INFLUENCE OF PHOSPHORUS ENRICHED ACIDIFIED CARBON ON MAIZE GROWTH CULTIVATED IN SALT AFFECTED SOIL

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Abstract

Proper management of phosphorus (P) in salt-affected soils is one of the major issues for the achievement of maximum maize yield. Disturbance in ionic homeostasis in soil due to the high amount of water-soluble salts in saline soils decreases the balance uptake of phosphorus. Organic amendments can play an imperative role in this regard. The use of acidified carbon is one such technology. Its application can decrease the soil pH, and thus can enhance the available P in salt-affected soils. That's why the current study was conducted to explore the effect of acidified carbon on maize growth in salt-affected soils. There were 3 levels of phosphorus enriched acidified carbon (PEC) i.e., 0, 2.5 and 5.0% applied in normal and saline soil (5.32 dS/m EC). Results showed that 5.0 PEC significantly improved shoot length, root length, shoot fresh and dry weight, chlorophyll a, chlorophyll b and total chlorophyll in maize compared to 0PEC in normal and saline soils. A significant improvement in leaves and root P concentration also validated the efficacious role of 5.0 PEC over 0PEC in normal and saline soils. In conclusion, 5.0PEC has the potential to improve phosphorus availability in salt-affected soils. It can also play an immense role in the improvement of maize growth in saline conditions. More, investigations are suggested at the field level under variable agroclimatic zones to declare 5.0PEC as the best application rate for enhancement of maize yield in saline soils.

Key words: Macronutrient; Chlorophyll contents; Photosynthetic rate; Transpiration Rate; Na concentration; Maize.

Introduction

Management of micro and macronutrients in salt affected soils is one of major hurdle. It is necessity for the achievement of high yield and better profit from agricultural commodities especially when cultivation is done in low or poor fertilize soils (Chhabra, 2021). Less uptake of macronutrients, not only minimize the yield but also deteriorates the commodity quality poor (Galani et al., 2022). Being the most important macronutrient, P plays a significant role in the metabolic processes of plants (Billah et al., 2019). In phospholipids, phosphoproteins, nucleic acid and coenzymes, P is present as vital elements (Tamburini et al., 2012). Besides that P is also involved in respiration, photosynthesis, signaling, nucleic acid and synthesis, enzyme activities, carbohydrate metabolism and redox reaction (Fahad et al., 2016; Vance et al., 2003). Excessive application of phosphorus can cause eutrophication when it is lost by the action of running water. On the other hand, the limited availability of phosphorus also minimizes the yield of crops (Onodera et al., 2020; Xiong et al., 2020). In saline soils, high concentration of calcium (Ca) restricts the uptake of P. This Ca makes complexes with the P thus, make it insoluble in water. When concentration of sodium (Na) is increased, this P is up taken in plants in the form of sodium phosphate. Accumulation of Na in plants while uptake of P, hampered the crops yield (Chhabra, 2021). Judicious application of fertilizers can overcome this issue to some extent. However, high pH and specific ion toxicity of salt affected soils also decrease their

bioavailability of inorganic P fertilizers (Chhabra, 2021). To tackle this problem, scientists suggest the addition of such amendments which can decrease the soil pH but increase soil carbon pool (Haider Sultan et al., 2020). Acidified carbon is one of such amendment. It can decrease the soil pH which can help in the solubilization of immobilized P in soil. Furthermore, balance soil carbon facilitate the microbial proliferation which also played imperative role in the regulation of nutrients (Ahmad Rahi et al., 2021; Ahmed et al., 2022; Haider Sultan et al., 2020). After rice and wheat, maize is 3rd most important cereal crop of Pakistan. It is cultivated due to highest amount of energy i.e., ME 3350 Kcal/kg among all cereals. Maize is highly polymorphic. It also holds maximum genetic variability (Carpici et al., 2010). That's why current study was planned to explore the effect of phosphorus enriched acidified carbon on maize growth under saline conditions. This study is covering the knowledge gap regarding the use of phosphorus enriched carbon in soil. It is hypothesized that phosphorus enriched carbon is an effective amendment for improvement in phosphorus uptake and growth of maize in salt affected soils.

