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Research Article

Solubilization of phosphorus by low molecular weight organic acids and amino acids in calcareous soils

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Abstract
In order for plants to perform well in nutrient-deficient calcareous soils, they have an efficient adaptive technique of root exudate secretion, which contains low molecular weight organic acids. They enhance nutrient release and thereby increase the nutrient availability in calcareous soils. *The present study aimed to* investigate the effect of concentration and time of incubation of low molecular weight organic acids on P solubilization from calcareous soils collected from various locations of Coimbatore district with varying levels of calcareousness. An incubation experiment was conducted with five calcareous soils with varying levels of free $CaCO_3$ viz., (1, 7.5, 12.5, 17.5 & 21.5%) by incubating with seven different concentrations (0, 20, 40, 60, 80 & 100 mM) of four organic (citric, malic, oxalic and acetic acid) and two amino acids (glycine and lysine) for nine incubation time intervals (5, 10, 20, 30, 60, 120, 240, 960 & 1440 mins) on a factorial experiment based on completely randomized design (CRD). Available P was analyzed to find the solubilization efficiency of various organic and amino acids. The organic acids were more efficient when compared to amino acids in P solubilization, especially citric acid followed by oxalic and malic acids, at 100 mM concentration incubated for 1440 mins. Also, the solubilization increased with increasing concentration and incubation time, irrespective of the soil calcareousness, but the magnitude of phosphorus extraction decreased with increasing soil calcareousness. Incubating the calcareous soils with 100 mM of citric acid for 1440 min solubilized more amount of phosphorus. Hence it can be concluded that addition of 100 mM citric acid will influence the phosphorus release even from highly calcareous soils.

Keywords: Amino acids, Calcareous soils, Low molecular weight organic acids, Phosphorus, Solubilization

INTRODUCTION

Calcareous soils have a high concentration of free $CaCO_3$, which raises the pH of the soil (to around 8.3), rendering all nutrients insoluble and inaccessible for plants (Jalali and Zinli, 2011). In calcareous soils, low organic matter status and nutrient availability are two typical issues that limit microbial activity. Calcareous soils span 228.8 million hectares, or 69.4 percent of the country's total geographical area (TGA), and are found in 38 of the country's 60 Agro Ecological Sub Regions (Pal *et al*., 2000). In many dry and semi-arid regions of the world, particularly in the Indian subcontinent, the formation of $CaCO_3$ in soils is an important pedogenic activity (Pal *et al*., 2013).
Phosphorus (P) is a macronutrient that plants require in bulk quantity (0.2 to 0.8%) for appropriate growth and development (Pal, 2020). Since increasing soil pH re-

duces nutrient solubility, many nutrients, especially phosphorus, are inadequate in calcareous soil (pH 8.3). The lime in calcareous soil reacts with the nutrients in the soil solution to form a strong insoluble precipitate of calcium phosphate, carbonates, and bicarbonates on the surface of the lime, which is only sparingly soluble. As a result of the high soil pH, nutrients are mainly unavailable in calcareous soil, which is followed by the production of metal complexes that are sparingly soluble and have low bioavailability (Taalab *et al*., 2019).

Plants have some adaptive techniques to overcome nutrient deficiencies in calcareous soils in order to thrive. Some of them are (1) nutrients will be stored in roots through luxury consumption during abundance and will mobilize to shoots during deficiency (Liu *et al*., 2020); (2) maintaining a high root-to-shoot ratio through the regulation of plant C partitioning (Xie *et al*., 2021); (3) colonization of roots with mycorrhiza so as to explore a larger volume of soil and larger uptake (Hack *et al*., 2019); (4) exudation of nutrients (Chen and Liao, 2016).

Root exudation has been considered a vital process by which plants take up nutrients in calcareous soils among all adaptation approaches (Jiang *et al*., 2017). When compared to calcifuge crops, many research have shown that calcicole crops can grow well in calcareous soils due to a better root exudation pattern with substantial amounts of organic acids, particularly citric and oxalic acids (Liao *et al*., 2020). Exudates from plant roots contain enzymes, carbohydrates, amino acids, phenolics, vitamins, purines, nucleosides, proteins, flavonoids, and organic acids, among other complex substances. Organic acids and amino acids are the most common components (Adeleke *et al*., 2017). Root exudates are made up of a variety of organic acids , all of which have a low molecular weight, ranging from 46 DA (Dalton) to a few 100 DA (Perminova *et al*., 2003). Citric, oxalic, acetic, malic, succinic, malonic, and maleic acids are the most frequent low molecular weight organic acids (LMWOA) present in root exudates and usually have one to three carboxylic groups (Taghipour and Jalali, 2013).

Low molecular weight organic acids will release anions, such as P from the soil and are attributed to the desorption of inorganic anions and solubilization of P compounds which is achieved through complex formation between organic acids/anions. Low molecular weight organic acids have the ability to: (1) promote mineral dissolution, (2) modify pH and chemical equilibria in soil solution, (3) occupy ligand exchange sites, and (4) form complexes with Fe, Al, and Ca ions, thus inhibiting anion adsorption sites on soil particles (Gypser *et al*., 2021).

