobacter* sp strain on improve nutrients available and contents in cowpea productivity and quality in newly reclaimed saline soil conditions during two summer seasons 2016 and 2017. The studies treatments were disturbed among the experimental pelts in split design with six replicates. The obtained results cleared that the used ammonium sulphate fertilizer was increase effect with increasing rate in EC , pH, and available N, P, K , Fe, Mn and Zn content in soil. Moreover data recorded the applied 30 kg N /fed was increase of plant height, weight of 100 seeds, weight of pods (g/plant) , weight of seeds (g)/plant and seeds yield (ton fed^{-1}) have been affected by inoculation with bio-fertilizer combined with nitrogen sources fertilizers and different rates than other treatments. The highest values of N, P , K, Fe, Mn and Zn concentrations and uptake in seeds treated with ammonium sulphate at rate 30 kgN/fed than 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 sulphat at 30 kg N/fed combined with bio-fertilizer was improve soil properties and cowpea productivity and quality under saline soils conditions.

Key word: available nutrients, concentration and uptake, cowpea productivity , cowpea quality , and saline soils.

Introduction

Salinity and soil nutrient deficiencies are the main factors reducing plant productivity in arid and semiarid areas. Among the essential elements, nitrogen is usually the most growth limiting plant nutrient in saline or non-saline soils, **Irshad et al (2002).** Improving salt affected soils may be achieved using different practices such as sub soiling, mole drain, soil amendments, farm manure and biofertilizer. These previous practices are important tools for improving crop productivity and soil properties in salt affected soils at the North Delta, **Saied et al (2017).**

In Egypt, cowpea cultivation area according to Agricultural economic bulletin, 2013 was about 14830 feddan with production of about 17248 tons with (an average yield of 1.163 ton/feddan). 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 **(Khatab and Samah, 2013).** 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, **Bashandy and El-Shaieny (2016).** Decrease of pod fresh, seed yield and weight of 100 seeds of Cowpea with increasing soil salinity, **Ahmed et al (2005).** The yield significant increase of cowpea yield component with decreased soil salinity stress, **Taffouo et al (2009).**

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 NO3-N from agricultural systems has caused public concerns for decades (**Ersahin and Rustu Karaman, 2001).** 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 resulted in decrease of base saturation in N treatments and this effect was more intensive with higher doses of N fertilization, **Vladimir et al (2017).**

Calcium Nitrate is a white granular soluble fertilizer that has two kinds of nutrients and that is easily absorbed by the plant. It contains 15.5% nitrogen (N) and 26.5% calcium oxide (CaO). ~~14%~~ of nitrogen originates from nitrate (NO_3) and 15% of nitrogen originates from ammonium (NH_4). Calcium oxide that is completely soluble in water contains 19% calcium (Ca). Soluble calcium and nitrate nitrogen provides various advantages, which other fertilizers do not have, for the plants. Application of calcium to plants ~~was~~ decreased ~~of~~ Na^+ content in plants parts and increased the K content, **Dabuxilatu (2005).**

The bio fertilizers are environmental friendly and contain organisms 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. Bio fertilizers are defined as preparations containing live or latent cells of efficient strains of micro- organism used for applications to seed, soil or composting preparation. The seeds inoculation with bio fertilizers significantly influenced the total phosphorus, available phosphorus, dehydrogenase enzyme activity and alkaline phosphate activity in cowpea, **Sushila et al (2017).**

The aim the present work was to improve of salt affected soil and cowpea productivity using some mineral nitrogen fertilizer sources alternative bio-fertilizer is a trend optimization to reclaimed soil health and reduce pollution and increase their cowpea production.

Materials and Methods

Two field experiments were carried out in clay saline soil at village El-Rowad in Sahl El-Hussinia, El-Sharkia governorate during two successive summer seasons 2017 and 2018 respectively, to study the evaluation of applied for calcium nitrate (20 % N), ammonium sulphate (20 % N) and Urea (46 % N) different rates alone or combined with bio-fertilizer on some nutrient available contents in soil and cowpea production under newly reclaimed saline soil. The location lies between 32° / 00 to 32° / 15, N latitude and 30° / 50 to 31° / 15 E longitude.

The main physical and chemical sample soils before cowpea planting according to the methods described by **Page et al (1982) and Kult (1986).** The obtained ~~data~~ were recorded in Table 1.

