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# Phosphorus Releasing Potentials of Amino Acids and Low Molecular Weight Organic Acids from Highly Calcareous Soils

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#### Authors' contributions

This work was carried out in collaboration among all authors. Author PMB executed the incubation study and laboratory analysis. Author TC designed the study and supervised the work. Authors DS, US and PJ managed the analyses of the study. All authors read and approved the final manuscript.

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# ABSTRACT

**Aims:** The present study was conducted to investigate the potentials of amino acids and low molecular weight organic acids, two major components of plant root exudates on phosphorus release from highly calcareous soils.

**Study Design:** Factorial experiment based on completely randomized design was used. **Place and Duration of Study:** Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India between 21<sup>st</sup> and 22<sup>nd</sup> of December 2020. **Methodology:** An incubation experiment was conducted with two amino acids (glycine and lysine) and four low molecular weight organic acids (citric acid, malic acid, oxalic acid and acetic acid) at seven different concentrations (0, 20, 40, 60, 80 & 100 mM) for assessing their potentials of phosphorus release from highly calcareous soils (>15% free CaCO<sub>3</sub>). The soils were incubated for a time interval of 5, 10, 20, 30, 60, 120, 240, 960 & 1440 minutes. Available Phosphorus status in the soils treated with both acids were analyzed after the expiry of each incubation time. **Results:** Organic acids had higher impact on phosphorus release than amino acids. Kinetic data obtained from the experiment were adequately described by Simple elovich ( $r^2 \ge 0.90$ , P < .001) and Power function ( $r^2 \ge 0.80$ , P < .001) models for both acids. Glycine among amino acids and citric acid among low molecular weight organic acids were highly effective in releasing phosphorus from highly calcareous soils.

**Conclusion:** The experiment concludes that, incubating the highly calcareous soils with 100 mM citric acid for 1440 minutes increased the kinetics of phosphorus release. The Simple elovich model was the best fitted model to explain the phosphorus release from calcareous soils as influenced by amino acids and low molecular weight organic acids.

Keywords: Calcareous soils; low molecular weight organic acids; amino acids; kinetic models; phosphorus release.

# 1. INTRODUCTION

Phosphorus (P) deficiency in the soils hinders the crop productivity in many of the agricultural areas all over the world, due to its adsorption by oxides of Fe, AI in acid soils and precipitation as Ca compounds in calcareous soils [1]. Calcareous soils occupy more than 30% of the earth surface around 800 mha, mainly in arid and semiarid regions of the world. They have more amount of CaCO<sub>3</sub> and hence pH will be around 8.3, which will result in nutrient deficiency especially P [2]. Hence, these soils are supplied with plenty of P fertilizer sources, in order to increase the P supply and to sustain the crop nutrient requirements for achieving the targeted yield in calcareous soils [3]. However, the excessive addition of P in soils pollute the groundwater in agricultural areas [4]. Furthermore, the added P won't be utilized by plants due to lesser P use efficiency and high P fixing capacity in the calcareous soils, where P is fixed as insoluble Ca-P. Even though large amount of P is applied as soluble fertilizers, the bioavailable P status in soil solution is very low  $(< 5 \mu mol L^{-1})$  [5].

In order to overcome the non-availability of P in calcareous soils, plants also have some adaptive mechanisms such as acidification of root zone through secretion of root exudates and rootinduced accumulation of P ions [6]. Out of the various adaptive mechanisms, root exudation of low molecular weight organic acids (LMWOA) is proved to be the most efficient mechanism [7] in plants which has increased the P availability in calcareous soils through solubilization [8]. Wang et al. [1] reported that, about 21.3% - 39.7% of total P was released by LMWOA and citric acid released 35% of P in forest soil. LMWOA will release the locked-up P and make them bioavailable [9]. The organic acids in root exudates forms complexes with anions and ions of Fe, Al and Ca, hence the anion adsorption sites in soil particles is blocked. Organic acids influence the solubility of insoluble precipitates through complex formation and occupy the ligand exchange sites thus the anions become bioavailable [10].