Materials and Methods

Experimental design and treatments arrangement: The design of experiment was completely randomized design (CRD). Two factorial arrangements of treatment were made i.e., salinity levels and phosphorus enriched acidified carbon (PEC). **Production and characterization of PEC:** For the production of PEC, modification in methods of Sultan *et al.*, (2020) was done. Instead of using H_2SO_4 , a 2:1 mixture of H_3PO_4 and H_2SO_4 was used. When carbon was produced then it was passed through a 2 mm sieve. Finally, a fine powder of PEC was applied as per the treatment plan in the soil. For pH and EC assessment of PEC, it was mixed in a 1:20 w/v ratio in deionized water. Final readings were taken on pre-calibrated pH and EC meter (Shi *et al.*, 2017). Di-acid mixture HNO₃:HClO₄ in a 2:1 ratio was used for the digestion of PEC at 200°C on a hot plate (Miller, 1998). Yellow colour methods were used for the assessment of P in PEC on a spectrophotometer (Chapman & Pratt, 1961). Potassium,

sodium and calcium were examined in digested material by running it on a flamephotometer (Donald & Hanson, 1998). For determination of N in PEC, digestion was done with H₂SO₄ at 380°C. After that Kjeldhal's distillation apparatus was used for the assessment of total N in PEC (Bremner, 1996). Ash content (AC) and volatile matter (VM) in PEC were examined by heating the sample in a muffle furnace at 550°C and 450°C respectively (Danish *et al.*, 2019). The fixed carbon was calculated using the equation by Noor *et al.*, (2012).

Fixed carbon (%) = 100 - (% volatile matter + % Ash content)

The characteristic of PEC is provided in Table 1.

	PEC		Soil					
Attributes	Units	Values	Attributes	Units	Normal	Saline		
pН	-	6.24	Sand	%	30	30		
EC	dS/m	3.95	Silt	%	30	30		
Volatile matter	%	13.10	Clay	%	40	40		
Ash content	%	15.67	Texture	-	Clay Loam			
Fixed C	%	71.23	pH <i>s</i>	-	8.15	8.57		
Total N	%	0.01	ECe	dS/m	2.05	5.32		
Total K	%	178	Organic matter	%	0.40	0.35		
Total Na	%	0.05	Extractable P	mg kg⁻¹	5.21	2.43		
Total Ca	%	0.18	Extractable K	mg kg ⁻¹	121	85		

 Table 1. Characteristics of PEC and soi.

Salinity and soil characterization: Naturally normal and saline soils were collected from nearby research areas. Soil EC was used as the main factor for the assessment of salinity. After analysis, it was noted that normal soil EC was 2.05 dS/m while saline soil EC was 5.32 dS/m. The hydrometer method was used for the assessment of sand, silt and clay. The final soil texture was computed by using USDA textural triangle (Gee & Bauder, 1986). For examination of soil EC and pH, 1:10 and 1:1 w/v ratio of soil and deionized water was mixed. After that pH of the soil paste was analyzed in a pre-calibrated pH meter. However, extraction was done for EC and extracted solution was run on EC meter for final EC determination (Page et al., 1983; Rhoades, 1996). For analysis of total organic matter potassium dichromate and ferrous ammonium sulphate were utilized as per standard protocol (Sparks et al., 1996). Extracting Olsen's reagent was used for extraction of available P. Final values of P were computed on a spectrophotometer by taking absorbance at 880nm (Kuo, 1996). Assessment of extractable K was done by using ammonium acetate solution. Final readings were noted by running the extracting solution on a flamephotometer (Donald & Hanson, 1998).

Treatment plan and PEC application: There were six treatments with 3 replications. The treatments include control (No PEC)+ normal soil (2.05 dS/m EC), 2.05 dS/m EC+2.5%PEC (2.5PEC), 2.05 dS/m EC+5.0%PEC (5.0PEC), saline soil (5.32 dS/m EC), 5.32 dS/m EC+2.5PEC and 5.32 dS/m EC+5.0PEC. On w/w basis PEC was applied in soil as per treatment plan manually.

Irrigation characteristics and application: The moisture in the pots were maintained at 65% field

capacity of soil. For irrigation purpose tap water was used. The characteristics of tap water were pH = 6.89, EC = 0.34 dS/m, carbonates = 0.00 (meq./L), bicarbonates = (3.27 meq./L), chlorides = (0.40 meq./L) and Ca+Mg = (3.21 meq./L) (Estefan *et al.*, 2013).