Table 1 Physical and chemical properties in soil study before cowpea

Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)		Texture	O.M gkg^{-1}		$CaCO_3$ gkg^{-1}
5.29	25.90	30.10	38.71		Clay loam	5.9		107.4
F.C.	W.P.	A.W.	B.D (mg/m^3)			T.P (%)		
28.39	10.56	12.90	1.45			43.00		
Chemical properties								
pH (1:2:5)	EC (dS/m)	Cations ($mmolcl^{-1}$)				Anions ($mmolcl^{-1}$)		
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO^{-}_3	Cl^{-}	SO^{--}_4
8.09	10.23	15.29	21.10	65.06	0.85	10.30	53.90	38.10
Macronutrients ($mgkg^{-1}$)			Micronutrients ($mgkg^{-1}$)					
N	P	K	Fe	Mn	Zn	Cu		
38.59	4.23	175	3.78	2.12	0.63	1.02		

In both seasons, each experiment was carried out in split plot design six replicates. The used three rates of N fertilizers ~~mean~~ plot while the sources of nitrogen fertilizers sub ~~mean~~ plot. The area of each experimental plot was 5 X 10 m (5 m width and 10 m long) which divide two division, first division was bio-fertilizer *Rhizobium radiobacte* 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. Second division without bio-fertilizer and density between rows 65 cm.

Field experiments:-

The soils of all the studies experimental pilot units are subjected to some pretreatments processes as follows :- a) leveling the soil surface by using lazar technique. b) Deep sub-soiling plough. c) Establishment of filed drains at a distance of 10 m between each of tow drains and a deep of 90 cm at drain beginning , their drainage water flow towards the main collectors of 2 m in depth and d) establishment of an irrigation canal in the middle part of the experimental pilot unit. Area was ploughed twice after receiving phosphorus (31 % P_2O_5) using ordinary super phosphate (15.5 % P_2O_5) during seed bed preparation.

Seeds of cowpea Kafr El-Shakh 1 variety *(Vigna unguiclata* L.) were supplied from Veg. Res. Dept . Hort. Res. Agric. Res. Center ARC. Sowing was carried out 25 April 2017 and 2018. Two and three of coated seeds or no coated were sown in hole with 5 cm. After 30 days from planting were the plant of each hole thinned to one plant. The biofertilizer was applied at a rate of 750 g for 7 kg seeds wetted with 400 ml of adhesive liquid (Arabic gum). 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 biofertilization was added 3 periods at 31, 45 and 65 days after planting through liquid sprays on soil at a rate of 20 L mixed with 400 L water /fed. Also, the soil fertilizer by potassium sulphat (48 % K_2O) was applied at rate 75 kg/fed on three periods 31, 45 and 65 days from planting.

Nitrogen sources

Calcium nitrate (20 % N) with or without bio-fertilizer; ammonium sulphate (20 % N) with or without bio-fertilizer and urea (46 % N) with or without bio-fertilizer were applied on three times 21, 40 and 55 days from planting at rates 0; 20 , 30 and 40 kg N/fed.

Harvest was done on September, 20thand 25th for the two successive seasons of 2017 and 2018, respectively.

After harvest, sample of the surface soil layers (0-30cm) from each plot were taken. Samples were analyzed for EC (in soil past extract), pH (in 1:2.5 soils : water suspension) organic matter, calcium carbonate and available–N, P, K, Fe, Mn, Zn and Cu as described by **Coatine et al (1982) and Page (1982).**

Plant samples of three replicates were taken after 75 days from sowing. Sample of each experiment plot was prepared for some vegetative growth parameters and some physiological 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 oven dried at 70Co 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 **Chapman and Pratt (1961).** The plant content of N, P, K, Fe, Mn and Zn was determined in plant digestion using the methods described by **Cottenie *et al* (1982) and Page *et al*(1982).** Protein percentage of seeds was calculated by multiplying the nitrogen percentage by the factor 6.25, **Hymowitz et al (1972)**. Total chlorophyll was estimated in fresh shoot as described by **Witham et al (1972).** Proline (%) content was **Bates et al (1973).**

The obtained data were statistically analysis using the COSTAT program and L.S.D. test at the probability levels of 5% was calculated according to **Gomez and Gomez (1984).**

Results and Discussion

Effect of different sources of nitrogen fertilizers and different rates on saline soil properties.

Soil pH.

