Journal of Experimental Agriculture International

43(4): 56-63, 2021; Article no.JEAI.68867
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Yield and Total Nutrient Uptake Influenced by Soil Salinity, Phosphorus Sources and Biofertilizers in Cowpea (*Vigna unguiculata* L.)

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Authors' contributions

This work was carried out in cooperation among all authors. Author BLY designed the study, managed the analyses of the study performed the statistical analysis, wrote the protocol. Authors KD, RB and LJ managed the literature and wrote the first draft of the manuscript. All authors read and approved the final manuscript

Article Information

DOI: 10.9734/JEAI/2021/v43i430672
Editor(s):
(1) Prof. Mohamed Fadel, National Research Center, Egypt.
Reviewers:
(1) Tarek M. Abdelghany, AL-Azhar University, Egypt.
(2) Ruly E. K. Kurniawan, Jenderal Soedirman University, Indonesia.
(3) Mohammadreza Mahdikhani, University of Western Australia (UWA), Australia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/68867

Original Research Article

Received 23 March 2021
Accepted 03 June 2021
Published 14 June 2021

ABSTRACT

A pot experiment was laid out at Sri Karan Narendra College of Agriculture, Jobner in 2015 during *kharif* season using cowpea as a test crop to study the influence of soil salinity, phosphorus sources and biofertilizers on yield and total nutrient uptake by cowpea (*Vigna unguiculata* L.). The experiment was tested in completely randomized design in which three levels of each salinity (EC 1.22, 4.0 and 6.0 dS/m), phosphorus (single super phosphate, di ammonium phosphate and phosphorus rich organic manure) and biofertilizers (control, phosphorus solubilizing bacteria and phosphorus solubilizing bacteria + vesicular arbuscular mycorrhiza) were used with three replications. The experimental data showed that soil salinity (EC 1.22 dS/m) recorded significantly higher yield and total nutrient uptake by cowpea in comparison to other treatments. But nitrogen content lowest recorded at EC 1.22 dS/m. Result further revealed that phosphorus source phosphorus rich organic manure recorded significantly maximum yield and total nutrient uptake by cowpea over rest of the treatments. Furthermore, seed inoculation with phosphorus solubilizing bacteria + vesicular arbuscular mycorrhiza recorded the maximum yield and nutrient uptake by

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cowpea over rest of the treatments. It's concluded that combination of EC 1.22 dS/m + phosphorus rich organic manure + Biofertilizers (phosphorus solubilizing bacteria + vesicular arbuscular mycorrhiza) found superior in all these parameters over the control.

Keywords: Biofertilizers; cowpea; phosphorus; PSB; salinity; VAM.

1. INTRODUCTION

Low quality groundwater is used for irrigation in many areas of arid and semi-arid regions. Soil salinity or sodicity is caused by the constant use of low-quality water for irrigation. Due to a lack of high quality water, this issue began in areas where saline / sodic ground water was used as a major source of irrigation. Unscientific and indiscriminate use of saline water for irrigation results in an accumulation of soluble salts in the root region, which has a negative impact on the physical and chemical properties of irrigated soils, lowering crop productivity due to decreased water availability to plants [1]. Because of the excessive build-up of salinity in the soil caused by irrigation with saline water, plant growth is either slowed or completely stopped. Salt-affected soils cover approximately 13.8 million hectares in the country [2] and 1.24 million hectares in Rajasthan, and are found to varying degrees in almost every district of the state [3].

For pulse crops, phosphorus is the most important mineral nutrient. Phosphorus, like nitrogen, is an essential nutrient, but available phosphorus in Indian soils is low to moderate. Just about 30% of the phosphorus added to crops is usable for crops, with the rest being converted to insoluble phosphorus. Several researchers have published on crop response to phosphorus application on sodic soils. Phosphorus, like nitrogen, is an essential nutrient. Its deficiency is the single most important factor contributing to low couple yield on all forms of soils [4]. It is found in nucleic acids such as ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), nucleoproteins such as ADP and ATP, amino acids, proteins, phosphatides, phytin, and a number of co-enzymes such as thiamine, pyrophosphate and pyrodoxyl phosphate. Different sources of phosphorus, such as DAP, SSP, rock phosphate, phosphogypsum, and phosphocompost (PROM), are used to fulfill the phosphorus requirements for various pulses or cowpea crops. PROM must now be a more effective source of phosphate application.

