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EFFECT OF GRADED PHOSPHORUS AND CITRIC ACID LEVELS ON SOYBEAN GROWTH, YIELD AND NUTRIENT UPTAKE IN AN ALFISOL

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ABSTRACT

Phosphorus (P) is a vital nutrient for crop growth, but it is utilized inefficiently (10- 30%) in Alfisols due to high aluminum and iron oxide content. Citric acid, a low molecular weight organic acid exuded by plant roots, enhances P solubilization in soil. Soybean, being highly responsive to P, shows improved root growth and pod development with adequate P availability. The present study aimed to investigate the effects of graded P and citric acid levels on growth, yield attributes and nutrient uptake in soybean. A pot experiment was conducted at college of Agriculture, KSNUAHS, Shivamogga, using a factorial completely randomized design with varying P (0, 40, 60 and 80 kg ha⁻¹) and citric acid (CA) levels (0, 2.5, 5.0 and 7.5 kg ha⁻¹). The results revealed that sole application of P at 80 kg ha⁻¹ and CA at 7.5 kg ha⁻¹ significantly improved plant height, number of seeds per pod and nutrient uptake. The conjoint application (80 kg ha⁻¹ + 7.5 kg ha⁻¹) in T₁₆ treatment combination significantly improved plant height (47.54 cm) at 60 DAS and P uptake (161.07 mg pot⁻¹) in soybean. These findings suggest that integrated use of higher P levels with citric acid enhances soybean performance and nutrient uptake in Alfisols attributed to improved nutrient solubilization facilitated by citric acid application.

Key words : Phosphorus, Citric acid, P uptake, Soybean.

Introduction

Achieving the global goal of creating world free from hunger by 2030 (Chaudhary *et al.*, 2024) has significantly increased the reliance on chemical fertilizers, to sustain high agricultural productivity (Bisht and Chauhan, 2020). Among essential macronutrients, phosphorus (P) plays a crucial role in plant metabolism, root development, energy transfer and structural integrity (Bhatta *et al.*, 2021; Bindraban *et al.*, 2020). Despite being the second most important nutrient after nitrogen for the plant growth, phosphorus use efficiency (PUE) remains low (10-30%), mainly due to its rapid fixation and reduced mobility in soils, leading to low availability to plants. Increasing fertilizer costs, also limits its access to small and marginal farmers (Johan *et al.*, 2021; Roy *et al.*, 2016; Tiecher *et al.*, 2023).

Soybean, globally recognized as a “miracle crop” for its high protein (40-42%) and oil (18-20%) content, plays a vital role to eliminate hunger crisis. Phosphorus is critical for soybean as it supports root-shoot growth, facilitates symbiotic nitrogen fixation, enhances nutrient and water use efficiency, stimulates pod setting and reduces unfilled pods, thus directly impacting yield potential. However, several factors affect P availability to plants, in most soils, large proportion of P is bound to minerals or organic matter, restricting plant uptake and reducing crop productivity (Ch’ng *et al.*, 2017). To overcome phosphorus limitations, plants have evolved adaptive strategies such as mycorrhizal associations, longer root hairs, production of cluster roots, secretion of phosphatase enzymes and rhizosphere acidification through exudation of low molecular weight organic acids (LMWOA) (Marschner and Romheld, 1994). These organic acids, include amino

acids, sugars, phenolics and other metabolites, which competes for adsorption sites, acidify the rhizosphere and form soluble metal complexes, thereby mobilizing P and improving uptake (Bolan *et al.*, 1994; Wang *et al.*, 2016).

However, the natural concentration of these acids is often low and they are quickly degraded in soils, limiting their effectiveness. Among various LMWOAs, citric acid stands out due to its tricarboxylic structure, which enables it to effectively chelates metals, solubilize P and compete for adsorption sites, thereby improving P availability. Citric acid, naturally present in citrus fruits and exuded by plant roots, dissociates completely near neutral pH and reacts aggressively with aluminum and iron, promoting mineral weathering and further P release. Considering these mechanisms, the present study was undertaken to evaluate the effect of varied P and CA levels on soybean growth, yield and nutrient uptake, aiming to improve phosphorus uptake and soybean productivity under Indian soil conditions.