Soil pH is one the more important parameter which reflects the overall change in soil chemical. Data in Table 2 show that the application of different nitrogen fertilizers sources alone or combined with bio-fertilizer on soil pH was positive effect. The soil pH values ranged around 8.09 in initial soil and 7.98 after harvest. The lowest pH value 7.98 and 8.00 recorded with soil application calcium sulphate at rate 40 kg N/fed combined with or without bio-fertilizer. Concerning that increasing rates of mineral nitrogen sources fertilizer application combined with bio-fertilizer gave decreased of soil pH. This result is in agreement by **Shaban and Omar (2006)** found that the effect of different mineral nitrogen fertilizer combined with bio-fertilizer on Dehydrogenas 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. **Rasouli *et al.*, (2013)** 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.

Fageria et al (2010) reported that the soil pH was decreased with increasing N rate by ammonium sulphate and urea fertilizers. **Ayub et al (007)** found that the applied of ammonium sulphate on saline soil was decreased of soil pH from (8.5 – 7.8) after 20 week.

Table 2 Effect of different nitrogen sources with or without bio-fertilizer on some soil properties.

Treatments	Rate of N (kg/fed)	pH (1:2.5)		EC (dSm^{-1})		N ($mgkg^{-1}$)		P ($mgkg^{-1}$)		K ($mgkg^{-1}$)	
		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer	
		With	Non	With	Non	With	Non	With	Non	With	Non
Calcium nitrate	0	8.06	8.07	8.85	9.02	40.23	39.85	4.89	4.66	185.00	180.00
	20	8.04	8.05	7.54	8.60	41.50	40.22	5.97	4.85	193.00	183.00
	30	8.01	8.04	6.30	7.33	41.96	40.90	6.03	4.93	195.00	187.00
	45	8.00	8.02	5.26	7.00	42.22	41.30	6.10	5.02	198.00	190.00
Mean				6.99	7.99	41.48	40.57	5.75	4.87	192.75	185.00
Ammonium sulphate	0	8.04	8.05	7.10	8.30	40.78	40.13	4.95	4.75	187.00	183.00
	20	8.02	8.03	6.22	7.85	41.89	40.88	6.05	4.97	199.00	188.00
	30	8.00	8.02	5.00	6.20	42.55	41.50	6.12	5.07	205.00	193.00
	45	7.98	8.00	4.55	5.11	44.10	41.99	6.26	5.10	211.00	197.00
Mean				5.72	6.87	42.33	41.13	5.85	4.97	200.50	190.25
Urea	0	8.05	8.06	7.67	8.59	40.65	40.00	4.90	4.80	186.00	179.00
	20	8.03	8.04	6.50	7.30	41.77	40.99	6.00	4.90	195.00	183.00
	30	8.00	8.02	5.18	6.10	42.30	41.40	6.09	4.98	198.00	189.00
	45	7.99	8.01	5.00	5.84	43.76	41.88	6.18	5.06	204.00	194.00
Mean		--	--	6.09	6.96	42.12	41.07	5.79	4.94	195.75	186.25
LSD. 5 % sources		--	--	ns	ns	ns	ns	ns	ns	ns	ns
LSD 5 % Rate		---	--	0.33	1.26	ns	ns	ns	ns	1.49	ns
Interaction		--	--	**	**	ns	ns	**	ns	**	**

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

As for soil salinity, the obtained data in Table (2) suggested that the application of different nitrogen fertilizer sources caused an appreciated decrease in the EC values. Soil salinity was no significant as affected with nitrogen sources, 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 of 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 combined with bio-fertilizer. The relative decreases of mean values EC (dSm^{-1}) was 11.18 and 7.52 % for soil treated with calcium nitrate combined with or without bio-fertilizer compared mean values of soil without nitrogen sources. On the other hand, the relative decreases of mean values EC (dSm^{-1}) soil treated with ammonium sulphate different rates were 27.32 and 20.49 % for soil treated with ammonium sulphate different rates combined or without bio-fertilizer compared soil without nitrogen sources. The relative decreases of mean values soil salinity (dSm^{-1})

treated with urea fertilizer different rates combined with or without bio-fertilizer was 22.62 and 19.44 % than soil without nitrogen sources.

The efficiencies of nitrogen fertilizers source in decreasing soil EC arranged as follow: ammonium sulphate > urea > calcium sulphate > without nitrogen sources. This trend can be due to *Rhizobium* producing phyto-hormones such as indole acetic acid, cytokinines 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, **Helmy et al (2013).** **Sina et al (2012)** reported that the application of ammonium sulfate caused the decreased in soil salinity as compared to urea.