Next to LMWOA, amino acids are also considered to be the dominant component in plant root exudates [11]. The amino acids which are passively released by roots at all the times are very little studied. It was postulated that; the amino acids have pH dependent charges and it may have a role in the complexation of metals within the rhizosphere and has a vital role in nutrient solubilization as well [12]. In fact, amino acids could participate in complexation with metal in the same way as organic acids. Few authors suggested that, amino acids may also play an important role in nutrient acquisition due to the possession of active uptake carrier [6], which needs a lime light. Despites all these points, amino acids are so under rated and their role in releasing the locked-up nutrients from soils is less studied and only limited literatures are available [11].

In order to assess the changes in P availability, rate of P release which is an essential factor for increasing the P availability in calcareous soils, kinetic study is an appropriate method to quantify the rate of P release from soils [13] which controls the availability of soil P, thus influences soil P fertility and its uptake by crops [1]. Very few information is available on the impact of amino acids like glycine, lysine and LMWOA like citric, malic, oxalic and acetic acids on the kinetics of P release from calcareous soils. The above mentioned organic/amino acids are predominantly available in root exudates. But the mechanisms of P release induced by amino acids and LMWOA is not well explained, especially in highly calcareous soils (>15% free

 $CaCO_3$ ), where the excess level of  $CaCO_3$  may affect the P release from soil.

General hypothesis is that, LMWOA improves the mobilization of soil inorganic P, as they have the capacity to promote the dissolution of insoluble precipitates and have the potential to alter the pH of soils. But on the other hand, role of amino acids on the nutrient release from soil requires lime light, especially in highly calcareous soils. Hence the present study was carried out with the aim to evaluate the effect of various amino acids and organic acids on the availability of phosphorus in highly calcareous soils.

#### 2. MATERIALS AND METHODS

An incubation experiment was conducted in the Micro nutrients laboratory, Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University (TNAU), Coimbatore to study the kinetics of P release from highly calcareous soils as influenced by low molecular weight organic acid and amino acid addition. The experiment was conducted in a completely randomized block design and replicated twice. The calcareous soils collected from farmers field in Thondamuthur block (S1-17% free CaCO<sub>3</sub>) and from TNAU campus (S2-21.5% free CaCO<sub>3</sub>). There were two amino acids (glycine and lysine) and four low molecular weight organic acids (citric, malic, oxalic and acetic) treated at seven different concentrations (0, 10, 20, 40, 60, 80 and 100 mM). The kinetic evaluation was made at nine incubation time intervals (5, 10, 20, 30, 60, 120, 240, 960 & 1440 min) with two replicates. 2 g of soil sample (2 g) was weighed and taken into 50 ml shaking bottles, then 20 ml of respective amino acids and organic amino acids was added at seven different concentrations to each bottle and incubated. After the incubation of specific time, the samples were shaken on a mechanical shaker for 30 min at 3000 rpm and filtered through Whatman filter paper (No.1). The P content in the aliquot was determined using 0.5 M NaHCO<sub>3</sub> [2].

The cumulative P released by the organic acids into the soil solution was plotted against incubation time intervals for both the soils. Five kinetic models were used to fit the P release patterns as described by wang et al. [1] and Taghipour and Jalali [8]:

| 1 | Zero order          | $:q_t = q_0 - k_0 t$                                  | k <sub>0</sub> : zero order rate constant, mg P kg <sup>-1</sup> min <sup>-1</sup> |
|---|---------------------|-------------------------------------------------------|------------------------------------------------------------------------------------|
| 2 | First order         | $:q_t = q_0 e^{-k} t_1^{t}$                           | k <sub>1</sub> : first-order rate constant, min <sup>-1</sup>                      |
| 3 | Parabolic diffusion | $:q_t = q_0 + k_p t^{1/2}$                            | $k_p$ : diffusion rate constant, mg <sup>-0.5</sup> P kg <sup>0.5</sup>            |
| 4 | Simple Elovich      | $:q_t = (1/\beta) \ln(\alpha\beta) + (1/\beta) \ln t$ | α: initial P desorption rate, mg P kg <sup>-1</sup> min <sup>-1</sup> ,            |
|   |                     |                                                       | β: P desorption constant, mg <sup>-1</sup> P kg                                    |
| 5 | Power function      | $:q_t = q_0 + t^b$                                    | q₀: initial release amount, mg P kg <sup>-1</sup>                                  |
|   |                     |                                                       | b: P release rate constants, (mg P kg <sup>-1</sup> ) <sup>-1</sup>                |