Materials and Methods

Treatment details

A pot experiment comprising sixteen treatment combinations with three replications was conducted during the Kharif season at College of Agriculture, KSNUAHS, Navile, Shivamogga, to study the “**Effect of graded phosphorus and citric acid levels on soybean growth, yield attributes and nutrient uptake in an Alfisol**” using two factors viz. P (0, 40, 60 and 80 kg ha⁻¹) and citric acid (0, 2.5, 5.0 and 7.5 kg ha⁻¹). The treatment combinations were comprised of; T₁ - (P₁CA₁) ; T₂ - (P₁CA₂); T₃ - (P₁CA₃); T₄ - (P₁CA₄); T₅ - (P₂CA₁) ; T₆ - (P₂CA₂); T₇ - (P₂CA₃); T₈ - (P₂CA₄); T₉ - (P₃CA₁) ; T₁₀ - (P₃CA₂); T₁₁ - (P₃CA₃); T₁₂ - (P₃CA₄) ; T₁₃ - (P₄CA₁) ; T₁₄ - (P₄CA₂); T₁₅ - (P₄CA₃) and T₁₆ - (P₄CA₄). Individual pot having soil capacity of 12 kg, were set up for crop production. Further, calculated quantity of Rhizobium, PSB and FYM was applied to each pot seven days prior to sowing of crop. Soybean variety JS-335 was used for the experimentation. It is a determinate type of variety grows up to height of 30- 65 cm, matures in 95 to 100 days after planting. The recommended dose of nutrients used was 30 kg N, 80 kg P₂O₅ and 37.5 kg K₂O ha⁻¹. Nitrogen and phosphorus were applied through urea, DAP and potash was applied using MOP. All the fertilizers were applied as basal dose and mixed well with the soil before sowing of the seeds. Two seeds of soybean were sown in each pot at a depth of 3-5cm and maintained until the final harvest. Initial soil characterization revealed a pH of 5.9, electrical conductivity (EC) of 0.013 dS m⁻¹ at 25°C, and organic carbon content of 5.8 g kg⁻¹. The soil was low in nitrogen

(215.8 kg ha⁻¹), high in phosphorus (81.85 kg ha⁻¹), and medium in available potassium (256.15 kg ha⁻¹). It also had high DTPA-extractable iron (18.21 mg kg⁻¹) and low exchangeable aluminium (3.8 mg kg⁻¹).

Observations recorded

Plant height

The height of the plant was measured from the base to the tip of main shoot at 30 and 60 DAS (days after sowing), expressed in centimeter (cm).

Number of branches plant⁻¹

The number of branches arise on the main stem were counted at 30 and 60 DAS and expressed in number of branches per plant⁻¹.

Number of seeds pod⁻¹

Number of seeds were calculated by opening five random pods from each pot randomly and their seeds were counted and average seeds pod⁻¹ was worked out by dividing the total number of seeds by total number of pods.

Pod weight plant⁻¹

Pod weight was estimated by taking five pods randomly from each pot and average weight was measured by dividing total weight of five pods to the total number of pods.

Nutrient uptake

Plant samples were collected at the time of harvest were shade-dried and dried at 60°C in hot air oven. The dried samples were powdered using grinder fitted with stainless blades and preserved in polythene bags for further analysis. Total nitrogen in plant samples was determined by Kjeldhal's method of nitrogen determination, as described by Jackson (1973). Whereas, for total elemental analysis powdered plant sample (0.2 gram) was pre-digested with 8 ml HNO₃ and 2 ml H₂O₂ then digested in microwave digestion (make Milestone and model Ethos easy). The volume of the digest was made up to 100 ml with distilled water and used for total elemental analysis (P, K, Ca, Mg and S). However, the uptake of nutrients was worked out by multiplying dry matter of the crop to the nutrient concentration.