Many research has looked at the role of low molecular weight organic acids in releasing phosphorus from soils, although it is uncertain how effective organic acids are at solubilizing P from calcareous soils of different calcareousness (Mazinanian *et al*., 2015). Amino acids are also found in root exudates, although their significance in phosphorus solubilization is unclear, necessitating further research. This research aimed to see how effective four low molecular weight organic acids and two amino acids were at removing P from five different calcareous soils with variable levels of calcareousness.

MATERIALS AND METHODS

Soil and organic acids

Five soils with various levels of free $CaCO_3$ content (S1 - 1%; S2-7.5%; S3-12.5%; S4-17% & S5- 21.5%) were collected from the surface layer at farmers fields (S1-1% & S4-17%) located in the Thondamuthur block of Coimbatore district and from various fields of Tamil Nadu Agricultural University campus (S2-7.5%; S3-12.5% & S5- 21.5%). Bulk soil samples collected were air-dried, processed, removed the debris and sieved through 2 mm sieve and their initial properties were estimated. Standard analar grade salts of organic acids viz., citric, malic, oxalic, acetic and amino acids viz., glycine and lysine were chosen and the stock solution of 1000 mM was prepared. From this stock, desired working standards were prepared and used.

Extraction efficiency of organic acids

Various concentrations of organic acids and amino acids (0, 10, 20, 40, 60, 80, and 100 mM) were examined to investigate the concentration-dependent extraction efficiency of organic and amino acids in calcareous soils with varied calcium carbonate content. The researchers used a completely randomized block design as a factorial experiment with three replications. To 5.0 g of soil deposited in 50 ml polypropylene tubes, 12.5 ml of seven different concentrations of four organic acids and two amino acids were added. =Calcareous soils and organic/amino acids were introduced and examined at different incubation time intervals of 5, 10, 20, 30, 60, 120, 240, 960, and 1440 minutes to assess the time-dependency of organic and amino acids mediated P extraction. Following the conventional approach specified by Olsen *et al*. (1954) available P content in the soil solution was determined using a spectrophotometer after the corresponding incubation time period had expired.

Statistical analysis

The obtained data were subjected to homogeneity and statistical analysis using SPSS statistical tool. Two-way analysis of variance (ANOVA) and multiple mean comparison test (DMRT) was done to compare the mean values for different factors and variables analyzed ($P < 0.05$) and also to determine the variation among the acids in phosphorus solubilization, Principal Component Analysis (PCA) was carried out to understand the similarities and dissimilarities between the acids in solubilization of P using XLSTAT 2021.3.1.

RESULTS

Initial soil characteristics

The soils used in this experiment were analyzed for various physicochemical characteristics and depicted in table 1. Among the five soils, four soils (S2-S5) were calcareous (pH 8.3) and one soil was non-calcareous (pH 7.69). The free $CaCO_3$ content in calcareous soils varied from 1 to 21.5% and grouped as non-calcareous (1%, S1), slightly (7.5%, S2), moderately (12.5%, S3) and highly calcareous soils (17.5 & 21.5%, S4 & S5). The available P status of all five soils was medium (11.7 to 20.8 kg ha^{-1})and P availability decreases with increasing calcareousness, which was evident from the lowest P status in highly calcareous soils.

Concentration dependent phosphorus extraction efficiency of organic acid and amino acids

Different quantities of organic and amino acids were tested in five different calcareous soils to see how effective they were at extracting available phosphorus. The mean of all the incubation time intervals was used to evaluate the concentration dependent phosphorus extraction efficiency. Boosted concentration increased phosphorus extraction regardless of low molecular weight organic acids, but soil calcareousness hindered phosphorus extraction. The next sections go through the specifics of organic/amino acid nutrition extraction efficiency.

pH

Both organic and amino acids pH reducing capacity increased significantly as their concentrations increased (p=0.05), whereas lower concentrations of organic and amino acids at 10 mM could only induce pH reduction in non-calcareous soil, followed by slightly calcareous and moderately calcareous soil (Fig. 1). The pH reduction was minimal in extremely calcareous soils, such as S4 and S5, with 17.5 and 21.5% free $CaCO_3$. When the concentration reached 100 mM, citric acid showed the greatest fall in pH, followed by oxalic and malic acids. Although the addition of amino acids resulted in a decrease in soil pH, when compared to organic acids, its efficacy in lowering soil pH was poor. When comparing the amino acids, glycine decreased the soil pH more than lysine applied at different levels.

Available phosphorus

Changes in P availability due to the addition of organic/ amino acids at various concentrations are shown in Fig. 2.. The amount of available P increased significantly with increasing the acid concentrations ($p < 0.05$), particularly induced by organic acids with greater relative effect of concentration (100 mM) on the P solubilization. Citric acid was more effective in P extraction among the organic acids, especially at 100 mM concentration (31.0 mg kg^{-1}). Next to citric acid, oxalic acid (12.4 - 29.2 mg kg^{-1}) solubilized more amount of P irrespective of the soil calcareousness. The P solubilization by amino acids was poor when compared to low molecular weight organic acids, however glycine (11.6 to 24.7 mg kg^{-1}) performed far better than lysine (11.4 to 24.1 mg kg^{-1}) in solubilizing the P from calcareous soils.