Many microorganisms play an important role for phosphate solubilization [5,6]. Phosphate solubilizing bacteria are heterotrophic and aerobic in nature, and they play an important role in increasing the availability of phosphorus to plants in phosphorus-deficient soils. The fungus Vesicular Arbuscular Mycorrhiza (VAM) is important in phosphorus cycling and plant phosphorus uptake. Improved phosphorus absorption increases the growth and yield of most crop plants as native and added phosphate are mobilized in the soil. It also improves crop plant resistance to biotic and abiotic stresses [7]. *Bacillus megatherium*, *Pseudomonas straita*, and *Bacillus polymixa* inoculants were found to be suitable and usable for seed inoculation after extensive testing. Phosphate applied to soil and nature is solubilized by these bacteria, making it available to plants for healthy development. When seeds are inoculated with phosphate solubilizing bacteria inoculants, there may be a significant reduction in added phosphorus. In comparison to the uninoculated control, seed inoculation with PSB culture increases green pod yield [6]. Solubilize 20-30% of the insoluble phosphate present in soil under favourable conditions, potentially increasing crop yield by 10-30% [8] Pulses are a good source of dietary protein, and they have a remarkable ability to preserve and restore soil fertility through biological nitrogen fixation and the addition of a lot of residues. Cowpea [*Vigna unguiculata* (L.) Wilczek] known as lobia in India is important *kharif* season pulse crops. Cowpea has great importance due to high yielding, availability of short duration and quick growing crop. The vegetable cowpea pods contain moisture 84.6%, protein 4.3%, carbohydrate 8.0% and fat 0.2% and green tender pods are used for vegetable purpose. An attempt was therefore made to study the mitigate the harmful effect of salinity on crop yield and total nutrient uptake with the application of phosphorus sources (PROM) and biofertilizers (PSB+VAM).

2. MATERIALS AND METHODS

2.1 Experimental Site and Layout

A pot experiment was conducted at Department of Plant Physiology, College of Agriculture, Jobner during 2015 in cage house in manner of completely randomized design (CRD) with three replications in which three levels of each salinity (1.22, 4.0 and 6.0 dS/m), phosphorus (SSP, DAP and PROM) and biofertilizers (control, PSB and PSB + VAM) and thereby, making 27 treatment combinations. The soil texture was loamy sand, bulk density, particle density, Na, Ca, Mg, CEC, exchangeable Na and ESP (1.52 Mg/m^3, 2.54 Mg/m^3, 9.51 me/100 g, 1.3 me/100 gm, 1.1 me/100 g, 7.9 cmol /kg, 0.64 cmol/kg and 9.54, respectively) in experimental soil. The experiment soil was consist pH (8.41), organic carbon (0.354%), nitrogen (128.10 kg/ha), phosphorus (22.24 kg P_2O_5/ha) and potassium (148.50 kg K_2O/ha) before the sowing of cowpea.

2.2 Treatment Application

To make the saline soils were added Cl^- and SO_4^{-2} of Na, Ca and Mg as solution keeping the ratio of 3:1 of Cl: SO_4 the ECe level to attain 4 and 6 dS/m and thoroughly mix in the soil before seeding (Table 1). The soil based VAM inoculation (*Glomus fasciculatum*) containing hyphae, spores, sporacarp and infected root fragments were incorporated into the soil @ 30 mg kg-1 soil (13-15 viable spore g^{-1} inoculum) in a uniform layer at a depth of about 5 cm. For Phosphate solubilizing bacteria, the seeds were inoculated with microphosculture i.e. *Bacillus megatherium var. phosphaticum* @ 5 g/kg seed as per routine procedure 2-3 hours before sowing and sown in ear marked pots. Entire dose of (40 kg /ha i.e. 20mg/kg soil) phosphorus through single super phosphate, diammonium phosphate and PROM (phosphorus rich organic manure) were thoroughly mixed in soil before sowing of the crop. The PROM was prepared locally by composting high grade rock phosphate (37/74) i.e. rock phosphate containing 34% P_2O_5 in (74 micron particle size) by using fresh cow dung in 1:2 ratio on dry weight basis.