#### 2.1 Statistical Analysis

Linear forms of the five kinetic equations were fitted to the kinetic experimental data, separately for each soil with all the organic/amino acid used. The coefficient of determination ( $r^2$ ) values and P release rate parameters were subsequently obtained from fitted equations as follows: the " $\alpha$ " and " $\beta$ " from the simple elovich equation [14], "a" and "b" from power function model [15] were calculated using OriginPro 8.5.0. The obtained data were subjected to factorial completely randomized block design in SPSS software for windows (SPSS Inc., Chicago and U.S.A) in two-way analysis of variance (ANOVA) for each soil and acid studied. Comparison of mean was done using least significant difference (LSD) at p ≤ .001 and p ≤ .05.

# 3. RESULTS AND DISCUSSION

#### **3.1 Characteristics of Experimental Soil**

The properties of the collected soil samples has been presented in Table 1. The collected soil sample of two different sites were highly calcareous very low in organic carbon content and others. The available P status decreased with increasing soil calcareousness.

| Soil   | рН   | EC<br>(dS m <sup>-1</sup> ) | Free<br>CaCO <sub>3</sub> (%) | Organic<br>carbon (%) | Available N<br>(kg ha <sup>-1</sup> ) | Available K<br>(kg ha <sup>-1</sup> ) | Available P<br>(kg ha <sup>-1</sup> ) |
|--------|------|-----------------------------|-------------------------------|-----------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| Soil 1 | 8.42 | 0.37                        | 17.5                          | 0.26                  | 162                                   | 156                                   | 16.6                                  |
| Soil 2 | 8.38 | 0.40                        | 21.5                          | 0.32                  | 154                                   | 187                                   | 11.7                                  |

# Table 1. Initial characteristics of the selected soils

Table 2. Coefficient of determination (r<sup>2</sup>) for selected kinetic equations used to describe the kinetics of P release after 1440 min from highly calcareous soil (17.5% free CaCO<sub>3</sub>) incubated with different concentrations of organic acids and amino acids