Fig. 2 also shows the calcareousness-dependent effectiveness of organic and amino acids in solubilizing the locked-up phosphorus in soil, irrespective of the concentration. The extracted P values varied from 15.8 to 31.0 mg kg^{-1} in non-calcareous soil (S1), 16.9 to 28.2 mg kg^{-1} in slightly calcareous soil (S2), 16.6 to 24.7 mg kg^{-1} in moderately calcareous soil (S3), 15.6 to 25.8 and 11.4 to 23.4 mg kg^{-1} in highly calcareous soils (S4 and S5) respectively. It was revealed that the extraction of P was low from highly calcareous soil with all the organic acids, but the magnitude of reduction varied among the acids. In all the soils, the highest P solubilization was noted in 100 mM concentration of citric acid and the lowest values were recorded with 10 mM concentration of lysine.

Time dependent phosphorus extraction efficiency of organic acids:

The effects of different incubation time on calcareous soils with different organic and amino acids were tested for their efficiency in extracting available phosphorus. Irrespective of the acids, an increase in incubation time

Table 1. Initial characteristics of the selected soils

pH	EC (dSm^{-1})	Free $CaCO_3$ (%)	Organic carbon (%)	Available N (kg ha^{-1})	Available P (kg ha^{-1})
7.69	0.37	1.00	0.22	220	20.8
8.35	0.34	7.50	0.16	198	18.3
8.40	0.54	12.5	0.46	187	17.2
8.42	0.37	17.5	0.26	162	16.6
8.38	0.40	21.5	0.32	154	11.7

increased the phosphorus extraction and decreased with increasing soil calcareousness.

pH

The effect of incubation time intervals with different organic/amino acids on the reduction of soil pH was tested and presented in Table 2. It is clear that reduction was observed with all the incubation time intervals, but the magnitude of reduction varied with organic/amino acids. The pH reduction was more prominent by the addition of citric acid, which increased as the incubation time increased. Citric acid recorded a greater reduction in pH (8.18-7.45) from 5 min to 1440 mins of incubation time, followed by oxalic acid (8.23-7.57). Compared to organic acids, the pH reduction by amino acids was lesser; however, glycine reduced the pH from 8.27-7.66, which is far better than lysine (8.30-7.71).

The effect of soil calcareousness on pH reduction by organic/amino acids is depicted in Table 3. It was evident that, only in non-calcareous soil, pH reduction was evident when compared to the initial soil pH of 8.3, whereas in highly calcareous soils with 21.5% free $CaCO_3$, the reduction in pH was lesser. Among the acids, citric acid reduced the pH to a greater extent when compared to other organic acids, while among amino acids, glycine was observed to reduce pH better than lysine. However, organic acids were more efficient in reducing the soil pH when compared to amino acids.

Available phosphorus

The amount of P solubilized from the soil depended on the type of organic/amino acids and the incubation time is mentioned in Table 4. Both organic and amino acids promoted the solubilization of P, but the magnitude of solubilization was more in organic acids than in amino acids and the P release tended to increase significantly as the incubation time increased ($p < 0.05$). Table 4 also showed that amongst organic acids, the rate of citric acid-mediated P extraction was the greatest (20.4-25.6 mg kg^{-1}) followed by oxalic acid (19.3-23.9 mg kg^{-1}) in all the soils. The extraction efficiency was the lowest with amino acids and the mean values varied from 18.5-21.0 mg kg^{-1} and 17.8-20.5 mg kg^{-1} for glycine and lysine, respectively. The extraction efficiency of acids increased by 10 folds at 1440 min when compared to 5 min incubation time for organic acids, while for amino acids there was only 3 folds increase in the extraction efficiency at 1440 min, when compared to 5 min.

The P extraction efficiency of acids varied from soil to soil and is depicted in table 5. The extraction efficiency of acids decreases as the soil calcareousness increases. When compared to calcareous soils, non-calcareous soil recorded more P solubilization as expected by all the organic/amino acids tested. Among the soils, the extraction efficiency of citric acid was more evident in both calcareous and non-calcareous soils and the values ranged from 26.0 to 17.7 mg kg^{-1}, where the maximum extraction of 26.0 mg kg^{-1} was recorded in non-calcareous soil and 17.7 mg kg^{-1} in soil 5 with 21.5% free $CaCO_3$. Compared to amino acids, the efficiency of P extraction was more with organic acids.

PCA analysis

The principal component analysis (PCA) is a multivariate statistical technique, to extract and simplify a given set of data. PCA was performed for phosphorus to study the phosphorus solubilization pattern of organic/amino acids as influenced by soil calcareousness.