2.3 Crop Management

Before the sowing cylindrical ceramic pots (20 cm diameter and 28 cm height) was filled with soil. Each pot contained 10 kg soil. During filling the pots, to allow free drainage of water were placed the broken pieces of stone in the bottom hole. The cowpea cv. 'RC-19' was shown on 7th July, 2015 with a seed rate of 5 seeds per pot. After the physiological maturity harvest the cowpea on 15th September, 2015. Three plants of each pot were harvested at maturity and tied up and kept on threshing floor for sun drying. After complete sun drying the produce of each pot was weighed for recording yield. After threshing, winnowing and clearing the produce of each pot was weighted separately and the weight recorded as grain yield in g per pot. For estimation of nitrogen, phosphorus, potassium content in representative samples of grain and straw taken at the time of threshing were ground to fine powder.

3. RESULTS AND DISCUSSIONS

3.1Yield of Cowpea

3.1.1 Effect of soil salinity

The results revealed that higher levels of salinity significantly ($p<0.05$) decreased grain and straw yield of cowpea in comparison to control (Table 2). The highest grain (5.50 g/pot) and straw yield (8.25 g/pot) of cowpea were recorded under S_1 (EC 1.22 dS/m) and lowest was observed under S_6 (EC 6.0 dS/m). In the salinity levels, S_4 and S_6 decrease to the extent of grain yield (10.88 and 60.81%) and straw yield (10.88 and 60.81%) over S_1 (normal soil), respectively. The increase in EC of soil might be due to the decreased grain and straw yield of cowpea by causing a restricted availability of water and nutrients to the plant. The substantial decreased recorded in yield of cowpea under the influence of different salinity levels. Similar findings also supported by [9] in cowpea and [10] in chickpea with the increasing level of soil salinity.

3.1.2 Effect of phosphorus sources

The experiment data showed that application of different levels of phosphorus significantly ($p<0.05$) increased the grain and straw yield of cowpea (Table 2). In compared to SSP and DAP, PROM were recorded significantly higher grain yield (5.38 g/pot) and straw yield (8.06 g/pot) of cowpea. The increase in grain and straw yield were obtained due to PROM to the extent of 47.39, 10.92, 46.81 and 11.01 percent, respectively over DAP and SSP. This might be due to excess assimilates stored in the leaves and later translocated into grains at the time senescing being the closest sink. So that ultimately increased the grain and straw yield due to the results of overall development and growth of plants. These results were confirmed with the finding of [11,12].

Table 1. Different salts and their ionic composition uses in base for creating different salinities

EC (dS/m)	mmol/kg					Final ECe (dS/m)
	Na^+	Ca^{+2}	Mg^{+2}	Cl^-	SO_4^{-2}	
1.00	9.7	1.2	1.4	2.3	6.0	1.23
4.00	15.6	5.7	5.7	7.8	23.0	4.15
6.00	25.6	11.4	12.2	1.28	38.0	6.12

Table 2. Effect of salinity, phosphorus and biofertilizers on yield and total nutrient uptake by cowpea