Macronutrient available content in soil study.

~~Data~~ in Table 2 showed that the values of the available macronutrients N, P and K (mg kg^{-1}~~)~~content in soil as affected by N- sources i.e. calcium nitrate, ammonium sulphate and urea rates alone or combined with bio-fertilizer. Soil available N, P and K increased as increasing rate alone or combined with bio-fertilizer. The application of nitrogen fertilizers and different rates combined with or without bio-fertilizer on N and P available was no significant, while the K content in soil increase significant with increasing rate of nitrogen sources combined with bio-fertilizer. As well as, the interaction between nitrogen fertilizer sources and different rates on N and P content in soil was no significant, while the P and K in soil treated with nitrogen fertilizers sources and rates combined with bio-fertilizer was significant. These results are in agreement by **Shaban and Attia (2009)** 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.

The relative increases of mean values of N, P and K available in soil was 7.49 % for N , 35.93 % for P and 10.14 % ; while 5.13 % for N , 15.13 % for P and 5.71 for K content in soil treated with calcium nitrate different rate combined with bio-fertilizer compared with initial soil. On the other hand, the relative increase of mean values soil treated with ammonium sulphat different rate combined with bio-fertilizer was 9.69 % for N, 38.20 % for P and 14.57 % for K, while the soil without bio-fertilizer was 6.58 % for N, 17.49 % for P and 8.71% for K compared with initial soil. Also, the relative increases of mean values N, P and K contents in soil treated with urea combined bio-fertilizer was 9.15% for N , 36.88 % for P and 11.86 % for K , while the soil without bio-fertilizer was 6.43 % for N , 16.78 % for P and 6.43 % for K than initial soil. In general, the positive effects of the used different nitrogen sources different rate alone or combined with bio-fertilizer on available N, P and K could be arranged in the following order : ammonium sulphate > urea > calcium nitrate > 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. **Shaban and Omar (2006)** found that the application of mineral nitrogen fertilizer combined with bio-fertilizer had decreased soil pH and increased available N, P and K.

Micronutrients available in soil.

The availability of some micronutrients depended on the pH of soil date in Table 3 show that the variation in available micronutrient contents of soil namely Fe, Mn and Zn (mg/kg soil), results from nitrogen fertilizer sources and different rates alone or combined with bio-fertilizer were increase with increasing rate of nitrogen sources especially soil treated with ammonium sulphate alone or combined with bio-fertilizer. On the other had, the effect of different nitrogen sources alone or combined with bio-fertilizer on Fe, Mn and Zn available were no significant, while the different rates of nitrogen sources on Mn available was significant increase with increasing rates alone or combined with bio-fertilizer. As well As, the Zn available content in soil treated with different rates nitrogen fertilizers sources combined with bio-fertilizer was significant increase with increasing different rates. Interaction between nitrogen fertilizers sources and different rates on Fe, Mn and Zn available in soil was no significant for Fe, while, Mn and Zn were significant increase in soil as affected with interaction between nitrogen sources and different rates alone or combined with bio-fertilizer. **Gilliam *et al.* (2011)** indicated that the soil treated with bio-fertilizer improved soil microbial activity and increase availability of nutrients.

Table 3 Effect of different nitrogen sources on micronutrients available in soil.

Treatments	Rate of N (kg/fed)	Fe ($mgkg^{-1}$)		Mn ($mgkg^{-1}$)		Zn ($mgkg^{-1}$)	
		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer	
		With	Non	With	Non	With	Non
Calcium nitrate	0	3.85	3.80	2.20	2.15	0.69	0.66
	20	3.89	3.84	2.31	2.18	0.82	0.73
	30	3.96	3.87	2.36	2.22	0.84	0.77
	45	4.02	3.89	2.40	2.28	0.88	0.79
Mean		3.93	3.85	2.32	2.21	0.81	0.74
Ammonium sulphate	0	3.87	3.83	2.25	2.17	0.72	0.69
	20	3.97	3.88	2.36	2.22	0.85	0.77
	30	4.03	3.92	2.44	2.29	0.88	0.80
	45	4.09	3.95	2.48	2.35	0.93	0.85
Mean		3.99	3.90	2.38	2.26	0.85	0.78
Urea	0	3.86	3.81	2.22	2.16	0.71	0.67
	20	3.94	3.86	2.34	2.21	0.83	0.75

