International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(4): 1433-1437

Received: 25-05-2017
Accepted: 26-06-2017

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Effect of soil salinity, sources of p and biofertilizers on soil microbial biomass p and salinity tolerance of cowpea [*Vigna unguiculata* (L.) Wilczek]

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Abstract
A pot experiment was conducted in 2015 at S.K.N. College of Agriculture, Jobner during *kharif* season using cowpea as a test crop to investigate the effect of phosphorus management in cowpea grown on saline soils. Three levels each of saline soils (EC 1, 4.0 and 6.0 dS/m), phosphorus sources (SSP, DAP and PROM), and biofertilizers (control, PSB and PSB + VAM), were tested in completely randomized design with three replications. The results indicated that application of soil salinity having EC 1dS/m recorded the maximum and significantly higher microbial biomass P of cowpea over rest of the treatments. Result further indicate that application of phosphorus source PROM recorded the maximum and significantly higher microbial biomass P of cowpea over rest of the treatments. Dual inoculation with PSB + VAM recorded the maximum and significantly higher microbial biomass P of cowpea over rest of the treatments. The results indicated that application of soil salinity having EC 1dS/m recorded the lowest Na/K and Ca/Mg ratio and highest Na/Ca and Na+K/Ca ratio over the rest treatments. Application of phosphorus source PROM recorded the maximum and significantly lowest Na/K, Ca/Mg ratio, Na/Ca and Na+K/Ca ratio over the rest treatment. Dual inoculation with PSB + VAM recorded also lowest Na/K and Ca/Mg ratio and highest Na/Ca and Na+K/Ca ratio over the rest treatments.

Keywords: biofertilizers, cowpea, phosphorus, psb, prom, salinity

Introduction

Cowpea [*Vigna unguiculata* (L.) Wilczek] commonly known in India as *lobia* is one of the important *kharif* pulse crops grown for vegetable, grain, forage and green manuring. This crop has great importance because of availability of short duration, high yielding and quick growing variety. Green tender pods are used as vegetable; the vegetable cowpea pods contain moisture 84.6%, protein 4.3%, carbohydrate 8.0% and fat 0.2%. In many parts of arid and semi-arid regions, groundwater which is often of poor quality is used as major source of irrigation. The continuous use of such water for irrigation creates salinity or sodicity in soil. The problem is aggravated in the areas where saline / sodic ground water is used as a chief source of irrigation owing to shortage of good quality water. Salt affected soils cover an area of nearly 13.8 Mha in the country (Yadav *et al.* 2007) [20] and 1.24 M ha in Rajasthan and occurs to a greater or lesser extent in practically all the district of state (Sharma *et al.*, 2004) [13]. Unscientific and indiscriminate usages of saline water for irrigation causes an accumulation of soluble salts in root zone and adversely affect the physical and chemical properties of irrigated soils which in turn decrease crop productivity due to reduced water availability to plants (Chauhan *et al*., 1988) [2].

Plant growth is either depressed or entirely prevented due to excessive build-up of salinity in soil due to irrigation with saline water. Phosphorus is most indispensable mineral nutrient for pulse crop. Response of crops to phosphorus application on sodic soils has been reported by several workers (Tomar *et al.*1996 and Yadav *et al.* 2009) [17, 19]. Phosphorus is an important nutrient next to nitrogen. Its deficiency is most important single factor, which is responsible for poor yield of cowpea on all types' soils. It is a constituent of nucleic acids as ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), ADP and ATP nucleoprotein, amino acids, proteins, phosphatides, phytin, several co-enzymes viz., thiamine, pyrophosphate and pyrodoxyl phosphate.

VAM can play an important role in enhancing phosphorus availability to plant in P deficient soils. VAM fungi can save P-fertilizer by 25-30%. It is well known that VAM fungi improve plant growth through increased uptake of relatively immobile nutrients such as P, Zn Cu etc. (Tarafdar and Rao, 1997) [16]. The inoculation of seeds with PSB culture increases the green pod yield over uninoculated control (Vaisya *et al.*, 1983) [18]. The phosphate solubilizing bacteria are aerobic and heterotrophic in nature. Under favourable conditions, they can solubilize 20-30% of insoluble phosphate and may increase yield of crops by 10-30 % (Tilak and Annapurna, 1993).