| Sources of  | Concentration | Zero-order         | First order    | Simple -       | Power          | Parabolic      |
|-------------|---------------|--------------------|----------------|----------------|----------------|----------------|
| acids       | levels        | kinetics           | kinetics       | elovich        | function       | function       |
|             | (mM)          | r <sup>2</sup>     | r <sup>2</sup> | r <sup>2</sup> | r <sup>2</sup> | r <sup>2</sup> |
| Citric acid | 0             | 0.594 <sup>ª</sup> | 0.324**        | 0.984**        | 0.843**        | 0.782**        |
|             | 10            | 0.601**            | 0.327**        | 0.985**        | 0.846**        | 0.789**        |
|             | 20            | 0.605**            | 0.330**        | 0.986**        | 0.849**        | 0.793**        |
|             | 40            | 0.604**            | 0.330**        | 0.986**        | 0.848**        | 0.792**        |
|             | 60            | 0.604 <sup>a</sup> | 0.329**        | 0.985**        | 0.848**        | 0.791**        |
|             | 80            | 0.603**            | 0.329**        | 0.985**        | 0.847**        | 0.791**        |
|             | 100           | 0.605**            | 0.331**        | 0.986**        | 0.849**        | 0.793**        |
| Malic acid  | 0             | 0.590**            | 0.320**        | 0.983**        | 0.839**        | 0.779**        |
|             | 10            | 0.606**            | 0.331**        | 0.986**        | 0.849**        | 0.793**        |
|             | 20            | 0.605**            | 0.330**        | 0.986**        | 0.849**        | 0.793**        |
|             | 40            | 0.604 <sup>a</sup> | 0.330**        | 0.986**        | 0.848**        | 0.792**        |
|             | 60            | 0.604 <sup>a</sup> | 0.330**        | 0.985**        | 0.848**        | 0.791**        |
|             | 80            | 0.603**            | 0.329**        | 0.985**        | 0.848**        | 0.791**        |
|             | 100           | 0.602**            | 0.328**        | 0.985**        | 0.847**        | 0.790**        |
| Acetic acid | 0             | 0.595**            | 0.324**        | 0.983**        | 0.842**        | 0.783**        |
|             | 10            | 0.606**            | 0.331**        | 0.986**        | 0.849**        | 0.793**        |
|             | 20            | 0.605**            | 0.330**        | 0.986**        | 0.849**        | 0.793**        |
|             | 40            | 0.604 <sup>a</sup> | 0.330**        | 0.986**        | 0.848**        | 0.792**        |
|             | 60            | 0.604 <sup>ª</sup> | 0.330**        | 0.986**        | 0.848**        | 0.791**        |
|             | 80            | 0.603**            | 0.329**        | 0.985**        | 0.848**        | 0.791**        |
|             | 100           | 0.602**            | 0.328**        | 0.985**        | 0.847**        | 0.790**        |
| Oxalic acid | 0             | 0.594**            | 0.324**        | 0.984**        | 0.843**        | 0.783**        |
|             | 10            | 0.606 <sup>ª</sup> | 0.331**        | 0.986**        | 0.849**        | 0.793**        |
|             | 20            | 0.605**            | 0.330**        | 0.986**        | 0.849**        | 0.793**        |
|             | 40            | 0.604**            | 0.330**        | 0.986**        | 0.848**        | 0.792**        |
|             | 60            | 0.604**            | 0.329**        | 0.985**        | 0.848**        | 0.791**        |
|             | 80            | 0.603 <sup>ª</sup> | 0.329**        | 0.985**        | 0.848**        | 0.791**        |
|             | 100           | 0.602**            | 0.328**        | 0.985**        | 0.847**        | 0.790**        |
| Glycine     | 0             | 0.596**            | 0.325**        | 0.984**        | 0.843**        | 0.784**        |
| -           | 10            | 0.606 <sup>ª</sup> | 0.331**        | 0.986**        | 0.849**        | 0.794**        |
|             | 20            | 0.606**            | 0.331**        | 0.986**        | 0.849**        | 0.793**        |
|             | 40            | 0.605**            | 0.330**        | 0.986**        | 0.848**        | 0.792**        |
|             | 60            | 0.604**            | 0.330**        | 0.986**        | 0.848**        | 0.792**        |
|             | 80            | 0.603**            | 0.329**        | 0.985**        | 0.848**        | 0.791**        |
|             | 100           | 0.602 <sup>ª</sup> | 0.328**        | 0.985**        | 0.847**        | 0.790**        |
| Lysine      | 0             | 0.596**            | 0.325**        | 0.984**        | 0.843**        | 0.784**        |
| -           | 10            | 0.606**            | 0.331**        | 0.986**        | 0.849**        | 0.794**        |
|             | 20            | 0.606**            | 0.331**        | 0.986**        | 0.849**        | 0.794**        |
|             | 40            | 0.605**            | 0.330**        | 0.986**        | 0.848**        | 0.792**        |
|             | 60            | 0.604**            | 0.330**        | 0.986**        | 0.848**        | 0.791**        |
|             | 80            | 0.603**            | 0.329**        | 0.985**        | 0.848**        | 0.791**        |
|             | 100           | 0.602**            | 0.328**        | 0.985**        | 0.847**        | 0.790**        |

<sup>a</sup> not significant at P< 0.05, \* significant at P< 0.05, \*\* significant at P< 0.01