Treatments	Grain Yield (g/pot)	Straw Yield (g/pot)	Total N uptake (mg/pot)	Total P uptake (mg/pot)	Total K uptake (mg/pot)
Salinity					
S_1(1.22 dS/m)	5.50	8.25	235.0	38.5	191.4
S_4 (4 dS/m)	4.96	7.44	226.1	29.0	160.5
S_6 (6 dS/m)	3.42	5.13	161.0	17.9	110.1
SEm. ±	0.06	0.09	4.4	0.9	2.9
C. D. (P=0.05)	0.18	0.24	12.6	2.8	8.3
Phosphorus sources					
P_1(SSP)	3.65	5.49	153.0	18.1	121.3
P_2 (DAP)	4.85	7.26	217.6	27.0	158.5
P_3(PROM)	5.38	8.06	253.4	40.0	190.2
S. Em. ±	0.06	0.09	4.4	0.9	2.9
C. D. (P=0.05)	0.18	0.24	12.6	2.8	8.3
Biofertilizers					
B_0 (Control)	3.68	5.52	155.0	18.5	121.9
B_1 (PSB)	4.72	7.08	212.0	28.0	152.7
B_2 (PSB+VAM)	5.48	8.22	247.5	38.3	187.5
S. Em. ±	0.06	0.09	4.4	0.9	2.9
C. D. (P=0.05)	0.18	0.24	12.6	2.8	8.3

dS/m= Deci siemens per meter, SSP=Single super phosphate, DAP= Di Ammonium Phosphate, PROM=Phosphorus rich organic manure, PSB= Phosphorus solubilizing bacteria, VAM= Vesicular arbuscular mycorrhiza, S.Em= Standard error of mean and CD= critical difference

3.1.3 Effect of biofertilizers

The experimental data revealed that dual inoculation with PSB + VAM recorded significantly ($p<0.05$) maximum grain (5.48 g/pot) and straw yield (8.22 g/pot) of cowpea in comparison to seed inoculation with PSB alone and no inoculation (Table 2). PSB + VAM showed an increase of 16.10 and 48.91 percent in grain yield over PSB alone and no inoculation. In case of straw yield represent an increase of 16.20 and 48.92 per cent over PSB and no inoculation, respectively. Phosphate solubilizing bacteria solubilize insoluble fixed P in soil by producing organic acids in solubilizing minerals and phosphorylated minerals, which aids in the release of phosphorus from stable complexes with cations such as calcium and magnesium. Such reaction also prevents the fixation of phosphate ions. The results are similarly supported by [13,14].

3.2 Total Nitrogen Uptake by Cowpea

3.2.1 Effect of soil salinity

The results presented in Table 2 revealed that total nitrogen uptake by grain and straw were significantly ($p<0.05$) decreased with increasing levels salinity. The higher total nitrogen uptake by cowpea (235.0 mg/pot) was observed under S_1 treatment, which was at par with S_4 treatment. The increasing levels of salinity significantly reduced the total nitrogen uptake by cowpea. The S_4 and S_6 reduced the total nitrogen uptake by cowpea an extent of 3.79 and 30.82 per cent over control, respectively. This increase may be explained by [15] hypothesis that in plants grown in higher salinity, protoplast contraction breaks intercellular connections in many plant sections, resulting in a reduction in the exchange of water and nutrients between cells. The accumulation of nitrogen in chickpea grain has also been recorded by [16].

3.2.2 Effect of phosphorus sources

Further results revealed that total uptake of nitrogen by cowpea was significantly ($p<0.05$) increased with the application of PROM over to DAP and SSP (Table 2). The highest total uptake (253.4 mg/pot) of nitrogen by cowpea was recorded under PROM application. An increase in total nitrogen uptake by cowpea was observed 16.45 and 65.62 % over the DAP and SSP, respectively (Table 2). The increase in total nitrogen uptake may be attributable to a well-developed root system that increased the supply of phosphorus to soil microbes, resulting in increased *Rhizobium* bacteria multiplication and, in turn, increased atmospheric N_2-fixation through improved nitrogen utilization [17].

3.2.3 Effect of biofertilizers

The experimental data revealed that total nitrogen uptake by cowpea was significantly ($p<0.05$) improved with the application biofertilizers. The maximum total nitrogen uptake (247.5 mg/pot) was recorded under B_2 and lowest in control (without biofertilizers). The seed inoculation with PSB and soil treatment with VAM was observed an increase of 36.77 and 59.68 % over PSB and control, respectively (Table 2). It may be attributed to improved root growth as a result of increased phosphorus availability caused by PSB + VAM, as well as the secretion of growth-promoting substances [18]. VAM improved nutrient uptake [19] by shortening the distance nutrients must diffuse to plant roots and increasing the rate of nutrient absorption and concentration at the absorption surface and finally be chemically modifying the availability of nutrients for uptake by plants through mycorrhizal hyphae [20].