Materials and Methods

A pot experiment was conducted at cage house of Department of Plant Physiology, College of Agriculture, Jobner during 2015 in completely randomized design (CRD) with three replications. The soil was loamy sand in texture, to attain the ECe level of 4 and 6 dS/m Cl- and SO_4^{-2} of Na, Ca and Mg were added as solution keeping the ratio of 3:1 of Cl: SO_4 and thoroughly mix in the soil before seeding (Table 1), alkaline in reaction (pH 8.40), low in organic carbon (1.85 g/kg), and available nitrogen (128.10 kg/ha), medium in available phosphorus (20.25 kg P2O5/ha) and potassium (145.80 kg K2O/ha) content. Bulk density, particle density, Na, Ca, Mg, CEC, exchangeable Na and ESP (1.50 Mgm^{-3}, 2.60 Mg m^{-3}, 9.60 me/L, 1.2 me/L, 1.2 me/L, 6.8 cmol (P+) kg/soil, 0.65 cmol/kg and 9.55, respectively) of experimental soil. The experiment was consisted of three levels of soil salinity (l, 4.0 and 6.0 dS/m), three sources of phosphorus (SSP, DAP and PROM), and three levels of biofertilizers (control, PSB and PSB + VAM) and thereby, making 27 treatment combinations.

Table 1: Amount of different salts and their ionic composition added 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(base soil)	9.6	1.2	1.2	2.2	6.0	1.22
4.00	16.6	5.6	5.6	7.8	24.0	4.14
6.00	25.6	11.2	11.2	1.28	39.0	6.10

Soil was filled in cylindrical ceramic pots (20 cm diameter and 28 cm height). Each pot contained 10 kg of soil. At the time of filling the pots, the broken pieces of stone were placed on the bottom hole to allow free drainage. The cowpea cv. 'RC-19' was sown on 7th July, 2015 with a seed rate of 5 seeds per pot. The crop was harvested on 15th September, 2015. Fully mature and developed pods from randomly selected five plants from each plot were plucked and number of seeds was counted. The average number of pods and seeds per plants was worked out. After threshing and winnowing the weight of seeds for each pot was recorded in gm per pot and then converted in to kg/ha.

Results and Discussion

A) Microbial biomass Pat one month interval from sowing to harvest

Effect of soil salinity

The perusal of data given in Table 2 revealed that different levels of soil salinity significantly decreased the microbial biomass P at all stages of sampling and decreased with the advancement of crop growth. The S_4 and S_6 decreased the microbial biomass P to the extent of 8.32 and 22.57 per cent over normal soil at one month after sowing, respectively. The corresponding decrease at second month after sowing was 9.00 and 19.98 per cent and at harvest, it was 10.41 and 23.98 per cent. The availability of phosphorus in soil decreased significantly with the increasing level of soil salinity. Magnitude of decrease was more pronounced in Ca dominated soil than that of Na dominated soil. The findings of present investigation get support from the work of Dinesh *et al.* (1998) [3].

Effect of Sources of P

A critical examination of data further revealed that application of sources of phosphorus significantly influenced the microbial biomass P at all stages of sampling and decreased with the advancement of crop growth. Application of phosphorus as PROM increased the microbial biomass P by 9.54 and 28.35 per cent over DAP and SSP at one month after sowing, respectively. The corresponding increase at second month after sowing was 10.12 and 23.06 per cent and at harvest was 12.04 and 30.27 per cent over DAP and SSP. PROM is prepared from high grade rock phosphate and organic matter. The organic matter released organic acids, which converted unavailable phosphate into available phosphate (Kumawat *el al*., 2013) [5] and significantly increased the available P in soil compared to SSP and DAP. Organic acids released through PROM increased the dehydrogenase, alkaline phosphatase activity and microbial biomass P in soil after harvest compared to SSP and DAP application. It provided