Table 2. Changes in the soil pH due to the addition of organic/amino acids at different incubation time intervals

Organic/ amino acids	Incubation time intervals (minutes)									Mean
	5	10	20	30	60	120	240	960	1440	
Citric acid	8.18 (±0.12)	8.05 (±0.18)	7.98 (±0.09)	7.95 (±0.18)	7.83 (±0.15)	7.81 (±0.01)	7.70 (±0.05)	7.56 (±0.01)	7.45 (±0.02)	7.83
Malic acid	8.20 (±0.13)	8.13 (±0.21)	8.05 (±0.14)	8.03 (±0.20)	7.91 (±0.20)	7.89 (±0.03)	7.82 (±0.20)	7.71 (±0.19)	7.58 (±0.13)	7.92
Acetic acid	8.25 (±0.06)	8.16 (±0.22)	8.08 (±0.08)	8.04 (±0.02)	8.05 (±0.18)	7.92 (±0.04)	7.84 (±0.13)	7.75 (±0.08)	7.61 (±0.16)	7.97
Oxalic acid	8.23 (±0.04)	8.13 (±0.06)	8.04 (±0.18)	8.02 (±0.18)	7.91 (±0.04)	7.89 (±0.18)	7.79 (±0.04)	7.69 (±0.13)	7.57 (±0.03)	7.92
Glycine	8.27 (±0.04)	7.84 (±0.08)	8.10 (±0.09)	8.07 (±0.16)	8.04 (±0.05)	7.94 (±0.03)	7.84 (±0.07)	7.76 (±0.19)	7.66 (±0.09)	7.95
Lysine	8.30 (±0.04)	8.23 (±0.07)	8.15 (±0.14)	8.11 (±0.02)	8.07 (±0.13)	7.98 (±0.02)	7.87 (±0.14)	7.79 (±0.11)	7.71 (±0.18)	8.02
Mean	8.24	8.09	8.07	8.04	7.97	7.91	7.81	7.71	7.60	7.94
	O	T	OXT							
SEd	0.005	0.019	0.017							
CD (P=0.05)	0.011	0.014	0.033							

* Values in the parenthesis indicates ± SE (n=3).

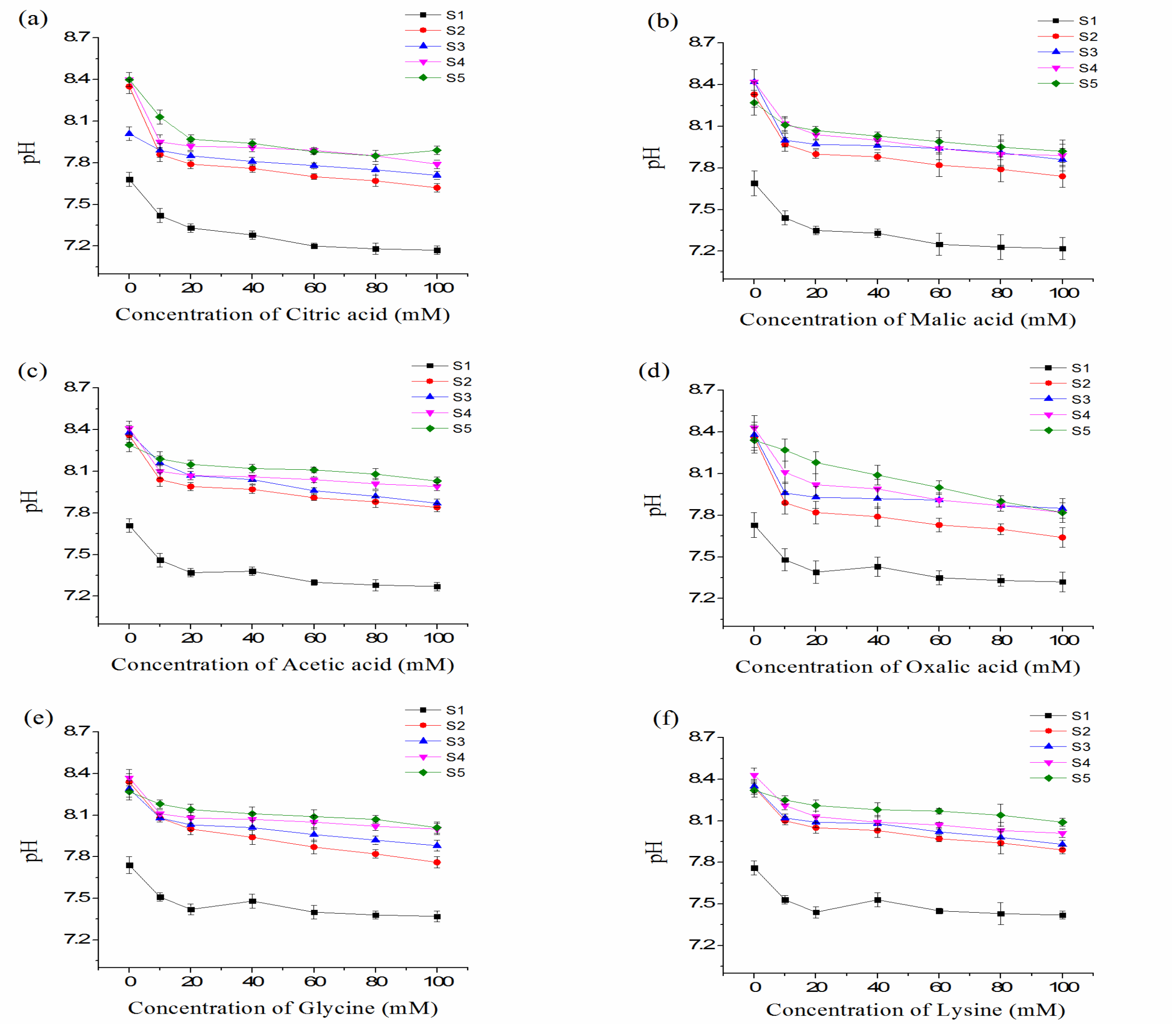

Fig. 1. *Changes in soil pH due to the addition of different concentrations of organic/amino acids [S1-Non-calcareous (1%); S2- Slightly calcareous (7.5%); S3-Moderately calcareous (12.5%); S4- Highly calcareous (17%) & S5- Highly calcareous (21.5%); Error bars represents mean of three replications]*