	30	3.98	3.95	2.41	2.24	0.86	0.78
	45	4.04	3.98	2.44	2.32	0.91	0.82
Mean		3.96	3.90	2.35	2.23	0.83	0.76
LSD. 5 % sources		ns	ns	ns	ns	ns	ns
LSD 5 % Rate		ns	ns	0.06	0.02	0.016	ns
Interaction		ns	ns	**	**	**	**

The relative increases of mean values Fe, Mn and Zn available in soil were 3.97, 9.43 and 28.57 % for soil treated with calcium nitrate combined with bio-fertilizer, while the Fe, Mn and Zn content in soil treated with calcium nitrate alone was 2.91, 4.25 and 17.46 %. Concerning, the relative increases of mean values Fe, Mn and Zn available in soil treated with ammonium sulphate different rates combined with bio-fertilizer were 5.56, 12.26 and 34.92 %, while soil treated with ammonium sulphate alone was 3.17 % for Fe, 6.60 % for Mn and 23.81 % for Zn available in soil than initial soil. Also, the relative increases with mean values of micronutrients available in soil treated with urea fertilizer different rates combined with bio-fertilizer were 4.76 for Fe, 10.85 for Mn and 31.75 % for Zn compared with initial soil, while, mean values of Fe, Mn and Zn were 3.17, 5.19 % and 20.63 % contents in soil treated with urea fertilizer alone compared with initial soil. **Ewees and Osman (2013)** reported that the soil with treated with bio- fertilizer in combination with N-mineral fertilizer caused progressive significant increases in all the studied available macronutrients and micronutrients than without bio-fertilizer.

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 bio-fertilizer could be arranged in the following order: ammonium sulphate > urea and > calcium nitrate > initial soil.

Effect of different sources nitrogen fertilizer and different rates alone or with bio-fertilizer on yield component.

Results from the present study indicated that plant height, weight of 100 seeds, weight of pods (g/plant) , weight of seeds (g)/plant and seeds yield (ton fed^{-1}) have been affected by inoculation with bio-fertilizer combined with nitrogen sources fertilizers and different rates were show in Table 4 indicated that there was no significant effect of different nitrogen sources alone or combined with bio-fertilizer , while the weight of pod/plant(g) was significant as affected with bio-fertilizer. The plant height (cm), weight seed (g)/plant and weight seeds yield (ton/fed) were significant increase with increasing different rates of nitrogen fertilizers sources combined with or without bio-fertilizer , while the 100 seeds (g) was no significant. The interaction between nitrogen sources and different rates combined with or without bio-fertilizer were significant increases all parameters of plants, except weight of 100 seeds was no significant. On the other hand, the relative increases of mean values were 14.29and 10.30 % for plant height (cm); 2.43 and 5.36 % for 100 seeds (g); 23.13 and 18.42 % for weight pod/plant (g); 28.90 and 10.46 % for seeds/plant (g) and 18.93 and 16.38 % for yield seeds (ton/fed) respectively as affected by calcium nitrates different rates combined with or without bio-fertilizer compared with without nitrogen fertilizers sources.

Table 4 Effect of different nitrogen sources on yield and yield component.

Treatments	Rate of N (kg/fed)	Plant length (cm)		100- seeds weight (g)		Pods weight g/plant		Seeds weight g/plant		Seeds yield Kg/fed	
		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer		Bio -fertilizer	
		With	Non	With	Non	With	Non	With	Non	With	Non
Calcium nitrate	0	75.90	71.57	14.80	11.92	16.65	14.33	13.20	11.89	720.10	650.30
	20	83.78	77.54	14.95	12.20	18.90	15.89	15.90	12.41	873.00	744.50
	30	92.85	79.34	15.34	12.65	22.87	16.97	18.36	13.85	910.74	795.00
	45	88.90	83.70	15.29	12.85	22.10	18.30	17.80	13.33	890.38	800.10
Mean		85.36	78.04	15.10	12.41	20.13	16.37	16.32	12.87	848.56	747.48
Ammonium sulphate	0	78.57	73.88	14.85	11.95	18.23	14.51	13.44	11.96	755.00	675.00
	20	88.70	79.44	15.38	12.60	22.40	15.96	17.30	13.20	920.00	789.00
	30	98.88	83.29	15.75	13.00	23.10	17.33	18.69	14.85	985.00	803.98
	45	95.97	87.77	15.44	13.40	22.78	19.29	17.99	16.00	943.88	919.00
Mean		90.53	81.10	15.36	12.74	21.63	16.77	16.86	14.00	900.97	796.75
Urea	0	77.85	72.66	14.83	11.93	16.98	14.42	13.75	12.00	773.00	685.00
	20	84.99	78.20	14.99	12.45	19.22	15.90	17.22	13.00	897.00	775.30
	30	94.80	80.55	15.49	12.89	23.00	17.00	18.45	14.66	940.00	795.00
	45	89.50	84.20	15.32	13.22	22.89	17.85	17.85	15.33	901.00	840.00
Mean		86.79	78.90	15.16	12.62	20.52	16.29	16.82	13.75	877.75	773.83
LSD. 5 % sources		ns	ns	ns	ns	0.71	ns	ns	ns	ns	ns
LSD 5 % Rate		0.67	1.57	ns	ns	1.41	ns	1.26	0.54	6.21	31.40
Interaction		**	**	ns	ns	*	**	**	**	**	**