| Sources of  | Concentration | Zero-order                  | First order    | Simple - | Power          | Parabolic      |
|-------------|---------------|-----------------------------|----------------|----------|----------------|----------------|
| acius       |               | r <sup>2</sup>              | r <sup>2</sup> |          | r <sup>2</sup> | r <sup>2</sup> |
| Citric acid | 0             | 0.573**                     | 0.317**        | 0.977**  | 0.832**        | 0 778**        |
|             | 10            | 0.575                       | 0.317          | 0.977    | 0.002          | 0.770          |
|             | 20            | 0.505                       | 0.303          | 0.070    | 0.002          | 0.705          |
|             | 40            | 0.505                       | 0.318**        | 0.001    | 0.000          | 0.781*         |
|             | 40<br>60      | 0.591                       | 0.310**        | 0.903    | 0.842**        | 0.701          |
|             | 80            | 0.552                       | 0.320**        | 0.000    | 0.042          | 0.702          |
|             | 100           | 0.555                       | 0.320          | 0.000    | 0.042          | 0.769**        |
| Malic acid  | 0             | 0.576                       | 0.317**        | 0.987**  | 0.832**        | 0.768*         |
|             | 10            | 0.568**                     | 0.305**        | 0.007    | 0.002          | 0.763**        |
|             | 20            | 0.500                       | 0.303          | 0.070    | 0.002          | 0.705          |
|             | 40            | 0.502                       | 0.318**        | 0.001    | 0.841**        | 0.775          |
|             | 40<br>60      | 0.550                       | 0.319**        | 0.000    | 0.842**        | 0.782**        |
|             | 80            | 0.593*                      | 0.320**        | 0.983**  | 0.842**        | 0.783*         |
|             | 100           | 0.576**                     | 0.310**        | 0.980**  | 0.834**        | 0.768**        |
| Acetic acid | 0             | 0.570                       | 0.315**        | 0.000    | 0.004          | 0.785**        |
|             | 10            | 0.507                       | 0.311**        | 0.000    | 0.021          | 0.775*         |
|             | 20            | 0.505                       | 0.377**        | 0.902    | 0.000          | 0.770          |
|             | 20<br>40      | 0.000                       | 0.325**        | 0.905    | 0.045          | 0.703          |
|             | 40<br>60      | 0.000                       | 0.325**        | 0.900    | 0.845**        | 0.795          |
|             | 80            | 0.000                       | 0.325**        | 0.900    | 0.045          | 0.701          |
|             | 100           | 0.002                       | 0.323          | 0.900    | 0.045          | 0.701          |
| Ovalic acid | 0             | 0.505<br>0.609 <sup>a</sup> | 0.312          | 0.301    | 0.850**        | 0.774          |
|             | 10            | 0.005                       | 0.333**        | 0.907    | 0.000          | 0.703          |
|             | 20            | 0.000                       | 0.330**        | 0.900    | 0.000          | 0.784**        |
|             | 20<br>40      | 0.007                       | 0.328**        | 0.900    | 0.846**        | 0.704          |
|             | 40<br>60      | 0.000                       | 0.328**        | 0.900    | 0.846**        | 0.702          |
|             | 80            | 0.004                       | 0.327**        | 0.900    | 0.040          | 0.702          |
|             | 100           | 0.003                       | 0.323**        | 0.300    | 0.040          | 0.785**        |
| Glycine     | 0             | 0.537                       | 0.325          | 0.304    | 0.040          | 0.705          |
| Orycine     | 10            | 0.570                       | 0.310**        | 0.900    | 0.000          | 0.775          |
|             | 20            | 0.573                       | 0.310**        | 0.001    | 0.000          | 0.778**        |
|             | 20<br>40      | 0.505<br>0.584 <sup>a</sup> | 0.308**        | 0.301    | 0.004          | 0.778**        |
|             |               | 0.586**                     | 0.300          | 0.982**  | 0.833**        | 0.779**        |
|             | 80            | 0.500                       | 0.312**        | 0.002    | 0.000          | 0.775          |
|             | 100           | 0.587*                      | 0.317**        | 0.002    | 0.004          | 0.700          |
| Lysine      | 0             | 0.581**                     | 0.334**        | 0.987**  | 0.851**        | 0.775*         |
| Lysinc      | 10            | 0.584*                      | 0.30-          | 0.981**  | 0.832**        | 0.770**        |
|             | 20            | 0.588*                      | 0.312**        | 0.982**  | 0.833**        | 0.774*         |
|             | 40            | 0.588*                      | 0.310**        | 0.982**  | 0.830**        | 0 775**        |
|             | 60            | 0.590*                      | 0.312**        | 0.982**  | 0.832**        | 0 776**        |
|             | 80            | 0.590 <sup>a</sup>          | 0.313**        | 0.982**  | 0.833**        | 0 777*         |
|             | 100           | 0.590*                      | 0.319**        | 0.982**  | 0.838**        | 0.777**        |