Table 3. Changes in the soil pH due to the addition of organic/amino acids in soils with varying intensities of calcareousness

Organic/amino acids	**Soil Calcareousness (% Free $CaCO_3$)**					**Mean**
	1 %	7.5 %	12.5 %	17.5 %	21.5 %	
Citric acid	7.32 (±0.01)	7.80 (±0.12)	7.96 (±0.17)	8.00 (±0.01)	8.08 (±0.15)	7.83
Malic acid	7.43 (±0.16)	7.98 (±0.13)	7.97 (±0.18)	8.04 (±0.18)	8.19 (±0.11)	7.92
Acetic acid	7.40 (±0.11)	8.02 (±0.07)	8.09 (±0.21)	8.08 (±0.20)	8.24 (±0.04)	7.97
Oxalic acid	7.36 (±0.04)	7.99 (±0.19)	8.02 (±0.09)	8.06 (±0.16)	8.17 (±0.01)	7.92
Glycine	7.47 (±0.05)	7.90 (±0.12)	7.99 (±0.06)	8.10 (±0.02)	8.28 (±0.16)	7.95
Lysine	7.51 (±0.06)	8.02 (±0.13)	8.09 (±0.12)	8.15 (±0.01)	8.35 (±0.00)	8.02
Mean	7.42	7.95	8.02	8.07	8.22	7.94
	O	S	OXS			
SEd	0.005	0.005	0.013			
CD (P=0.05)	0.011	0.010	0.025			

*O-Organic/amino acids; S-Soil calcareousness. Values in the parenthesis indicates ± SE (n=3)