Also, the relative increases of mean values were 22.06 and 14.86 for plant height (cm); 4.65 and 8.97 % for 100 seeds (g); 31.64 and 21.57 % for weight pod/plant (g); 33.66 and 22.66 % for seeds/plant (g) and 26.72 and 24.96 % for yield seeds (ton/fed) respectively as affected by ammonium sulphate different rates combined with or without bio-fertilizer compared with without nitrogen fertilizers sources. As well as the relative increases of mean values were 15.91 and 11.39 for plant height (cm); 2.97 and 7.71for 100 seeds (g); 25.51 and 17.34 % for weight pod/plant (g); 32.54 and 19.92 % for seeds/plant (g) and 21.79 and 19.90 % for yield seeds (ton/fed) respectively as affected by urea different rates combined with or without bio-fertilizer compared with without nitrogen fertilizers sources. This results may be due to the ammonium salphate 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 . **Ghodia (2012)** reported that the inoculation of cowpea seeds with rhizobia significant increased number pods/plant , number of seeds/pod, seed yield/plant and seed yield/fed compared un inoculation seed.

Macronutrients contents in seeds.

~~Data~~ presented in Table 5 illustrate ~~data~~ of macronutrients concentration and uptake by of seeds cowpea under different nitrogen sources alone or 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 4.04, 0.53 and 2.13 (%) and 39.97, 5.22 and 20.98 (kg/fed) uptake in seeds for seeds treated with bio-fertilizers combined ammonium sulphate rate with 30 kg/fed combined with other treatments. The results ~~are~~ in agreement by **Sina et al (2012)** indicated that the application of the maximum 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 sources on N, P and K concentrations were ~~on~~ significant while, the N uptake was significant for seeds treated without bio-fertilizer than other nutrients uptake. The ~~efferent~~ rates of nitrogen sources combined with or without bio-fertilizer were no 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 or without 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 sources and rates combined with or without bio-fertilizer for N, P and K concentrations and uptake in seeds cowpea were significant increases. **Ghodia (2012)** found that the increases in N, P and K content may be due to the interaction effect between rhizobial which consequently increased the uptake of nutrients in cowpea plant.

Table 5 . Effect of different nitrogen sources on macronutrients concentration and uptake in seeds of Cowpea

N-sources	Rate of N kg/fed	Concentration of N (%)		Uptake of N Kg/fed		Concentration of P (%)		Uptake of P (kg/fed		Concentration of K (%)		Uptake of K (kg/fed)	
		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer	
		With	Without	With	Without	With	Without	With	Without	With	Without	With	Without
Calcium nitrate	0	2.78	2.33	20.02	18.08	0.29	0.23	2.09	1.89	1.86	1.58	13.39	12.10
	20	3.54	2.89	30.90	26.36	0.42	0.35	3.67	3.13	1.95	1.66	17.02	14.52
	30	3.98	3.12	36.24	31.64	0.45	0.38	4.10	3.58	2.05	1.84	18.67	16.30
	45	3.20	3.49	28.49	25.60	0.33	0.42	2.94	2.64	1.90	1.89	16.92	15.20