Table 2: Effect of soil salinity, sources of P and biofertilizers on soil microbial biomass P (µg g-1 soil) at one month interval from sowing to harvest

Treatments	Month I	Month II	At harvest
Soil salinity			
S_1 (1 dS/m)	28.71	25.87	21.89
S_4 (4 dS/m)	26.32	23.54	19.61
S_6 (6 dS/m)	22.23	20.70	16.64
SEm±	0.51	0.38	0.35
CD (P=0.05)	1.43	1.08	1.00
Sources of P			
P_1 (SSP)	22.36	20.94	16.78
P_2 (DAP)	26.20	23.40	19.51
P_3 (PROM)	28.70	25.77	21.86
SEm±	0.51	0.38	0.35
CD (P=0.05)	1.43	1.08	1.00
Biofertilizers			
B_0 (Control)	22.31	20.88	16.85
B_1 (PSB)	26.29	23.35	19.30
B_2 (PSB + VAM)	28.66	25.88	21.99
SEm±	0.51	0.38	0.35
CD (P=0.05)	1.43	1.08	1.00

Substances essential for microbial growth and activity, which in turn was responsible for increase in soil microbial biomass P. Reason attribute in reduction/ death of microbial cell due to absence of any phosphate substrate. The addition of higher level of phosphorus through external source might have influenced the metabolism of micro-organism which is responsible for higher level of soil microbial biomass P. Similar rise in soil microbial biomass P was also reported by Majumdar *et al*. (2007) [8] and Mahanta and Rai (2008) [7].

Effect of biofertilizers

It is apparent from the data given in (Table 2) that the microbial biomass P increased significantly with each treatment of inoculation of biofertilizers over no inoculation at all stages of sampling and decreased with the advancement of crop growth. PSB alone and PSB + VAM increased the microbial biomass P to the extent of 9.01 and 28.46 per cent over control at one month after sowing, respectively. The corresponding increase at second month after sowing was 10.83 and 23.94 per cent and at harvest, it was 13.93 and 30.50 per cent. Use of biofertilizers significantly increased the crop productivity and thereby provided substrate essential for microbial growth and activity which probably responsible for this increase in soil microbial biomass P. The low content in control could be due to no addition of any external input into the soil and over by poor crop productivity. Inoculation of PSB+VAM significantly increased soil microbial biomass P compared to no inoculation. Maximum soil microbial biomass P in soil was recorded under treatment receiving dual inoculation of PSB+VAM and minimum in soil under no inoculation. Similar evaluation in SMB-P with the inoculation of PSB+VAM was also observed by Nath *et al.* (2012) [11] and Singh *et al.*, (2012) [14].

B) Salinity tolerance (Ionic Ratio)