Nutrient uptake (mg pot⁻¹) = Nutrient content (%) × Wt. of dry matter (mg pot⁻¹).

Results and Discussion

Effect on growth parameters

Plant height

Results of the our study (Table 1) revealed significant variation in the plant height of soybean at distinct growth

Table 1 : Effect of varied phosphorus and citric acid levels on growth attributes of soybean at different growth stages.

Treatments	Plant height (cm)		Number of branches plant ⁻¹	
	30 DAS	60 DAS	30 DAS	60 DAS
P ₁ (0 kg ha ⁻¹)	17.94	38.70	5.82	9.05
P ₂ (40 kg ha ⁻¹)	19.79	42.07	6.36	9.10
P ₃ (60 kg ha ⁻¹)	23.23	43.28	7.10	9.12
P ₄ (80 kg ha ⁻¹)	27.23	45.02	8.09	9.26
S. Em±	0.33	0.09	0.16	0.05
CD (5 %)	0.95	0.25	0.47	0.13
CA ₁ (0 kg ha ⁻¹)	20.85	41.27	6.54	9.08
CA ₂ (2.5 kg ha ⁻¹)	21.40	41.84	6.67	9.10
CA ₃ (5.0 kg ha ⁻¹)	22.44	42.70	7.03	9.17
CA ₄ (7.5 kg ha ⁻¹)	23.49	43.26	7.12	9.17
S. Em±	0.33	0.09	0.17	0.05
CD (5 %)	0.95	0.25	NS	NS
P ₁ CA ₁	17.23	37.93	5.63	9.013
P ₁ CA ₂	17.49	38.43	5.72	9.053
P ₁ CA ₃	17.93	38.90	5.87	9.080
P ₁ CA ₄	19.12	39.53	6.08	9.067
P ₂ CA ₁	19.00	41.03	6.22	9.087
P ₂ CA ₂	19.33	41.47	6.16	9.097
P ₂ CA ₃	20.05	42.70	6.43	9.107
P ₂ CA ₄	20.75	43.07	6.62	9.093
P ₃ CA ₁	21.44	42.13	6.79	9.110
P ₃ CA ₂	21.99	43.00	6.81	9.123
P ₃ CA ₃	24.04	43.79	7.32	9.120
P ₃ CA ₄	25.42	44.20	7.46	9.110
P ₄ CA ₁	25.73	43.97	7.53	9.127
P ₄ CA ₂	26.79	44.47	8.00	9.130
P ₄ CA ₃	27.73	45.40	8.52	9.363
P ₄ CA ₄	28.67	47.54	8.55	9.403
S. Em±	2.18	0.16	0.33	0.09
CD (5 %)	NS	0.50	NS	NS

Note: P- Phosphorus, CA- Citric acid, DAS- Days after sowing, NS- Non significant

Table 2 : Interaction effects of varied phosphorus and citric acid levels on yield parameter of soybean.

Levels P (kg ha ⁻¹)	Number of seeds pod ⁻¹				Pod weight plant ⁻¹			
	Levels CA (kg ha ⁻¹)							
	0	2.5	5.0	7.5	0	2.5	5.0	7.5
0	1.53	1.67	1.73	1.93	0.25	0.25	0.25	0.26
40	2.07	2.07	2.20	2.27	0.25	0.25	0.25	0.26
60	2.40	2.47	2.53	2.67	0.28	0.28	0.28	0.28
80	2.87	3.00	3.00	3.07	0.30	0.30	0.30	0.30
S E m ±	0.07				0.02			
CD (5%)	NS				NS			