Mean		**3.38**	**2.96**	**28.91**	**25.42**	**0.37**	**0.35**	**3.20**	**2.81**	**1.94**	**1.74**	**16.50**	**14.53**
Ammonium Sulphat	**0**	**2.80**	**2.60**	**21.14**	**18.90**	**0.32**	**0.25**	**2.42**	**2.16**	**1.88**	**1.59**	**14.19**	**12.69**
	20	**3.89**	**3.15**	**35.78**	**30.69**	**0.47**	**0.38**	**4.32**	**3.71**	**1.98**	**1.77**	**18.22**	**15.62**
	30	**4.04**	**3.70**	**39.79**	**32.48**	**0.53**	**0.44**	**5.22**	**4.26**	**2.13**	**1.87**	**20.98**	**17.12**
	45	**3.44**	**3.95**	**32.46**	**31.61**	**0.42**	**0.48**	**3.96**	**3.86**	**1.94**	**1.95**	**18.31**	**17.83**
Mean		**3.54**	**3.35**	**32.29**	**28.42**	**0.44**	**0.39**	**3.98**	**3.50**	**1.98**	**1.80**	**17.93**	**15.82**
	0	**2.85**	**2.55**	**22.03**	**19.52**	**0.34**	**0.24**	**2.63**	**2.33**	**1.87**	**1.63**	**14.46**	**12.81**
	20	**3.77**	**2.96**	**33.81**	**29.23**	**0.48**	**0.37**	**4.31**	**3.72**	**1.97**	**1.78**	**17.67**	**15.27**
	30	**4.00**	**3.26**	**37.60**	**31.80**	**0.50**	**0.40**	**4.70**	**3.98**	**2.06**	**1.85**	**19.36**	**16.38**
	45	**3.59**	**3.75**	**32.34**	**30.16**	**0.46**	**0.45**	**4.14**	**3.86**	**1.92**	**1.93**	**17.30**	**16.13**
Mean		**3.55**	**3.13**	**31.45**	**27.68**	**0.45**	**0.37**	**3.95**	**3.47**	**1.96**	**1.80**	**17.20**	**15.15**
LSD. 5 % sources		**ns**	**ns**	**ns**	**1.24**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
LSD 5 % Rate		**ns**	**ns**	**1.90**	**3.10**	**0.05**	**0.02**	**0.29**	**ns**	**0.02**	**0.05**	**0.64**	**ns**
Interaction		***	**	**	**	***	***	**	**	**	**	**	**

It is evident from the distribution patterns of N, P and K concentration and up take 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 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. **Kloepper (2003)** 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. **Massoud *et al.,* (2004)** who suggested, that inoculation with N_2-fixer bacteria increased uptake of N, P and K by pea plants.

Micronutrients concentration and uptake in seeds cowpea.

Data presented in Table 6 show the effect of different nitrogen fertilizers sources 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 30 kgN/fed 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 no significant . 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 of nitrogen fertilizers sources and nitrogen sources on Fe , Mn and Zn concretions and uptake in seeds were significant. These results are in agreement by **Helmy et al (2013)** 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.

Table 6 . Effect of different nitrogen sources on macronutrients concentration and uptake in seeds of Cowpea

N-sources	Rate of N kg/fed	Concentration of Fe (mg/kg)		Uptake of Fe (g/fed)		Concentration of Mn (mg/kg)		Uptake of Mn (g/fed		Concentration of Zn (mg/kg)		Uptake of Zn (g/fed)	
		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer	
		With	Without	With	Without	With	Without	With	Without	With	Without	With	Without
Calcium nitrate	0	65.30	62.83	470.23	424.65	49.83	45.49	358.83	324.04	32.59	29.94	234.68	211.93
	20	77.30	69.33	674.83	575.50	53.91	47.61	470.63	401.36	34.85	31.50	304.24	259.46
	30	79.10	74.30	720.40	628.85	55.30	49.72	503.64	439.64	35.87	32.88	326.68	285.17
	45	72.89	78.55	649.00	583.19	51.66	52.90	459.97	413.33	33.98	34.00	302.55	271.87
Mean		73.65	71.25	628.62	553.05	52.68	48.93	448.27	394.59	34.32	32.08	292.04	257.11
Ammonium Sulphat	0	68.20	64.98	514.91	460.35	50.51	46.77	381.35	340.94	33.20	30.22	250.66	224.10
	20	82.18	71.75	756.06	648.40	55.20	49.30	507.84	435.53	36.59	33.66	336.63	288.71
	30	85.33	77.39	840.50	686.04	58.99	54.20	518.05	474.27	39.21	35.71	386.22	315.57
	45	78.30	82.70	739.06	719.58	54.88	55.30	518.00	504.35	35.10	37.00	331.30	322.57
Mean		78.50	74.21	712.63	628.59	54.90	51.39	481.31	438.77	36.03	34.15	326.20	287.74
Urea	0	66.82	63.85	516.52	457.72	49.88	45.89	385.57	341.68	33.00	30.00	225.09	226.05
	20	80.22	69.98	719.57	621.95	54.30	48.22	487.07	420.99	34.99	32.91	313.86	271.28
	30	84.10	75.89	790.54	668.60	57.82	52.55	543.51	459.67	35.85	34.40	336.99	285.01
	45	80.77	80.66	727.74	678.47	53.11	54.20	478.52	446.12	34.29	35.11	308.95	288.04
Mean		77.98	72.60	688.59	606.69	53.78	50.22	473.67	417.12	34.53	33.11	296.22	267.60
LSD. 5 % sources		ns	ns	ns	1.19	ns	ns	1.86	ns	ns	ns	2.77	3.85
LSD 5 % Rate		4.33	4.10	ns	1.79	ns	2.00	2.94	ns	0.54	1.58	2.20	4.45
Interaction		***	**	**	**	***	***	**	**	**	**	**	**

