Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 1691-1695
Received: 01-05-2017
Accepted: 02-06-2017

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Effect of soil salinity, phosphorus and biofertilizers on physical properties of soil, yield attributes and yield 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 total and effective nodules, nodule index, number of pods per plant, number of seeds per pod, grain yield, straw yield and root mass of cowpea over rest of the treatments. Result further indicate that application of phosphorus source PROM recorded the maximum and significantly higher total and effective nodules, nodule index, number of pods per plant, number of seeds per pod, grain yield, straw yield and root mass of cowpea over rest of the treatments. However dual inoculation with PSB + VAM recorded the maximum and significantly higher total and effective nodules, nodule index, number of pods per plant, number of seeds per pod, grain yield, straw yield and root mass of cowpea over rest of the treatments but in case of nodule index remained at par with PSB. However, application the test weight and harvest index unchanged under different treatment levels.

Keywords: Biofertilizers, cowpea, phosphorus, PSB, PROM, salinity, yield

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) [32] 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) [21]. 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) [3].

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) [30, 33]. 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) [28].

The inoculation of seeds with PSB culture increases the green pod yield over uninoculated control (Vaisya *et al.*, 1983) [31]. 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) [29].

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 biofertilizer (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

Soil studies

Effect of soil salinity

The results indicated that application of soil salinity with EC ***6.0*** dS/m significantly increased the soil EC_e and significantly decrease pH_2 in cowpea as compared to other treatments. Application of soil salinity @***6.0*** dS/m recorded the maximum EC and lowest pH over other treatments (Table ***2).*** The pH of soil decreased significantly with increasing level of soil salinity. The observed decrease in soil pH may further be ascribed to the depression of thickness of the diffuse double layer at higher concentration of soluble salts in the soil (Russell, 1963). The decrease in soil pH with an increase in EC of irrigation water was also observed by Kumawat (2004) [9]. Application of low soil salinity level (***1.0*** dS/m) showed significantly higher total phosphorus, available phosphorus, dehydrogenase enzyme activity and alkaline phosphate activity over rest of treatments. Alkaline phosphatase enzyme in soil decreased significantly with the increasing level of soil salinity over normal soil. This might due to the accumulation of toxic ion in soil. This in conformity with the results of Batra and Manna (1997) [1] who reported lower activities of various enzymes in saline soil and contaminated soils irrigated with various industrial effluents. The findings of present investigation get support from the work of Dinesh *et al.* (1998) [4].

Table 2: Effect of soil salinity, sources of phosphorus and biofertilizers on yield and yield attributes

Treatments	Total nodules per plant	Effective nodules per plant	Nodules index	Plant height (cm)	Number of pods per plant	Number of grains per pod	Grain yield (g/pot)	Straw yield (g/pot)	Test weight (g)
Soil salinity									
S_1 (*1 dS/m)	25.11	23.56	2.57	68.71	13.81	9.05	5.50	8.25	77.02
S_4 (4 dS/m)	22.18	15.72	2.49	62.59	10.63	8.33	4.96	7.44	76.30
S_6 (6 dS/m)	16.64	12.42	2.45	55.52	8.34	6.70	3.42	5.13	74.81
S. Em. ±	0.29	0.25	0.04	0.86	0.20	0.13	0.06	0.09	1.28
C. D. (P=0.05)	0.83	0.70	0.11	2.43	0.56	0.38	0.18	0.24	NS
Sources of P									
P_1 (SSP)	20.11	16.20	2.46	60.31	9.83	7.79	3.65	5.49	74.54
P_2 (DAP)	20.83	16.48	2.50	60.54	10.16	7.90	4.85	7.26	76.50
P_3(PROM)	22.99	19.02	2.56	65.98	12.79	8.39	5.38	8.06	77.10
S. Em. ±	0.29	0.25	0.04	0.86	0.20	0.13	0.06	0.09	1.28
C. D. (P=0.05)	0.83	0.70	0.11	2.43	0.56	0.38	0.18	0.24	NS
Biofertilizers									
B_0(Control)	16.36	15.26	2.44	55.28	8.72	6.84	3.68	5.52	74.96
B_1(PSB)	22.65	17.32	2.51	62.61	10.68	8.38	4.72	7.08	76.28
B_2(PSB+VAM)	24.92	19.12	2.57	68.94	13.38	8.86	5.48	8.22	76.89
S. Em. ±	0.29	0.25	0.04	0.86	0.20	0.13	0.06	0.09	1.28
C. D. (P=0.05)	0.83	0.70	0.11	2.43	0.56	0.38	0.18	0.24	NS

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 (Minhas and Gupta, 1992) [14].