stages owing to graded levels of phosphorus and citric acid application. Phosphorus application at 80 kg P ha⁻¹ exhibited significantly higher plants of height 27.23 and 45.02 cm at 30 and 60 DAS, respectively, over the treatment where P fertilization was omitted. These results corroborated with the study of Islam *et al.* (2023), who showed that phosphorus fertilization at 40, 80 and 120 mg/kg soil significantly increased shoot and root biomass and plant height in industrial hemp. Moreover application of phosphorus sources significantly increased the plant height of soybean (Yadav *et al.*, 2024). Similar response were obtained at higher CA level (7.5 kg ha⁻¹) application with notable plant height 23.49 and 43.26 cm at 30 and 60 DAS respectively, attributed to improved plant nutrition and crop development due to solubilization of essential nutrients from the soil by citric acid. Similar results were reported by Abdullahi *et al.* (2004), who observed that citric acid effectively enhanced soybean seedling height.

The conjoint application showed non-significant interaction amidst treatment combination at 30 DAS, conversely produces significant response in enhancing plant height at 60 DAS, indicating a time dependent response. The taller plants of height 28.67 and 47.54 at 30 and 60 DAS of soybean respectively, were found in the treatment combination T₁₆ (80 kg P ha⁻¹ + 7.5 kg CA ha⁻¹), whereas the lowest was in the treatment combination (17.23 and 37.93 cm at 30 and 60 DAS respectively) devoid of phosphorus and citric acid application. The increase in soybean plant height with graded phosphorus and citric acid application is attributed to improved root development, enhanced rhizobial activity, increased symbiotic nitrogen fixation, and better availability of essential nutrients. Citric acid also contributed in solubilize soil nutrients, thereby supporting overall plant nutrition, throughout crop cycle. These results align with the findings of Karadihalli *et al.* (2022), who demonstrated that applying LMWOAs (oxalic and citric acid) at 10 mg kg⁻¹ soil along with phosphatic fertilizer resulted in an 18.77 per cent increase in plant height compared to the control.

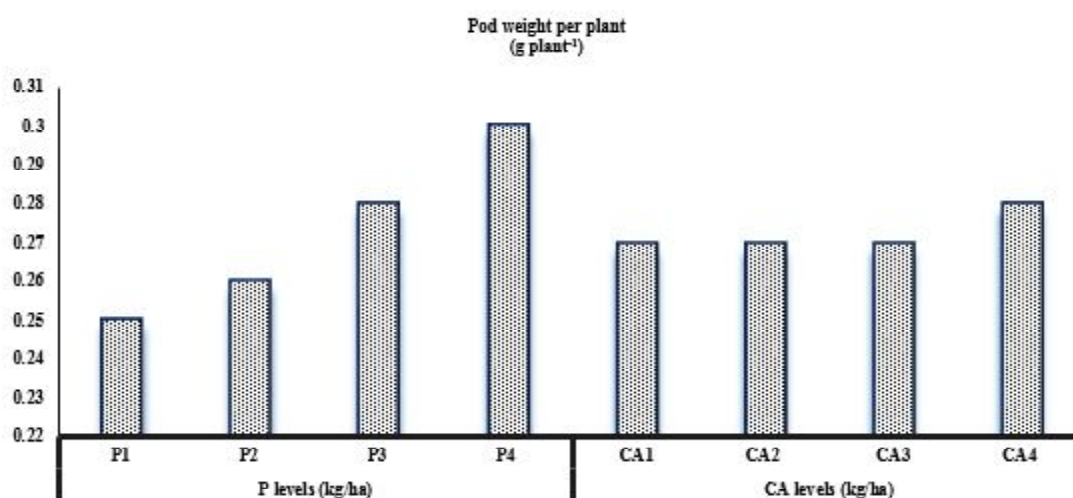


Fig. 1 : Effects of varied P and CA levels on pod weight per plant of soybean.