Effect of soil salinity

The Na/K ratio in grain and straw of cowpea increased significantly (Table 3) with increasing level of soil salinity. Under S_4 and S_6 levels of soil salinity, Na/K ratio was higher by 11.49 and 20.87 percent in grain and 13.21 and 29.60 percent in straw over S_1, respectively. The Ca/Mg ratio in grain and straw increased significantly with increase in salinity levels. Under S_4 and S_6 levels of soil salinity, Ca/Mg ratio was higher by 7.88 and 14.45 percent in grain and 19.93 and 27.33 percent in straw over S_1, respectively. The highest Na+K/Ca ratio in grain and straw was recorded with S_1 level of soil salinity. S_6 level decreased the Na+K/Ca ratio in grain to the extent of 8.60 and 16.24 percent and in straw decreased by 9.48 and 27.29 percent over S_4 and S_6, respectively. The highest Na/Ca ratio in grain and straw was recorded with S_1 level, which was at par with S_4 level. S_6 level decreased the Na/Ca ratio in grain to the extent of 10.42 percent and 10.29 percent in straw over S_1, respectively. This may be due to the fact that Na concentration increased with salinity by higher uptake of Na from soil solution and by using Na salts in the experiment for development of soil salinity, while the K concentration decreased. Salinity tolerance was associated with efficient K regulation in shoot. K concentration and K fluxes were highest in the control and decreased under salinity. The result finds support from the work of Misra (2001) [10] and Jat (2011) [4] in Chick pea who also observed increased Na/K ratio in grain and straw with increasing level of soil salinity. The Ca/Mg ratio in grain and straw of cowpea increased significantly with increasing level of soil salinity (Table 3). This may be due to the fact that Ca concentration increased with the increasing level of salinity, while the Mg concentration decreased. The results are in close conformity with Sharma and Manchanda (1989) [9], and Jat (2011) [4] in chickpea. Who reported increased Ca/Mg ratio in grain and straw of mungbean with increasing level of soil salinity. The Na+K/Ca and Na/Ca ratios in grain and straw decreased significantly with increase in levels of soil salinity (Table 3). This may be due to the fact that increases in salinity, the concentration of Ca increased in soil as well as in grain and straw of crop. The lower Na+K/Ca and Na/Ca ratios at higher levels of ECe showed more tolerance to salinity than higher Na+K/Ca and Na/Ca ratios at lower levels of EC. Pathan *et al.* (2000) and Misra (2001) [10] have also reported lower Na+K/Ca ratios in wheat, clusterbean and chickpea, respectively, at higher level of EC. The results are in conformity with the findings of Gupta and Srivastava (1989) [15], and Sharma and Manchanda (1989) [9] who reported lower Na+K/Ca and Na/Ca ratios in plant with increasing soil salinity.

Table 3: Effect of soil salinity, sources of P and biofertilizers on Na/K, Ca/Mg, Na/Ca and Na+K/Ca ratio in grain and straw (Ionic Ratio)

Treatments	Na/K		Ca/Mg		Na/Ca		Na+K/Ca	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
Soil salinity								
S_1 (1 dS/m)	0.321	0.152	1.722	7.328	1.180	0.437	3.928	3.121
S_4 (4 dS/m)	0.348	0.174	1.827	7.780	1.158	0.424	3.590	2.825
S_6 (6 dS/m)	0.388	0.197	1.971	9.331	1.057	0.392	3.290	2.269
S. Em. ±	0.008	0.004	0.038	0.156	0.026	0.011	0.049	0.041
C. D. (P=0.05)	0.021	0.012	0.106	0.442	0.075	0.032	0.137	0.116
Sources of P								
P_1 (SSP)	0.427	0.198	1.736	7.779	1.351	0.482	3.782	2.766
P_2 (DAP)	0.330	0.178	1.841	7.869	1.098	0.448	3.608	2.632
P_3(PROM)	0.300	0.147	1.943	8.791	0.945	0.343	3.419	2.617
S. Em. ±	0.008	0.004	0.038	0.156	0.026	0.011	0.049	0.041
C. D. (P=0.05)	0.021	0.012	0.106	0.442	0.075	0.032	0.137	0.116
Biofertilizers								
B_0(Control)	0.409	0.199	1.762	7.759	1.311	0.494	3.826	2.817
B_1(PSB)	0.345	0.171	1.824	7.915	1.117	0.426	3.525	2.691
B_2(PSB+VAM)	0.303	0.152	1.935	8.764	0.967	0.353	3.466	2.507
S. Em. ±	0.008	0.004	0.038	0.156	0.026	0.011	0.049	0.041
C. D. (P=0.05)	0.021	0.012	0.106	0.442	0.075	0.032	0.137	0.116