Table 3. Coefficient of determination (r<sup>2</sup>) for selected kinetic equations used to describe the kinetics of P release after 1440 min from highly calcareous soil (21.5% free CaCO<sub>3</sub>) incubated with different concentrations of organic acids and amino acids

<sup>a</sup> not significant: not significant at P< 0.05, \* significant at P< 0.05, \*\* significant at P< 0.01

# 3.2 Kinetics of P Release Pattern

The coefficients of determination  $(r^2)$  indicated that, time and concentration dependent kinetics of P release by amino acids/LMWOA in highly calcareous soils were better fitted with the

Simple elovich ( $r^2 \ge 0.90$ ) and power function ( $r^2 \ge 0.80$ ) models. Whereas parabolic ( $r^2 \ge 0.70$ ), zero- ( $r^2 \ge 0.60 - 0.50$ ) and first-order models ( $r^2 \ge 0.20 - 0.30$ ) performed very poorly (Tables 2 and 3). Among the two soils, the  $r^2$  values for highly calcareous soil (21.5% free CaCO<sub>3</sub>) was bit

lesser than highly calcareous soil with 17.5% free CaCO<sub>3</sub>. Among the acids tested, citric acid was found to be very efficient and hence the P release kinetics by citric acid in both the calcareous soils, fitted with all five kinetic models which is depicted in Figs. 1 and 2. Among all the models only in simple elovich, power and parabolic functions a single straight line covering the full time course was observed. Since the  $r^2$  values for parabolic function is lesser, the two models viz., simple elovich and power function are more suitable to describe and compare the P release kinetics of highly calcareous soils (>15 % free CaCO<sub>3</sub>) treated with various concentrations of amino acids/LMWOAs.

The dissolution of Ca<sup>2+</sup> that binds the maximum amount of P in calcareous soils by low molecular weight amino acids and organic acids may be the possible mechanism for P release according to

the well-fitted simple elovich model [1]. Whereas. power model was found as a well-fitting model to describe the P release in calcareous soils by Taghipour and Jalali [8]. The kinetics of P release by amino acids and organic acids decreased with increasing soil calcareousness as it was shown in Figs. 1 and 2. The P release followed a two phase pattern of initially rapid and later slowly, which was also reported by Jalali and Tabar [3] and Wang et al. [1]. Each curve in simple elovich model can be converted into two segments where, low is the chemical adsorption of gases on solid surfaces and the fractures in curves can point to the existence of different mechanisms controlling the speed of the processes [16]. Few researchers suggested that, fracture in simple elovich curve can be considered as an indicator of change from one type of binding place to another [17].



Fig. 1. The kinetics of P release by citric acid applied at 0, 10, 20, 40, 60, 80, 100 mM concentrations in highly calcareous soil having 17.5% free CaCO<sub>3</sub> as a function of time and similar data described by five kinetic models: Zero-order ( $q_t$  vs t) (a), First order ( $\ln q_t$  vs t) (b), Simple elovich ( $q_t$  vs  $\ln t$ ) (c), Power ( $\ln q_t$  vs  $\ln t$ ) (d) and parabolic functions ( $q_t$  vs Sqt t) (e), where  $q_t$  is quantity of P released in time t



Fig. 2. The kinetics of P release by citric acid applied at 0, 10, 20, 40, 60, 80, 100 mM concentrations in highly calcareous soil having 21.5% free CaCO<sub>3</sub> as a function of time and similar data described by five kinetic models: Zero-order ( $q_t$  vs t) (a), First order ( $\ln q_t$  vs t) (b), Simple elovich ( $q_t$  vs  $\ln t$ ) (c), Power ( $\ln q_t$  vs  $\ln t$ ) (d) and parabolic functions ( $q_t$  vs Sqt t) (e), where  $q_t$  is quantity of P released in time t