Number of branches plant⁻¹

Data concerning to number of branches plant⁻¹ of soybean given in Table 1 clearly showed that, higher phosphorus level (80 kg P ha⁻¹) significantly elevated the number of branches plant⁻¹ in soybean *i.e.*, 8.09 and 9.26 at 30 and 60DAS, respectively. The results supported by the findings of Bing *et al.* (2012), who reported that increased phosphorus application improved plant height, number of branches, stem diameter and leaf area of the crop. However, graded citric acid application had no notable impact. Although the treatment combinations failed to show significant impact in improving number of branches plant⁻¹, but treatment combination T₁₆ (80 kg P ha⁻¹ + 7.5 kg CA ha⁻¹) exhibited the highest numerical values (8.55 and 9.40), while the pots devoid of P and CA (T₁) application showed the lowest (5.63 and 9.01) at 30 and 60 DAS, respectively). Enhanced phosphorus availability through fertilization and soil P solubilization likely contributed to the increased number of branches per plant by promoting photosynthesis and plant development. This agrees with Dabesa and Tana (2021), who reported number of branches in soybean increased with P levels up to 69 kg ha⁻¹.

Effect on yield parameters

Pod weight plant⁻¹

The data summarized in Table 2. highlighted that varied phosphorus levels resulted in a significant enhancement of pod weight plant⁻¹. Application of phosphorus at 80 kg ha⁻¹ resulted in the maximum (0.30 g) pod weight per plant, with 20 per cent increase over the treatment devoid of P application (0.25 g). While increased citric acid levels had no significant effect on pod weight per plant. Despite statistical parity among the treatment combinations, T₁₆ (80 kg P ha⁻¹ + 7.5 kg CA ha⁻¹) recorded numerically

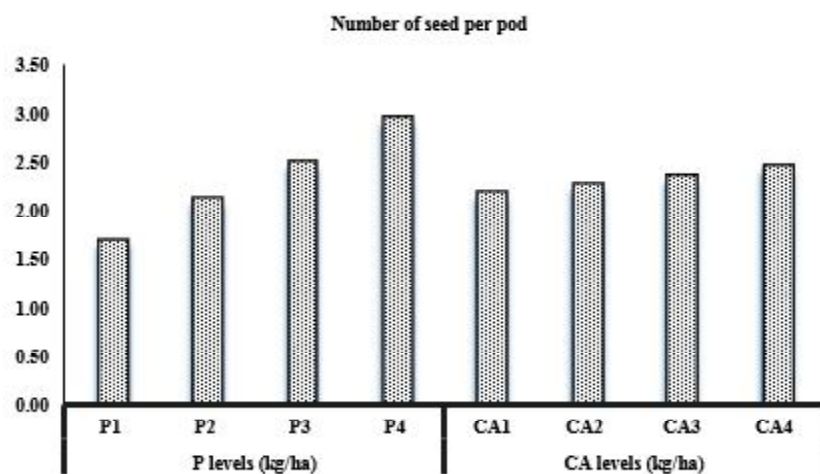
superior pod weight per plant (0.30 g) over the control omitted P and CA application might be due to improved phosphorus availability, which enhance overall crop growth and development. The findings of our study is in agreement with Akter *et al.* (2013), who reported that varying levels of phosphorus significantly influenced yield parameters, including the number of leaves, branches, pods, seeds per plant and thousand seed weight. Similarly, Prijambada and Proklamasiningsih (2010) found that the addition of organic acids such as malate and lactate significantly improved the number of pods in soybean, highlighting the beneficial role of organic acids in yield enhancement.