Effect of phosphorus: The application of phosphorus sources on ECe and pH_2 was found non-significant but application of phosphorus significantly increased total and available P. 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) [10] and significantly increased the available P in soil compared to SSP and DAP. Organic acids released through PROM increased the dehydrogenase and alkaline phosphatase activity in soil after harvest compared to SSP and DAP application. It provided substances essential for microbial growth and activity, which in turn was responsible for increase in soil microbial biomass P. Similar rise in soil microbial biomass P was also reported by Santhy *et al*. (2004) [22] and Majumdar *et al.* (2007) [12].

Effect of biofertilizers: The seed inoculation with biofertilizers significantly influnced the total phosphorus, available phosphorus, dehydrogenase enzyme activity and alkaline phosphate activity in cowpea. Maximum soil enzymatic activities in soil were recorded under treatment receiving dual inoculation of PSB+VAM and minimum in soil under no inoculation. Similar evaluation recorded with the inoculation of PSB+VAM was also observed by Nath *et al*. (2012) [15] and Singh *et al*., (2012) [26]. The inoculation of biofertilizers viz. PSB+VAM significantly increased the dehydrogenase activity in soil.Similar result were also reported by Fries *et al*. (1998) [5].

Data presented in Table 2 revealed that application of PSB+VAM significantly enhanced the alkaline phosphatase activity in soil at harvest of cowpea over no inoculation of biofertilizers. Increased microbial and root activity in the rhizosphere may generally account for higher activity including phoshpatase Nath *et al*., (2012) [15], and Meena *et al.* (2015) [13].

Yield attributes and yield

Effect of soil salinity: Significant reduction in total and effective nodules per plant, plant height, nodule index, pods per plant, and grains per pod, grain and straw yield with an increase in levels of soil salinity was observed in present study table 3. The test weight was found not effect by different level of soil salinity. This is due to the build-up of soil salinity with different saline soil containing excessive Cl- and SO42- of Na, Ca and Mg which affects the plant growth adversely results from high osmotic stress, low physiological availability of water and direct toxic effects of individual ions. This may be explained on the basis of fact that increasing levels of salinity in water increased the EC of soil resulting into decreased availability of N, P and K (Bajwa *et al.*, 1998). Soil salinity affects nutrient availability by modifying retention; fixation and transformation of nutrients in soils, interfering with the uptake and/or absorption of nutrients due to disproportionate ionic composition have reduced nutrient metabolisms mainly due to water stress which leads to poor plant growth and development (Shrinivasrao *et al.*, 2004) [25]. Further, poor symbiotic N fixation due to toxic effects of salts on rhizobia also leads to drastic reduction in nodulation.

Table 3: Effect of soil salinity, sources of Phosphorus and biofertilizers on soil properties

Treatments	ECe (dS/m)	pH_2	Total P content (%)	Available P_2O_5 (kg/ha)	Dehydrogenase activity (p kat kg^{-1} soil)	Alkaline phosphatase activity (µg PNP produced g^{-1} h^{-1})
Soil salinity						
S_1 (*1 dS/m)	1.47	8.40	0.032	22.07	6.04	10.09
S_4 (4 dS/m)	4.37	8.23	0.028	21.43	5.87	9.40
S_6 (6 dS/m)	6.44	8.03	0.025	20.74	5.54	8.60
S. Em. ±	0.05	0.13	0.0004	0.28	0.10	0.17
C. D. (P=0.05)	0.15	0.37	0.0011	0.80	0.28	0.48
Sources of P						
P_1 (SSP)	4.06	8.34	0.024	20.75	5.58	8.64
P_2 (DAP)	4.10	8.21	0.029	21.47	5.84	9.36
P_3(PROM)	4.13	8.12	0.033	22.02	6.02	10.09
S. Em. ±	0.05	0.13	0.0004	0.28	0.10	0.17
C. D. (P=0.05)	NS	NS	0.0011	0.80	0.28	0.48
Biofertilizers						
B_0(Control)	4.07	8.27	0.026	20.75	5.53	8.59
B_1(PSB)	4.09	8.21	0.029	21.46	5.83	9.35
B_2(PSB+VAM)	4.12	8.18	0.032	22.03	6.08	10.15
S. Em. ±	0.05	0.13	0.0004	0.28	0.10	0.17
C. D. (P=0.05)	NS	NS	0.0011	0.80	0.28	0.48

The higher amount of salts may also adversely affect the enzymatic activities and utilization of photosynthesis in plant. There are several evidences that cationic (Ca, Mg, Na, K) imbalance could lead to disturbances in photosynthesis and activity of stroma enzymes (Brand and Becker, 1984 and Jat (2011) [2, 6] in chickpea.