Effect of nitrogen fertilizers sources and rates combined with or without bio-fertilizer on cowpea quality.

~~Data~~ in Table 7 show that the increase of mean value of protein (%) , chlorophyll (mg/g f.w.) and proline (%) content in cowpea plants as affected with nitrogen fertilizers sources alone and with combined with bio-fertilizer. The highest values of protein (%), chlorophyll (mg/g f.w.) and proline (%) content in cowpea plants treated with 30 kgN/fed ammonium sulphate combined with bio-fertilizer, while all other sources alone were 40 kgN/fed. The effect of different nitrogen sources combined with or without bio-fertilizer on protein (%), chlorophyll (mg/g f.w.) and proline (%) content in cowpea plants were no significant, while the different rates of nitrogen sources alone on protein (%) while, the chlorophyll was significant increase with nitrogen sources combined with bio-fertilizer. The Proline (%) was significant as affected with different rates of nitrogen fertilization combined with bio-fertilize.

Table 7 . Effect of different nitrogen sources on quality of Cowpea

N-sources	Rate of N kg/fed	Protein (%)		Total Chlorophyll (mg/g f.w)		Proline (mg/g f.w)	
		Bio-fertilizer		Bio-fertilizer		Bio-fertilizer	
		With	Without	With	Without	With	Without
Calcium nitrate	0	17.38	14.56	20.14	17.30	58.93	65.90
	20	22.13	18.06	22.39	18.00	45.90	63.00
	30	24.88	19.50	23.85	18.57	35.20	50.13
	45	20.00	21.81	22.00	19.32	40.30	59.20
Mean		21.10	18.48	22.10	18.30	45.08	59.56
Ammonium Sulphat	0	17.50	16.25	21.67	18.22	55.20	63.89
	20	24.31	19.69	24.88	19.97	40.88	59.80
	30	25.25	23.13	25.55	20.87	28.78	55.30
	45	21.50	24.69	23.99	21.10	33.75	60.70
Mean		22.14	20.94	24.02	20.04	39.65	59.92
Urea	0	17.81	15.94	21.44	18.00	56.00	63.98
	20	23.56	18.50	23.98	19.23	42.97	60.45
	30	25.00	20.38	24.89	20.75	30.88	47.10
	45	22.44	23.44	24.59	21.44	33.00	52.90
Mean		22.20	19.57	23.73	19.86	40.71	56.11
LSD. 5 % sources		ns	ns	ns	ns	ns	ns
LSD 5 % Rate		ns	1.80	1.14	ns	3.71	1.43
Interaction		**	***	**	ns	***	***

The interaction between the nitrogen sources fertilizers and rates were significant for protein and proline contents in cowpea plant. **Mabrouk (2002)** found that bio-mineral fertilization was more effective in increasing protein content of peanut plants as 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 **Kapoor and Srivastava (2010)** 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 bio-fertilizer. These results may be decreased of soil salinity. These results ~~are~~ in agreement by **Siam et al (2013)** indicated that the increase of levels of nitrogen fertilizer application led to the increase of chlorophyll content in plants.

Conclusion

From the obtained results it could be concluded that the increasing the rate of ammonium sulphate combined with bio-fertilizer application is useful to obtain increase improve soil contents in nutrients under saline soil conditions and the application of 30 kg N/fed was increases of cowpea yield and quality.

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