Seeds collection and sowing: Seeds of maize YH 1898 variety was collected from a local certified seeds shop. Initially, weak and damaged seeds were screened out manually. After that, 4 seeds were sown in each pot. When seeds get germinated, thinning was done to maintain 2 seedlings per pot for further experiment.

Fertilizer application: Nitrogen fertilizer was applied at the rate of 227.24 kg ha⁻¹ in three separate aliquots. Phosphorus (143.26 kg ha⁻¹) and K (91.93 kg ha⁻¹) were applied as a basal dose at the time of sowing (Saboor *et al.*, 2021).

Harvesting and data collection: Plants were harvested at the vegetative phase of maturity (before tillering) (Saboor *et al.*, 2021). Shoot length, root length, shoot fresh and dry weight, root fresh and dry weight were recorded soon after harvesting. For dry weight analysis, samples were oven-dried at 65°C for 48 hours. After the achievement of constants weight, analytical grade balance was used for the collection of readings.

Gas exchange attributes: IRGA (infrared gas analyzer) was utilized for the determination of photosynthetic rate, transpiration rate and stomatal conductance on a sunny day (9 and 11 am) (Danish and Zafar-ul-Hye, 2019; Saboor *et al.*, 2021).

Chlorophyll contents: For the determination of chlorophyll contents, initially grinding and then extraction was done by using 80% acetone. After that absorbance was recorded on spectrophotometer at 645, 663 and 480 nm (Arnon, 1949; Kirk & Allen, 1965; Sims & Gamon, 2002).

Chlorophylla a (mg g⁻¹) = $\frac{12.7 (\text{OD } 663) - 2.69 (\text{OD } 645) \text{ x V}}{1000 (\text{W})}$ Chlorophylla b (mg g⁻¹) = $\frac{22.9 (\text{OD } 645) - 2.69 (\text{OD } 663) \text{ x V}}{1000 (\text{W})}$

Total chlorophyll (mg g⁻¹) = Chlorophyll a + Chlorophyll b

where, OD = Optical density (wavelength) V = Final volume made W = Fresh leaf weight (g)

Electrolyte leakage: Electrolyte leakage (EL) was measured using the method by Lutts *et al.*, (1996). Leaf discs of equal size (1g) were dipped in 15ml of deionized water (DI) water and incubated for 2 hours at 25°C in test tubes. Initial EC of the solution (EC1) was taken after incubation. Samples were again autoclaved at 120°C for 20 minutes and final EC (EC2) was measured after equilibrium at 25°C.

$$EL(\%) = \left(\frac{EC1}{EC2}\right) \times 100$$

Statistical analysis

All the data were processed by using standard statistical procedure (Steel *et al.*, 1997). Two factorial ANOVA and Tukey's test were applied for the comparison of treatments. Origin2021Pro software was used for making paired comparisons and Pearson correlation graphs (OriginLab Corporation, 2021).

Results

The effect of treatment was significant on maize shoot length and root length cultivated under salinity stress. Treatments 2.5PEC and 5.0PEC caused a significant increase in shoot length compared to control (0PEC+No salinity stress). Application of 5.0PEC remained significantly better than 2.5PEC for improvement in maize shoot length under control (no salinity stress). No significant change was noted between 0PEC and 2.5PEC for shoot length in salinity stress (5.32 dS/m). However, 5.0PEC caused a significant increase in shoot length compared to 0PEC under salinity stress (5.32 dS/m) (Fig. 1A). In the case of root length, 2.5PEC did not bring any significant change over control (0PEC+No salinity stress). Treatments 5.0PEC significantly enhanced root length compared to control (0PEC+No salinity stress). It was observed that both 2.5 and 5.0PEC caused a significant improvement in root length than 0PEC under salinity stress (5.32 dS/m) (Fig. 1B).



Fig. 1. Effect of phosphorus enriched chemically produced carbon (PEC) different application rates on shoot (A) and root length (B) of maize under normal (2.05 dS/m EC) and saline (5.32 dS/m EC) soil. Different values on bars are p-values computed by paired comparison Tukey test; $p \le 0.05$. OPEC (control having no PEC); 2.5PEC (2.5% w/w PEC applied in soil); 5PEC (5.0% w/w PEC applied in soil).