# 3.3 Kinetic Parameters of Simple Elovich Model

From the coefficient of determination  $(r^2)$  values it is evident that simple elovich is the best fitted model for describing the P release rate from calcareous soils in both acids. Hence the kinetics parameters, initial P release rate ( $\alpha$ ) (mg P kg<sup>-1</sup> min<sup>-1</sup>) and P release rate constant ( $\beta$ ) [(mg P kg<sup>-1</sup>)<sup>-1</sup>] of simple elovich model were calculated and depicted in Tables 4 and 5. The kinetic parameter,  $\alpha$  of amino acids ranged from 11.10

to 16.15 mg P kg<sup>-1</sup> min<sup>-1</sup> and from 7.10 to 14.43 mg P kg<sup>-1</sup> min<sup>-1</sup> in the calcareous soil having 17.5% and 21.5% free CaCO<sub>3</sub> respectively. Whereas the  $\beta$  values ranged from 0.033 - 0.057 mg P kg<sup>-1-1</sup> in soil with 17.5% free CaCO<sub>3</sub> and from 0.065 to 0.037 mg P kg<sup>-1-1</sup> in soil with 21.5% free CaCO<sub>3</sub>. As regards to LMWOA, the kinetic parameters  $\alpha$ , and  $\beta$  for highly calcareous soil ranged from 11.17 to 16.54 mg P kg<sup>-1</sup> min<sup>-1</sup> and 0.028 to 0.050 mg P kg<sup>-1-1</sup> in the soil with 17.5% free CaCO<sub>3</sub>. The  $\alpha$  and  $\beta$  values ranged from 7.463 to 15.59 mg P kg<sup>-1</sup> min<sup>-1</sup> and 0.030 to 0.064 mg P kg<sup>-1-1</sup> pertaining to organic acids in soil having 21.5% free CaCO<sub>3</sub>.

Irrespective of soils and amino/organic acids tested,  $\alpha$  value of simple elovich function increased with increasing concentration of amino/organic acid and the maximum values were registered with 100 mM concentration of all the amino/organic acids. The magnitude of initial P release rate ( $\alpha$ ) was higher with citric acid (16.54 mg P kg<sup>-1</sup> min<sup>-1</sup>) among LMWOA and in glycine (16.15 mg P kg<sup>-1</sup> min<sup>-1</sup>) among amino acids. The values of  $\beta$  decreased with increasing concentration and the lowest value of 0.028 mg P kg<sup>-1-1</sup> was recorded with 100 mM concentration of citric acid. Taghipour and Jalali [8] also found that, the kinetics of P release from calcareous soils by citric, oxalic and malic acids were more at the maximum concentration of acids (50 mM).

An increase in the values of initial P release rate  $(\alpha)$  and a decrease in the values of P release rate constant (B) are indicative of an increase in the rate of P release [18,16]. When compared to calcareous soil with 17.5% free CaCO<sub>3</sub>, the soil with 21.5% free CaCO3 recorded lesser initial P release rate ( $\alpha$ ) and higher P release rate constant (B) values in both amino acids and LMWOA. This proves that increasing soil Р calcareousness restricts the release irrespective of amino/organic acids tested. However even in soil with 21.5% calcareousness, LMWOA were found to have greater initial P release rate ( $\alpha$ ) values and lower P release rate constant ( $\beta$ ) values thereby registering their superiority in influencing the kinetics of P release from highly calcareous soils.

#### 3.4 Kinetic Parameters of Power Function Model

The kinetics of P release from calcareous soils was best fitted to power function next to simple elovich model and hence, the estimated values of initial release amount  $(q_o)$  and P release rate coefficient (b) were calculated and presented in

Tables 4 and 5. In case of amino acids, the maximum kinetic parameter values were recorded with glycine and in LMWOAs, citric acid recorded the highest values. The quantity of initial P release  $(q_o)$  by amino acids ranged from 11.49 to 22.78 and 9.871 to 19.21 mg P kg<sup>-1</sup>, whereas in LMWOA, the values ranged from 12.57 to 23.30 mg P kg<sup>-1</sup> and 10.10 to 21.34 mg P kg<sup>-1</sup> in soils with 17.5% free CaCO<sub>3</sub> and 21.5% free CaCO<sub>3</sub> respectively. From the values, it was clear that the initial P release amount was more in LMWOA than amino acids. The P release rate coefficient (b) of amino acids varied from 0.349 to 0.366 mg P kg<sup>-1-1</sup> in calcareous soil with 17.5% free CaCO<sub>3</sub> and from 0.355 to 0.369 mg P kg<sup>-1-1</sup> in soil having 21.5% free CaCO3. In LMWOA treatment, it ranged from 0.331 to 0.366 and 0.369 to 0.350 mg P kg<sup>-1-1</sup> in soils with 17.5% and 21.5% free CaCO<sub>3</sub> respectively. The P release rate coefficient (b) decreased with increase in amino/organic acid concentration but the decrease in P release rate coefficient (b) was much higher in organic acids than amino acids. Irrespective of the acids, increasing soil calcareousness decreases the initial P release amount  $(q_0)$  and increased the P release rate coefficient (b) significantly.