Effect of Sources of P

The Ca/Mg ratio in grain and straw of cowpea increased significantly and decreased in Na/K ratio with the application of P (Table 3). Under application of PROM, Na/K ratio was reduced by 22.71 and 29.74 percent in grain and 10.10 and 25.75 percent in straw over DAP and SSP, respectively. The application of PROM as source of phosphorus significantly increased the Ca/Mg ratio in grain and straw. Under application of PROM, Ca/Mg ratio was higher by 5.54 and 11.92 percent in grain and 11.71 and 13.00 percent in straw over DAP and SSP, respectively. Application of PROM decreased the Na+K/Ca ratio in grain to the extent of 4.60 and 9.59 percent and 4.84 and 5.38 percent in straw over DAP and SSP, respectively. The application of PROM decreased the Na/Ca ratio in grain to the extent of 18.72 and 30.05 percent and 7.05 and 28.83 percent in straw over DAP and SSP, respectively. The decreased in Na/K and increased Ca/Mg ratios could be due to the fact that P application increases the cation exchange capacity of roots by increasing surface area which helped in increasing absorption of nutrients from the soil. Increased CEC of roots by P application, helps in less absorption of K^+ and Mg^{2+} and their translocation to upper parts of plants than Na^+ and Ca^{2+} consequently increased Na^+ regulation as well as Na/K and Ca/Mg ratios in grain and straw. The present investigation is targeted to investigate the beneficial effect of phosphorus application on inducement of salinity tolerance and is concerned with the use of saline soils with P application. It is suggested that application of phosphorus reduced the Na/K ratio and induced the salinity tolerance in cowpea. Additional application of P helps in mitigating the adverse effect of salinity by lowering the Na/K ratio in grain of wheat was also stated by Lal and Lal (1992) [6]. This may be explained to the fact that P application decreased the concentration of Ca^{2+} more than of concentration of Na^+ in grain and straw and K^+ concentration increased significantly with increasing levels of P application. Due to increase in CEC of roots, there was more absorption and translocation of K^+ ion and Na^+ ions to upper parts of plants including grains. Therefore, high concentration of K^+, Na^+ ions in plant cells leading to higher Na/Ca and Na+K/Ca ratios in grain and straw due to P application. The results get support from the findings of, Awad *et al*. (1990) and Jat (2011) [4] who reported that increasing the solution P concentration reduced the effect of salinity.

Effect of Biofertilizers

Inoculation of biofertilizers significantly increased Ca/Mg ratio and decreased Na/Ca, Na/K and Na+K/Ca ratios in cowpea (Table 3). Under dual inoculation with PSB+VAM, Na/K ratio was reduced by 15.64 and 25.91 percent in grain and 14.07 and 23.61 percent in straw over PSB and control, respectively. Dual inoculation with PSB + VAM registered significantly differs in respect to Ca/Mg ratio in grain and straw. Under dual inoculation with PSB+VAM, Ca/Mg ratio was higher by 6.08 and 9.81 percent in grain and 11.87 and 12.95 percent in straw over PSB and control, respectively. Dual inoculation with PSB+VAM decreased the Na+K/Ca ratio in grain to the extent of 7.86 and 9.40 percent over PSB and control and in straw decreased was 4.47 and 11.00 percent over PSB and control, respectively. Dual inoculation with PSB+VAM decreased the Na/Ca ratio in grain to the extent of 14.79 and 26.23 percent and in straw decreased was 13.76 and 28.54 percent over PSB and control, respectively. Grain inoculated with PSB and soil inoculated with VAM (VAM+PSB) decreased the Na^+ content and increase Ca^{2+} and Mg^{2+} content so the Ca/Mg ratio increased and Na/Ca, Na/K and Na+K/Ca ratios decreased because VAM+PSB increased phosphorus availability through increase phosphorus solubility from fixed phosphate then Na which react with soil-P and get precipitated in their insoluble form (Na-phosphate) by which availability of Na^+ to plant become very less and higher Ca and Mg availability. Similar results were also reported by Parsad *et al* (2012).

Conclusion

On the basis of one year field experimentation, it seems quite logical to conclude that soil microbial biomass P increased with application of phosphorus source PROM and seed inoculation with PSB and VAM. However, higher levels of soil salinity adversely affect the soil microbial biomass P.

Acknowledgements

The authors are heartily thankful to Department of Soil Science and Agricultural Chemistry, SKN College of Agriculture, Jobner (Rajasthan) for providing field trial facilities and also thankful to the Dean, SKN College of Agriculture, Jobner for providing the facilities for the investigation.

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