The influence of treatment was significantly different on maize shoot fresh and dry weight grown saltaffected and normal soils. Application of 5.0PEC significantly enhanced shoot fresh weight compared to control (0PEC+No salinity stress). The addition of 5.0PEC and 2.5PEC also differed significantly better than 0PEC for the increase in maize shoot fresh weight under salinity stress (5.32 dS/m). No significant change was noted between 2.5PEC and 5.0PEC for shoot fresh weight in salinity stress (5.32 dS/m) but 5.0 performed significantly better than 2.5PEC under control (0PEC+No salinity stress) (Fig. 2A). For shoot dry weight, 2.5 and 5.0 PEC bring significant increase compared to control (OPEC+No salinity stress). The addition of 5.0PEC significantly increased shoot dry weight than 2.5PEC under salinity stress (5.32 dS/m) (Fig. 2B). It was observed that 5.0PEC caused significant improvement in shoot dry weight compared to 2.5PEC under salinity stress (5.32 dS/m). However, both 2.5 and 5.0PEC remained statistically alike to each other for shoot dry weight in control (0PEC+No salinity stress).

Application of treatments differed significantly on maize root fresh and dry weight under normal and saltaffected soils. Application of 5.0PEC caused a significant enhancement in root fresh weight than control (0PEC+No salinity stress). Treatments 2.5PEC did not differ significantly for root fresh weight over control (0PEC+No salinity stress). The addition of 5.0PEC and 2.5PEC also remained significantly better than OPEC for improvement in maize root fresh weight under salinity stress (5.32 dS/m). No significant change was observed between 2.5PEC and 5.0PEC for root fresh weight under salinity stress (5.32 dS/m) and control (0PEC+No salinity stress) (Fig. 3A). In root dry weight, 2.5 and 5.0 PEC remained statistically alike with control (0PEC+No salinity stress). The addition of 2.5 and 5.0PEC significantly enhanced root dry weight compared to 0PEC salinity stress (5.32 dS/m) (Fig. 3B). It was observed that 5.0PEC did not cause a significant enhancement in root dry weight than 2.5PEC under salinity stress (5.32 dS/m).

For chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, the impact of applied treatments was significant. It was observed that 2.5 and 5.0 caused a significant increment in chlorophyll a, chlorophyll b, total chlorophyll compared to PEC under control (2.05 dS/m EC) and saline (5.32 dS/m EC) soil. Treatment 5.0PEC differed significantly better under control (2.05 dS/m EC) but remained non-significant in saline (5.32 dS/m EC) soil over 2.5PEC for chlorophyll a (Fig. 4A), chlorophyll b (Fig. 4B), total chlorophyll (Fig. 4C). In the improvement of carotenoids (Fig. 4D), 5.0PEC performed significantly better compared to 2.5PEC under control (2.05 dS/m EC) and saline (5.32 dS/m EC) soil. However, compared to 0PEC under normal (2.05 dS/m EC) and saline (5.32 dS/m EC) soil, both 2.5 and 5.0 remained significantly better for enhancement in carotenoids.

Results showed that 0, 2.5 and 5.0 remained statistically alike to each other in control (2.05 dS/m EC) for leaves and root Na. A significant decrease in root and leaves Na was noted where 2.5 and 5.0 PEC were applied

over 0PEC in saline (5.32 dS/m EC) soil. In saline (5.32 dS/m EC) soil, the performance of 5.0PEC remained significantly better than 2.5PEC for a significant decrease in Na in roots and leaves. For roots and leaves P, a significant increase was observed where 2.5 and 5.0 PEC was applied over 0PEC in control (2.05 dS/m EC). Under control (2.05 dS/m EC), no significant change was noted between 2.5 and 5.0PEC for leaves and root P. Both 0 and 2.5 PEC remained statistically alike to each other for leaves and root P under saline (5.32 dS/m EC) soil. However, the addition of 5.0PEC performed significantly better than 0PEC for enhancement in root and leaves P in saline (5.32 dS/m EC) soil. The maximum increase of 83 and 40% was observed in root and leaves of P where 5.0PEC was applied over 0PEC under saline (5.32 dS/m EC) soil respectively (Table 2).