3.3 Total Phosphorus Uptake by cowpea

3.3.1 Effect of soil salinity

The experimental results showed that total phosphorus uptake by cropwas decreased significantly ($p<0.05$) with increasing levels of salinity (Table 2). The maximum decreased in total phosphorus uptake by cowpea (17.9 mg/pot) was observed under S_6 and it was lower by 24.68 and 53.51% over S_4 and control treatments. This decrease in total phosphorus uptake may be due to a synergistic relationship between SO_4^{2-} and PO_4^{-3} ions, as well as antagonistic relationships between Cl and PO_4^{3-} ions. Cl^- and P have been found to be antagonistic in wheat chickpea [21,16].

3.3.2 Effect of phosphorus sources

The total phosphorus uptake was significantly ($p<0.05$) improved with different levels of phosphorus (Table 2).The total phosphorus uptake by cowpea significantly increased with PROM (40.0 mg/pot) by cowpea due to application of PROM was recorded by 48.14 and 120.99 percent over the DAP and SSP, respectively. The balanced nutrient status of soil, which was deficient in N and P and medium in K, could be attributable to the increased availability of phosphorus status in soil, which increased nutrient absorption both macro and micro with P fertilization. The availability of improved the root system of the plant, resulting in more P accumulation in the crop. These results were also reported by [22,23].

3.3.3 Effect of biofertilizers

Moreover, the seed inoculated with PSB and soil inoculated with VAM was observed significantly ($p<0.05$) higher total phosphorus uptake by grain over PSB and no inoculation. Total phosphorus uptake was recorded an increase of 51.35 and 107.03 % over PSB alone and control (Table 2). When grains were inoculated prior to sowing, nutrient absorption by crops was increased, which can be explained by increased basic activities of isocitric and malic dehydrogenase, the source of electrons for fixation [24], resulting in a better nutritional setting. The interaction of two or more species, as well as improved phosphorus absorption due to solubilization efficiency of two or more organisms and increased uptake of phosphorus under VAM treated pots was also reported by [7].

3.4 Total Potassium Uptake by Cowpea

3.4.1 Effect of soil salinity

The perusal of data show that total potassium uptake was decreased significantly ($p<0.05$) with increasing levels of salinity (Table 2). The highest total potassium uptake (191.4 mg/pot) was recorded under S_1 (Normal soil). Total potassium uptake was decreased to the extent 16.14 and 42.48% due to S_6 over S_1 and S_4, respectively. This is because the concentration of Na in the soil solution has increased. Since Na competes with K for absorbing sites, increased Na concentration in soil solution causes more Na absorption by plants and lowers K uptake. According to the hypothesis of [25] the antagonistic, a crop's ability to develop under high Na saturation is due to the toxic effect of Na itself and K deficiency caused. These same findings also supported from the work of [16,25] who reported a reduction in K content with increasing level of soil salinity.

3.4.2 Effect of phosphorus sources

Furthermore, the total potassium uptake was significantly ($p<0.05$) improved with different levels of phosphorus (Table 2). The highest total potassium uptake (190.2 mg/pot) was observed under PROM application and lowest observed under control. An increase in total potassium uptake due to PROM application by cowpea was recorded in 30.67 and 56.80 % over the DAP and SSP, respectively. The Na^+ ion reacts with soil-P and forms an insoluble form (Na-phosphate), reducing the amount of Na available to plants as the level of phosphorus rises. Plants can also substitute Na^+ cation with $H_2PO_4^-$ anion from exchangeable sites, resulting in a decrease in Na absorption ultimately K uptake increase and Na content decrease in grain and straw [16].