Number of seed pod⁻¹

Our study revealed that individual factors exerted a significant effect on the number of seeds pod⁻¹ (Fig. 2). Amidst the tested P doses, treatment receiving 80 kg ha⁻¹ resulted the highest number of seeds pod⁻¹ (2.98) followed by 60 kg ha⁻¹ (2.52), while pots lacking P application registered the lowest number of seeds pod⁻¹ (1.72). These findings are consistent with Khanam *et al.* (2016), who reported a significant increase in the number of seeds per pod with phosphorus application up to 175 kg ha⁻¹ of phosphorus fertilizer (TSP). Similar pattern was recorded with varied CA levels, where the highest number of seed pod⁻¹ was recorded with the application of 7.5 kg CA ha⁻¹ (2.48) followed by 5.0 kg CA ha⁻¹ (2.37) compared to the treatment received no CA (2.22). The current findings are in line with Karadihalli *et al.* (2022) observed that soil application of low molecular weight organic acids (LMWOAs), such as oxalic and citric acid, significantly enhanced the number of seeds per pod and pods per plant compared to the control that might be due to solubilization of native P and other nutrient that helps in growth and development of the crop.

Table 3 : Effect of varied phosphorus and citric acid levels on total nutrient uptake by soybean.

Treatments	Total Uptake (mg pot ⁻¹)					
	N	P	K	Ca	Mg	S
P ₁ (0 kg ha ⁻¹)	427.96	57.61	262.22	176.51	91.13	51.67
P ₂ (40 kg ha ⁻¹)	544.15	73.22	303.63	189.14	104.76	60.54
P ₃ (60 kg ha ⁻¹)	693.22	116.85	408.17	264.84	135.79	80.06
P ₄ (80 kg ha ⁻¹)	819.18	148.20	469.78	282.81	151.66	93.21
S. Em±	15.08	0.99	3.10	3.71	1.42	0.59
CD (5 %)	43.45	2.84	8.94	10.68	4.08	1.69
CA ₁ (0 kg ha ⁻¹)	567.19	90.23	334.16	212.50	113.76	66.57
CA ₂ (2.5 kg ha ⁻¹)	605.23	93.74	353.81	225.89	118.48	70.50
CA ₃ (5.0 kg ha ⁻¹)	639.37	101.64	369.13	234.36	122.42	73.38
CA ₄ (7.5 kg ha ⁻¹)	672.73	110.27	386.71	240.55	128.68	75.03
S. Em±	15.08	0.99	3.10	3.71	1.42	0.59
CD (5 %)	43.45	2.84	8.94	10.68	4.08	1.69
P ₁ CA ₁	360.96	57.65	235.41	160.82	85.68	47.77
P ₁ CA ₂	435.75	51.84	252.85	169.24	90.75	50.11
P ₁ CA ₃	431.35	57.64	271.33	186.65	92.46	52.88
P ₁ CA ₄	483.78	63.30	289.30	189.33	95.64	55.93
P ₂ CA ₁	504.84	64.04	286.12	178.35	98.82	57.17
P ₂ CA ₂	544.35	68.22	295.70	181.15	100.80	58.98
P ₂ CA ₃	554.83	77.83	312.29	193.37	103.54	62.78
P ₂ CA ₄	572.60	82.78	320.41	203.71	115.89	63.22
P ₃ CA ₁	674.26	101.81	365.77	236.84	127.79	74.72
P ₃ CA ₂	657.55	109.46	404.27	266.91	134.57	78.97
P ₃ CA ₃	686.44	122.22	415.43	275.60	138.18	82.72
P ₃ CA ₄	754.64	133.92	447.22	280.00	142.63	83.85
P ₄ CA ₁	728.71	137.42	449.34	273.98	142.77	86.62
P ₄ CA ₂	783.27	145.43	462.43	286.26	147.81	93.96
P ₄ CA ₃	884.85	148.89	477.45	281.84	155.51	95.15
P ₄ CA ₄	879.88	161.05	489.91	289.15	160.55	97.13
S. Em±	30.17	1.97	6.20	7.41	2.83	1.18
CD (5%)	NS	5.68	NS	NS	NS	NS