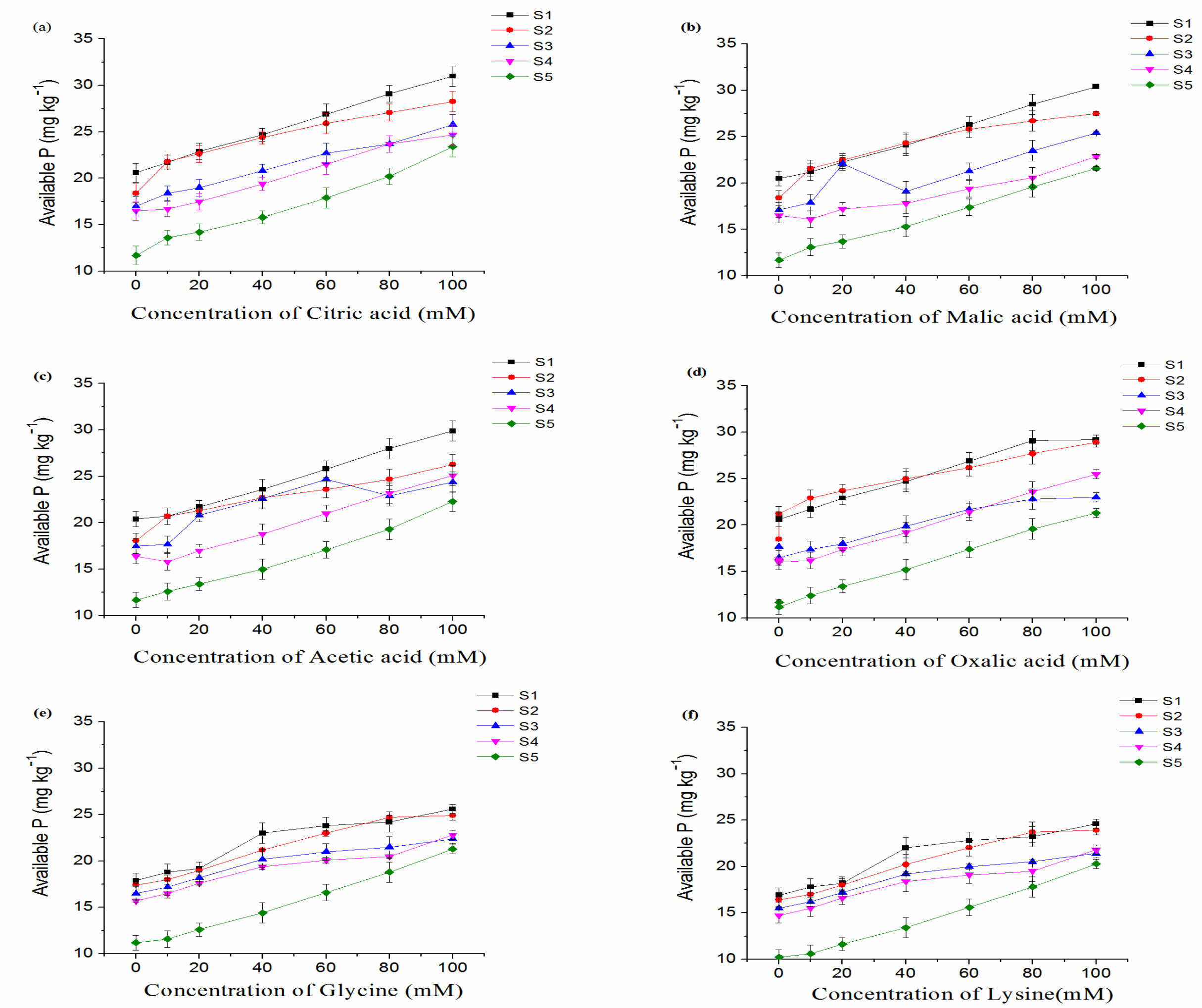

Fig. 2. *Changes in P availability due to the addition of organic/amino acids at various concentrations [S1-Non-calcareous (1%); S2- Slightly calcareous (7.5%); S3-Moderately calcareous (12.5%); S4- Highly calcareous (17%) & S5- Highly calcareous (21.5%); Error bars represents mean of three replications].*

Table 4. Changes in P availability due to the addition of organic/amino acids in calcareous soils at different incubation time intervals

Organic/ amino acids	Incubation time intervals (minutes)									Mean
	5	10	20	30	60	120	240	960	1440	
Citric acid	20.4 (±0.41)	20.7 (±0.24)	21.1 (±0.07)	21.4 (±0.25)	21.8 (±0.06)	22.8 (±0.02)	23.8 (±0.09)	24.7 (±0.43)	25.6 (±0.15)	22.5
Malic acid	19.3 (±0.40)	20.4 (±0.19)	20.6 (±0.19)	20.9 (±0.51)	21.2 (±0.10)	21.7 (±0.40)	22.0 (±0.26)	23.0 (±0.49)	23.6 (±0.33)	21.4
Acetic acid	19.8 (±0.15)	20.2 (±0.29)	20.5 (±0.42)	20.8 (±0.25)	21.1 (±0.04)	21.6 (±0.54)	21.8 (±0.47)	22.7 (±0.43)	23.5 (±0.51)	21.3
Oxalic acid	19.3 (±0.01)	20.3 (±0.45)	20.8 (±0.24)	20.9 (±0.13)	21.3 (±0.30)	21.9 (±0.07)	22.2 (±0.05)	23.1 (±0.55)	23.9 (±0.11)	21.5
Glycine	18.5 (±0.26)	19.3 (±0.38)	19.4 (±0.43)	19.7 (±0.24)	19.9 (±0.43)	20.1 (±0.51)	20.5 (±0.00)	20.9 (±0.42)	21.0 (±0.20)	19.9
Lysine	17.8 (±0.12)	17.9 (±0.18)	18.5 (±0.39)	19.0 (±0.21)	19.4 (±0.30)	19.6 (±0.04)	19.9 (±0.06)	20.1 (±0.46)	20.5 (±0.01)	19.2
Mean	19.2	19.8	20.2	20.5	20.8	21.3	21.7	22.4	23.0	21.0
	O	T	OXT							
SEd	0.09	0.11	0.26							
CD (P=0.05)	0.17	0.21	0.52							

* O-Organic/amino acids; T-Time intervals.Values in the parenthesis indicates ± SE (n=3).

In the present study, six acids (four organic and two amino acids) were tested for their phosphorus solubilizing potentials in five soils with varying levels of calcareousness. From the results it was noted that only one component had eigen value of more than 1, hence it was alone considered and PCs having eigen values less than one are considered non-significant. The factor scores, eigen values, variability percentage and cumulative percentage obtained for P in PCA are depicted in Table 6. Total variance of 97.1% was contributed by all the three extracted factors of PCA in combination and Factor 1 (F1) contributed the maximum to the total variability in solubilization of P (71.7%). Factor scores were maximum for citric acid (2.87) in P solubilization, followed by oxalic acid (1.52) and malic acid (1.23). Whereas, the factor scores for acetic acid (-0.24), glycine (-1.65) and lysine (-3.71) were very low.

DISCUSSION

Due to its low bioavailability, phosphorus deficiency is a serious issue that inhibits plant growth in calcareous soils (Vahedi *et al*., 2022). The total P concentration in calcareous soils, on the other hand, is generally higher, but it resides in the precipitate and inaccessible forms. In the area of their roots, calcicole crops release larger amounts of organic acids, primarily citric and oxalic acids. Many researchers have proposed that low molecular weight organic acids are involved in phosphorus solubilization within the rhizosphere region, with citric acid being particularly effective in this regard (Mazinanian *et al*., 2015). Baranimotlagh *et al*. (2013) postulated that crops exudate larger amounts of low molecular weight organic acids in order to solubilize P and overcome its deficiency which was in accordance with these findings.