3.4.3 Effect of biofertilizers

The seed treatment with PSB and soil treatment with VAM recorded significantly ($p<0.05$) higher total potassium uptake by cowpea over control and PSB (Table 2). The highest total potassium uptake was obtained under PSB+VAM and lowest in control. An increase in total potassium uptake by cowpea was extent of 25.27 and 53.81 % in PSB+VAM over PSB and no inoculation. When grains were inoculated before to sowing, nutrient absorption by crops was increased due to resulting in a better nutritional setting which can be explained by increased basic activities of isocitric and malic dehydrogenase the source of electrons for fixation [26]. The interaction of two or more species, as well as improved phosphorus absorption due to solubilization effect of two or more organisms and increased uptake of phosphorus was also reported by [7]. These findings are in confirmation with findings of [27].

3.5 Interaction Effect

3.5.1 Soil salinity and phosphorus sources

The interactive effect of saline soils and phosphorus sources on grain and straw yield was found significant (Table 3) and data revealed that the grain and straw yield of cowpea increased significantly ($p<0.05$) with combination of EC 1.22 dS/m (S_1) and PROM application (P_3) over the rest of treatments. The highest grain (6.40 g/pot) and straw yield (9.59 g/pot) were observed under treatment combination S_1P_3 (EC 1.22 dS/m and PROM application) and the lowest grain and straw yield obtained under S_6P_1 (EC 6 dS/m and SSP application). This might be due to the harmful effect of salinity can be mitigated by applying PROM application [16].

3.5.2 Soil salinity and biofertilizers

The interactive effect of soil salinity and biofertilizers on grain and straw yield was noted significant (Table 4) and data cleared that the grain and straw yield increased significantly ($p<0.05$) with normal soil (S_1) and dual inoculation (PSB+VAM) over the other treatments. The highest grain (6.51 g/pot) and straw yield (9.78 g/pot) were recorded under treatment combination S_1B_2 (EC 1.22 dS/m and dual inoculation PSB+VAM and the lowest grain and straw yield obtained under S_6B_0 (EC 6 dS/m and no inoculation). This may be due to the detrimental effect of salinity can be reduced by applying PSB+VAM [24]. Because PSB+VAM increased nutrient supply through increase nutrients solubility from fixed sites than Na. React with soil-P and get precipitated in their insoluble form (Na- phosphate) by which availability of Na to plant becomes very less and higher Ca and Mg availability [25,27] so that improved the plant production.

Table 3. Interactive effect of soil salinity and phosphorus sources on grain and straw yield

Treatments	Grain yield (g/pot)			Straw yield (g/pot)		
	S_1	S_4	S_6	S_1	S_4	S_6
P_1	4.34	3.91	2.70	6.53	5.89	4.06
P_2	5.76	5.20	3.58	8.64	7.79	5.37
P_3	6.40	5.77	3.98	9.59	8.65	5.96
S.Em. ±	0.11					
C. D.(P=0.05)	0.31					

Table 4. Interactive effect of soil salinity and biofertilizers on grain and straw yield

Treatments	Grain yield (g/pot)			Straw yield (g/pot)		
	B_0	B_1	B_2	B_0	B_1	B_2
S_1	4.37	5.61	6.51	6.56	8.42	9.78
S_4	3.95	5.06	5.88	5.92	7.59	8.81
S_6	2.72	3.49	4.05	4.08	5.23	6.07
S.Em. ±	0.11					
C. D.(P=0.05)	0.31					

4. CONCLUSION

Based on the results of the experiments, it seems to assume that the application of phosphorus (PROM) and seed inoculation with PSB and soil inoculation with VAM increased cowpea productivity and improved total nutrient uptake. On the other hand, higher salinity levels have a negative impact on the cowpea yield and nutrient uptake by cowpea.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Soil Science and Agricultural Chemistry, SKN College of Agriculture, Jobner (Rajasthan), for providing experimental facilities, as well as the Dean, SKN College of Agriculture, Jobner, for providing investigational facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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DOI: 10.4172/2157-7471.1000305
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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/68867