Pods per plant and grains per pod decreased with increase in level of soil salinity. This is because of the fact that high soil salinity resulting into high EC of soil which decreased the physiological availability of water to plants. The normal cell division which is responsible for production of yield attributes may also be slowed down under saline condition. The reduction in number of pods per plant and grains per pod may also be due to destruction of naturally accruing hormones under saline conditions. Results of this investigation are in similar line with those of Netwal (2003) [16] in cowpea and Jat (2011) [6] in chickpea.

In general, the significant decrease in yield under influence of different salinity levels was due to the increase in EC of soil which intern responsible for the reduction in grain and straw yield by causing a restricted availability of water and nutrients to the plant. Several workers have also observed the significant yield reduction (Manchanda *et al.*, 1991, Sharma and Manchanda, 1989) [11, 23] with the increasing level of soil salinity.

Effect of phosphorus: The application of phosphorus source PROM recorded significantly increased total and effective nodules, nodule index, number of pods per plant, grain per pod, grain and straw yield over rest of treatments (Table 3). The test weight was found not effect by different sources of phosphorus. Phosphorus has been recognized as an essential constituent of all living organisms and plays an important role in the conservation and transfer of energy in the metabolic reaction of living cell including biological energy transformation. The increase in grain yield under application of PROM might be due to an organic source of nutrition and organic matter and various essential nutrients. The beneficial effect of PROM addition is also related to the improvement in soil physical properties and soil health. This might be due to the fact that excess assimilates stored in the leaves and later translocated into grains at the time senescing being the closest sink. Thus, ultimately increased the grain yield and straw yield was also increased due to the result of overall increased growth and development of plants.
These results were confirmed with the finding of Shanmugam (2001) [20] and Shekhawat and Sharma (2001) [24].

Effect of biofertilizers: The seed inoculation of biofertilizer significantly influenced nodulation, yield attributes and yield of cowpea. The seed inoculation with PSB + VAM significantly increased total and effective, nodule index, number of pods per plant, grain yield and straw yield as compare to other treatments (Table 3). Inoculation of seed with PSB and soil with VAM improved the effective and total nodules per plant, nodule index (Table 3) which may be due to solubilization of native phosphorus mediate by phosphate solubilizing micro-organism through production of organic acids like glutamic, succinic, lactic, oxalic, malic, fumaric, tartaric, ketobutyric, propionic and formic acid.Some of these acids (hydroxy acids) may form chelates with cations such as Ca2+ and Fe2+, resulting in effective solubilization of phosphorus. Such improvement under increased availability of P might have promoted metabolic processes in plants resulting in greater meristematic activities and apical growth, thereby improving effect and total nodules per plants, nodule index and number of pods per plant and also improved photosynthetic surface of the plant. Konde *et al.* (1998) [8] also reported that VAM fungi enhanced the P availability in soil and this had favourable effect on the nodule formation and survival of *Rhizobium* in the rhizosphere of green gram. Increased availability of P helped in nodulation and nitrogen fixation was also reported by Nirmalnath and Badanur (2001) [17]. The results obtained in present investigation are in line with the findings of Rathore *et al*. (2010) [19], Singh *et al*. (2012) [26] and Khan *et al*. (2015) [7]. Organic acids solubilize more P than inorganic acids at same pH due to the chelating effect of former (Somani, 2002) [27]. Production of CO_2 by soil microorganisms and plant roots leads to formation of carbonic acid which also encourages solubilization of insoluble P. The solubilization of insoluble phosphatase without production of acid may be due to release of protons accompanying respiration or ammonium assimilation and insoluble phosphate, thus directly solubilized at the microbial cell surface (Somani, 2002) [27]. The observed additive influence of biofertilizers is attributed to mutually benificial and synergistic role played by each group of biofertilizers used. Such mutually beneficial synergistic effect has also been reported by Rathore *et al*. (2010) [19] and Pramanik *et al.* (2012) [18].

Conclusion
On the basis of one year field experimentation, it seems quite logical to conclude that grain and straw yield increased with application of phosphorus source PROM and seed inoculation with PSB and VAM. However, higher levels of soil salinity adversely affect the nodulation and yield of cowpea.

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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