Maximum phosphorus was recovered in soil with low $CaCO_3$ content by both organic and amino acids in both concentration and time-dependent extraction. The amount of phosphorus solubilized by acids reduced as soil calcareousness increased, with the lowest phosphorus extraction found in highly calcareous soil with 21.5% free $CaCO_3$. There was no doubt that increasing concentration increases phosphorus solubilization, but even at 100 mM, solubilization was lower in highly calcareous soil (21.5% free $CaCO_3$- S5) than in non-calcareous soil (21.5% free $CaCO_3$- S5). It was obvious that low molecular weight organic acids effectively extract more phosphorus from calcareous soils, but their solubilization magnitude reduces as the calcareousness of the soil increases. Because the free $CaCO_3$ content of the soils used in this experiment ranges from 1 to 21.5%, Ca^{2+} is the dominant cation in these soils, all calcareous (free $CaCO_3$ > 5%). In addition, a greater proportion of soil P precipitated as insoluble and inaccessible forms. By substituting P in the Ca-P precipitate, LMWOAs such as citrate, oxalate, malate, and acetate readily generate Ca-oxalate, Ca-citrate, Ca-malate, and Ca-acetate (Jones, 1998).

According to the findings, low molecular weight organic acids had a higher phosphorus extraction efficiency than amino acids in concentration-dependent extrac-

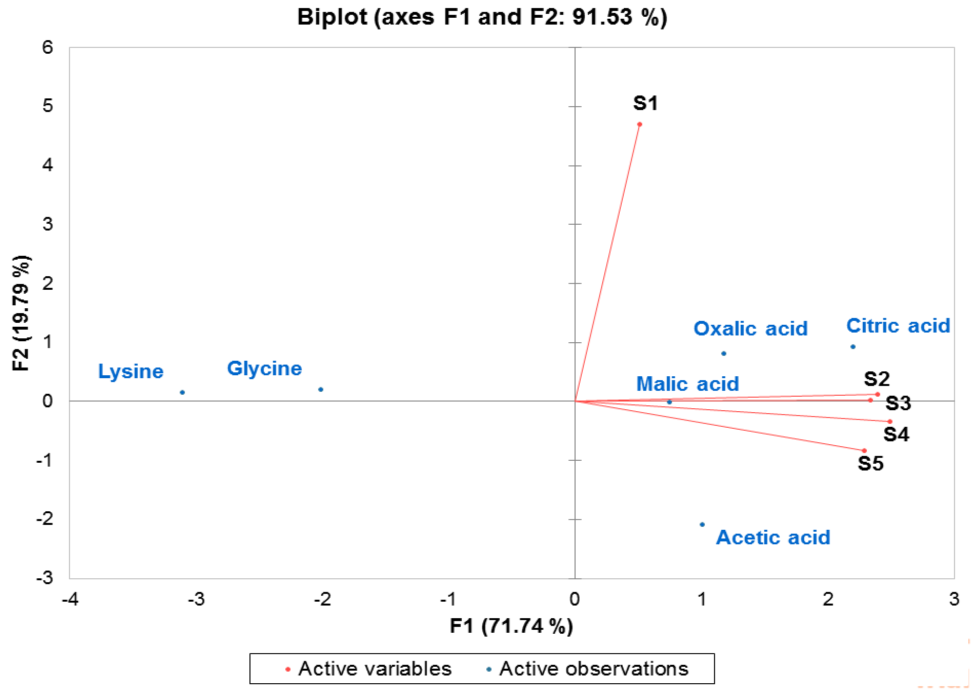

Fig. 3. *Bi-plot obtained for P solubilization as influenced by organic/amino acids and soil calcareousness [S1-Non-calcareous (1%); S2- Slightly calcareous (7.5%); S3-Moderately calcareous (12.5%); S4- Highly calcareous (17%) & S5-Highly calcareous (21.5%); per cent variance explained by each component is given in parenthesis].*

Table 5. Changes in P availability due to the addition of organic/amino acids in soils having varying levels of calcareousness

Organic/ amino acids	Soil Calcareousness (% Free $CaCO_3$)					Mean
	1 %	7.5 %	12.5 %	17.5 %	21.5 %	
Citric acid	26.0 (±0.15)	25.0 (±0.03)	22.8 (±0.51)	20.9 (±0.07)	17.7 (±0.35)	22.5
Malic acid	25.6 (±0.28)	24.6 (±0.36)	20.5 (±0.16)	20.4 (±0.36)	16.3 (±0.14)	21.5
Acetic acid	25.2 (±0.42)	24.8 (±0.05)	20.3 (±0.13)	19.8 (±0.47)	16.7 (±0.04)	21.4
Oxalic acid	24.8 (±0.14)	24.4 (±0.15)	21.9 (±0.07)	20.0 (±0.40)	16.4 (±0.11)	21.5
Glycine	22.5 (±0.42)	21.3 (±0.28)	19.4 (±0.06)	19.1 (±0.10)	15.7 (±0.31)	19.6
Lysine	21.6 (±0.04)	20.8 (±0.05)	19.8 (±0.11)	17.6 (±0.45)	15.0 (±0.04)	19.0
Mean	24.3	23.5	20.8	19.6	16.3	20.9
	O	S	OXS			
SEd	0.09	0.08	0.20			
CD (P=0.05)	0.17	0.16	0.39			

*Include O-Organic/amino acids; S-Soil calcareousness. Values in the parenthesis indicates ± SE (n=3).