**Fig. 2 :** Effects of varied P and CA levels on number of seed per pod of soybean.

Conversely, conjoint application did not exhibit any significant impact (Table 2), with numerically higher number of seeds pod⁻¹ (3.07) was recorded with 80 kg P ha⁻¹ + 7.5 kg CA ha⁻¹ (T₁₆) followed by 80 kg P ha⁻¹ + 5.0 kg CA ha⁻¹ (3.00) and the lowest was in control (1.53) which might be attributed to the higher availability of phosphorus at elevated application levels (i.e., 80 kg P ha⁻¹ as RDF), leading to an increased number of seeds per pod. Similarly Jat and Meena (2014) revealed that application of citric acid (2 kg ha⁻¹) along with farmyard manure (2 t ha⁻¹) significantly enhanced groundnut (*Arachis hypogaea*)

productivity, increasing pod yield by 28.6 compared to FYM alone. Furthermore, the combined use of citric acid and single superphosphate (SSP) improved soil available phosphorus and enhanced phosphorus uptake in both pods and haulm.

Nutrient uptake

Significant variations in nutrient uptake characteristics were reported owing to varied P and citric acid application (Table 3). Amidst the varied P and CA levels, 80 kg ha⁻¹ and 7.5 kg ha⁻¹ resulted in notably greater nutrient uptake. The significantly higher total N (819.18 mg pot⁻¹), P (148.20 mg pot⁻¹), K (469.78 mg pot⁻¹), Ca (282.81 mg pot⁻¹), Mg (151.66 mg pot⁻¹) and S (93.21 mg pot⁻¹) was recorded with 80 kg ha⁻¹ phosphorus application compared to the P omitted pots. Analogous pattern was observed with CA, where 7.5 kg CA ha⁻¹ resulted in marked uptake of total N (672.73 mg pot⁻¹), P (110.27 mg pot⁻¹), K (386.71 mg pot⁻¹), Ca (240.55 mg pot⁻¹), Mg (128.68 mg pot⁻¹) and S (75.03 mg pot⁻¹). Our findings were similar to those of Dalshad *et al.* (2013), who revealed that phosphatic fertilizer application by SSP and DAP significantly increased the N, P, K and protein content in soybean. Additionally Karadihalli *et al.* (2022) showed that application of P fertilizer along with LMWOA significantly increased phosphorus content in soybean. Lingaraju *et al.* (2016) revealed that higher P content of soybean was found with combined application of phosphorus solubilizing bacteria and foliar spray of organic acids.

However, the tested treatment combinations showed statistical parity with each other in enhancing the total uptake of N, K, Ca, Mg, and S. Treatment combination T₁₆ significantly outperformed others in terms of total P uptake. In 80 kg ha⁻¹ + 7.5 kg ha⁻¹ treated pots, total P uptake rose significantly from 51.84 mg pot⁻¹ (T₂) to 161.05 mg pot⁻¹. Besides, treatment combinations T₁₆, T₁₅, T₁₄ and T₁₃ recorded 179.35, 158.26, 152.26 and 138.37 per cent increased total P uptake, respectively. The results are in agreement with the findings of Tusei (2019), who reported a significant increase in the availability and uptake of nutrients- N, P, K, Ca and Mg in wheatgrass following citric acid application, compared to the control. The increased nutrient content and uptake in soybean might be attributed to better supply of essential plant nutrient due enhanced root growth, root hair and root surface area by the application of phosphatic fertilizer hence, more will be the availability and uptake of nutrient by soybean roots. These outcomes corroborate with the findings of Dalshad *et al.* (2013).

Conclusion

The results of this research highlighted that citric acid application solely or in combination with phosphatic fertilizers enhances nutrient availability to the plant roots by solubilizing native fixed phosphorus alongside reduce its adsorption onto soil colloids. Applying 80 kg ha⁻¹ of phosphorus and 7.5 kg ha⁻¹ of citric acid had boosted the growth and yield attributes of soybean and total nutrient uptake compared to the control.

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Conflict of interest

The authors declare that they have no conflict of interest.

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