It was noted that photosynthetic rate and transpiration rate did not show any significant change where 2.5PEC was applied than OPEC in control (2.05 dS/m EC). A significant increase was noted in 2.5PEC over 0PEC for stomatal conductance in control (2.05 dS/m EC). However, under control (2.05 dS/m EC), 5.0PEC remained significantly best for enhancement in photosynthetic rate, transpiration rate and stomatal conductance compared to OPEC. Furthermore, the addition of 2.5 and 5.0PEC differed significantly for improvement in transpiration rate and photosynthetic rate in saline (5.32 dS/m EC) soil. However, 2.5 and 5.0PEC remained statistically alike to 0PEC for stomatal conductance in saline (5.32 dS/m EC) soil (Table 3). Pearson correlation showed that electrolyte leakage, leaves and root Na were significant negatives in correlation with all other growth attributes. Chlorophyll contents and gas exchange attributes i.e., photosynthetic rate, transpiration rate and stomatal conductance were significantly positive in correlation with leaves and root P. It was also noted that leaves and roots P showed a significant positive correlation with a fresh and dry weight of roots and shoot along with shoot and root length (Fig. 5).

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Salinity	Treatmonts	L	eaves Na (m	g/g DW)	Root Na (mg/g DW)			
	Treatments	Mean	SE	Labelling	Mean	SE	Labelling	
Control	0PEC	3.68	0.18	d	2.46	0.07	d	
	2.5PEC	3.71	0.08	d	2.21	0.12	d	
	5.0PEC	3.37	0.18	d	1.78	0.09	d	
5.32dS/m	0PEC	10.49	0.46	а	12.36	0.45	а	
	2.5PEC	7.33	0.25	b	10.25	0.34	b	
	5.0PEC	5.78	0.50	с	7.31	0.61	с	
Salinity	Treatments	Leaves P (%)			Roots P (%)			
Control	0PEC	0.11	0.0033	b	0.17	0.005	b	
	2.5PEC	0.14	0.0055	а	0.23	0.009	а	
	5.0PEC	0.15	0.0022	а	0.25	0.004	а	
5.32dS/m	0PEC	0.05	0.0021	d	0.06	0.003	d	
	2.5PEC	0.06	0.0035	cd	0.08	0.005	d	
	5.0PEC	0.07	0.0038	с	0.11	0.006	с	

Table 2. Effect of phosphorus enriched chemically produced carbon (PEC) different application rates on leaves
Na (A), root Na (B), electrolyte leakage (C) and leaves N (D) of maize under
(2.05 dS/m EC) and saline (5.32 dS/m EC) soil.

Different letters are showing significant differences computed by paired comparison Tukey test; $p \le 0.05$. Means are an average of three replicates. SE (Means standard error); 0PEC (control having no PEC); 2.5PEC (0.50% w/w PEC applied in soil); 5.0PEC (1.00% w/w PEC applied in soil). Red bars are indicating salinity stress. Green bars are indicating normal soil conditions







Fig. 3. Effect of phosphorus enriched chemically produced carbon (PEC) different application rates on root fresh (A) and dry weight (B) of maize under normal (2.05 dS/m EC) and saline (5.32 dS/m EC) soil. Different values on bars are p-values computed by paired comparison Tukey test; $p \le 0.05$. OPEC (control having no PEC); 2.5PEC (2.5% w/w PEC applied in soil); 5PEC (5.0% w/w PEC applied in soil).

Table 3. Effect of phosphorus enriched chemically produced carbon (PEC) different application rates on photosynthetic rate = Pn (A), transpiration rate = E (B) and stomatal conductance = gs (D) of maize under (2.05 dS/m EC) and saline (5.32 dS/m EC) soil.