tion when compared to amino acids. Citric acid was found to release more P than all other organic acids when compared to all other acids in all soils regardless of calcareousness, which could be explained by greater pH reduction with the addition of citric acid when compared to all other acids in all soils regardless of calcareousness. The pH of the solution declined as the concentration of citric acid increased, regardless of the soil (Fig. 1), with the pH reduction being greater in non-calcareous soil. As expected, the pH reduction was lowest in highly calcareous soil (21.5% free $CaCO_3$). After adding organic acids, the soil's pH was substantially lower than the pH of the native soil, allowing phosphorus to be released from the cation complex more easily (Zhang *et al*., 2020). Citric acid appears to be a more potent extracting agent than all other organic acids due to three hydroxyl groups (Lazo *et al*., 2015). Geng *et al*. (2020) discussed the acidification of calcareous soil due to organic/amino acid addition in calcareous soils with the addition of citrate and oxalate at 10mM concentration. Ma *et al.* (2020) previously reported that citric acid had a higher capacity for releasing phosphorus than malic, oxalic, and acetic acids.

Organic acids such as citric acid, malic acid and oxalic acid are useless in phosphorus solubilization unless present in sufficient amounts (Vengavasi *et al*., 2021; Wang and Lambers, 2020). P extraction rose when the citric and oxalic acid concentrations increased (Strom et al.,2005) while Keskinen et al. (2013) discovered a significant increase in P extraction when the concentration reached 80 Mm. The maximum phosphorus solubilization was observed in present study at 100 mM concentrations, regardless of acidity, and even in soil with 21.5% free $CaCO_3$ there was more phosphorus solubilization at 100 mM concentrations. These data suggest that high levels of organic acids in the rhizosphere may be required to impact phosphorus solubilization in calcareous soils significantly.

In the case of time-dependent phosphorus extraction, a higher pH reduction at 1440 minutes using a citric acid mediated reaction demonstrated the maximum phosphorus release rate. The fall in soil pH justifies the increase in phosphorus release due to a prolonged incubation period with organic acids, and Strom *et al*. (2005) and Oral *et al*. (2005) found comparable results. Citric acid, oxalic acid, malic acids and non-calcareous and slightly calcareous soils (S1- 1% $CaCO_3$ and S2-7.5% free $CaCO_3$) all shifted towards the positive quadrant of the study's PCA bi-plot, indicating that they had a positive link with P solubilization (Fig. 3). Citric, oxal-

Table 6 Factor scores, eigen value, variability percentage and cumulative percentage of factors obtained for organic acids and amino acids for phosphorus solubilization**.** Factor scores, eigen value, variability percentage and cumulative percentage of factors obtained for organic acids and amino acids for phosphorus solubilization

Organic/amino acids	Available P		
	F1	F2	F3
Citric acid	2.19	0.92	-0.21
Malic acid	1.00	-2.08	0.24
Acetic acid	0.74	-0.01	-0.88
Oxalic acid	1.17	0.81	0.86
Glycine	-2.01	0.19	-0.14
Lysine	-3.11	0.15	0.13
Eigen value	3.58	0.98	0.27
Variability %	71.7	19.7	5.58
Cumulative %	71.7	91.5	97.1

*F1-Principal component 1; F2-Principal component 2 and F3-Principal component 3 ic, and malic acids are the principal drivers, but acetic acid, as well as the amino acids glycine and lysine, are in the negative quadrant, demonstrating their inefficiency in solubilizing P. According to the PCA data, citric acid performs better at a concentration of 100 mM, and solubilization was more pronounced in non-calcareous soil than in soil with 7.5% free $CaCO_3$.

According to our findings, P was more efficiently solubilized by citric and oxalic acids than malic and acetic acid, at a concentration of 100 mM incubated for 1440 minutes (24hrs). Citric acid is a strong complexing agent that binds to metals and releases the trapped P (Mazinanian *et al*., 2015). The extraction efficiency of soil accessible P, both in terms of concentration and time, was: Citric acid > Oxalic acid > Malic acid > Acetic acid > Glycine > Lysine. When cultivated in calcareous soils, plant exudation of citric and oxalic acids may help with phosphorus solubilization. However, supplementary additions of organic acids boosted phosphorus availability as an additive effect on calcareous soils.

Conclusion

The low molecular weight organic acids are thought to be capable of solubilizing locked-up phosphorus in calcareous soils. In line with this, the present study's data implies that low molecular weight organic acids had a role in the solubilization of P. Regardless of the organic/amino acids or soils, the amount of P released increases as the incubation duration increases. Organic acids were more successful than amino acids at extracting phosphorus from calcareous soils. More P was soluble in calcareous soils after 1440 minutes of incubation with 100 mM citric acid, which was followed by oxalic acid > malic acid > acetic acid > glycine > lysine. The solubilization of phosphorus by low molecular weight organic acids through soil acidification was linked to this increase in P release due to increased incubation duration and concentration. Thus, incubating calcareous soils with 100 mM citric acid for 1440 minutes will aid in locking-up phosphorus solubilization in calcareous soils, and the chemistry behind the phosphorus release requires further research.

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Conflict of interest

The authors declare that they have no conflict of interest.

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