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Salinity	Treatments	Pn (µmol/m²/s¹)		E (μmol/m²/s¹)			gs (mmol/m ² /s ¹)			
		Mean	SE	Labelling	Mean	SE	Labelling	Mean	SE	Labelling
Control	0PEC	14.19	0.19	b	2.07	0.06	bc	0.08	0.0015	с
	2.5PEC	14.99	0.15	b	2.28	0.07	b	0.10	0.0038	b
	5.0PEC	17.01	0.46	а	2.57	0.08	а	0.12	0.0025	а
5.32dS/m	0PEC	9.55	0.15	d	1.48	0.04	e	0.07	0.0063	с
	2.5PEC	12.83	0.22	с	1.79	0.03	d	0.08	0.0013	с
	5.0PEC	16.27	0.28	а	2.02	0.04	cd	0.08	0.0020	с

Different letters are showing significant difference computed by paired comparison Tukey test; $p \le 0.05$. Means are average of three replicates. SE (Means standard error); 0PEC (control having no PEC); 2.5PEC (0.50% w/w PEC applied in soil); 5.0PEC (1.00% w/w PEC applied in soil). Red bars are indicating salinity stress. Green bars are indicating normal soil conditions



Fig. 4. Effect of phosphorus enriched chemically produced carbon (PEC) different application rates on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and carotenoids (D) of maize under normal (2.05 dS/m EC) and saline (5.32 dS/m EC) soil. Different values on bars are p-values computed by paired comparison Tukey test; $p \le 0.05$. 0PEC (control having no PEC); 2.5PEC (2.5% w/w PEC applied in soil); 5PEC (5.0% w/w PEC applied in soil).

Discussion

Results of the current study showed that root and shoot length was significantly low in OPEC treatment compared to 5.0PEC. Improvement in 5.0PEC was due to better uptake of P in the plants. The deficiency of P caused a significant decrease in the primary roots. It also minimizes root elongation due to restricted cell division in the apical meristem (Sánchez-Calderón et al., 2005; Ticconi et al., 2009). During this phase, differentiation processes in root tips are initiated which played a major role in sensing P deficiency (Chacón-López et al., 2011). Balance P availability promotes cell division in the root cortex (Gentili et al., 2006). A significant improvement in root and shoot fresh weight was associated with the enhancement in root length. An increase in the root length of plants also enhanced the rhizosphere area which resulted in better uptake of mineral nutrients and water (Li et al., 2016). These mineral nutrients played an imperative role in the enhancement of dry weight in plants (Chatzistathis & Therios, 2013). It was also observed that the application of 2.5 and 5.0PEC played a vital role in the significant increase of chlorophyll contents in the plants. Such improvement in the chlorophyll was associated with an increase in the P concentration of leaves. Phosphorus is an important

substrate of the energy-rich compound ATP present in the stroma of chloroplast. Under deficiency of P, ATP synthase activity become restricted resulting in the limited synthesis of ATP and CO₂ fixation (Carstensen et al., 2018). Our results are also in line with above argument. A significant decrease in the photosynthetic rate was noted where 0PEC was applied over 2.5 and 5.0PEC. Improvement in the photosynthetic rate was linked with the better uptake of P in the plants. Better uptake of P in the plants also facilitate the biosynthesis of carbohydrates. Under P deficit conditions, RuBisCO activity and RuBP regeneration capacity are also become restricted that negatively influenced the photosynthesis metabolism in the plants (Brooks, 1986; Thuynsma et al., 2016). A significnt decrease in electrolyte leakage also validated the effectiveness of 5.0 PEC compared to 0PEC. This reduction in leaves electrolyte leakage was also due to limited uptake of Na and better uptake of P in the plants. Under salinity conditions, a higher uptake of Na caused oxidative stress. Production of reactive oxygen species resulted in poor plant growth (Liu et al., 2021). Furthermore, a higher accumulation of slats in plants also caused damage to the plasma membrane. That's why electrolyte leakage from the plasma membrane is considered one of the most noticeable effects of salinity stress (Ashraf & Ali, 2008).



Fig. 5. Pearson correlation for different studied attributed amended with different levels of phosphorus enriched chemically produced carbon (PEC) under normal (2.05 dS/m EC) and saline (5.32 dS/m EC) soil. The blue colour is indicating a negative while the red colour is indicating a positive correlation.

Conclusion

In conclusion, PEC has potential to improve P uptake in plants.It also can enhance growth and improve the gas exchange attributes when applied at the rate of 5.0% compared to 0%. Application of 5.0PEC can increase chlorophyll contents in maize under salinity stress. Farmers are recommeded to apply 5.0% PEC for the achievement of better maize growth under salt affected soils. More investigations are suggested at field level under variable soil texture are suggested to declare 5.0% PEC as best amendmnt for alleviation of salinity stress and better uptake of P in maize.

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