Between the acids studied, organic acids recorded the least P release rate coefficient (b) and the maximum initial P release amount  $(q_0)$ when compared to amino acids, but the magnitude of increase in initial P release rate  $(q_0)$ and the decrease in P release coefficient (b) decreased with increasing soil calcareousness. Citric acid at 100 mM concentration among the organic acids and glycine among the amino acids recorded the least P release rate coefficient and maximum initial P release amount  $(q_0)$  in both the calcareous soils having 17.5% free CaCO<sub>3</sub> and 21.5% free CaCO<sub>3</sub>. Successful description of P release by the power function equation was also reported by Nafiu [19] and Jalali and Khanlari [20]. Safarzadeh et al. [17] proposed that, an increase in the value of  $q_{\text{o}}$  and decrease in the value of b in power function probably indicates an increase in the rate of nutrient release from soils. In the present study, highly calcareous soils treated with 100 mM concentration of citric acid among LMWOAs and glycine among amino acids, recorded the highest qo and least b values.

The P release data were best fitted in simple elovich and power function models but not with parabolic diffusion equation based on the  $r^2$  value. This suggests that, dissolution of Fe<sup>3+</sup>/Al<sup>3+</sup> or Ca<sup>2+</sup> compounds binding the phosphorus by

LMWOA may be a possible mechanism for P release according to the well-fitted simple elovich model [21]. However, the release of P by organic acids through dissolution and chelation may be described better by power function [8]. Enhanced P release in the presence of LMWOA were likely due to the accelerated desorption and dissolution

process, which depends on the strength of the acid used [22,23]. The mechanism behind P release kinetics of amino acid may be their pH dependent charges by which they would form complexes [12] or due to the possession of active uptake carrier which may help in nutrient acquisition [6].

Table 4. Kinetic parameters of the selected simple elovich and power function models describing the kinetic release of P after 1440 min from highly calcareous soil (17.5% free CaCO<sub>3</sub>) treated with different concentrations of organic acids and amino acids

| Sources of  | Concentrations | Simple elovich      |                     | Pow                  | Power function       |  |  |
|-------------|----------------|---------------------|---------------------|----------------------|----------------------|--|--|
| acids       | of acids (mM)  | α                   | β                   | b                    | q <sub>0</sub>       |  |  |
| Citric acid | 0              | 11.35 <sup>ae</sup> | 0.047 <sup>b</sup>  | 0.356 <sup>ac</sup>  | 14.77 <sup>ae</sup>  |  |  |
|             | 10             | 10.63 <sup>ag</sup> | 0.045 <sup>ab</sup> | 0.353 <sup>ac</sup>  | 14.98 <sup>ag</sup>  |  |  |
|             | 20             | 11.04 <sup>af</sup> | 0.043 <sup>bc</sup> | 0.352 <sup>ab</sup>  | 15.63 <sup>af</sup>  |  |  |
|             | 40             | 12.30 <sup>ad</sup> | 0.037 <sup>bd</sup> | 0.350 <sup>a</sup>   | 17.40 <sup>ad</sup>  |  |  |
|             | 60             | 13.71 <sup>ac</sup> | 0.033 <sup>be</sup> | 0.349 <sup>ade</sup> | 19.39 <sup>ac</sup>  |  |  |
|             | 80             | 15.21 <sup>ab</sup> | 0.030 <sup>bf</sup> | 0.348 <sup>ad</sup>  | 21.48 <sup>ab</sup>  |  |  |
|             | 100            | 16.54 <sup>a</sup>  | 0.028 <sup>bg</sup> | 0.331 <sup>ae</sup>  | 23.30 <sup>a</sup>   |  |  |
| Malic acid  | 0              | 11.24 <sup>be</sup> | 0.049 <sup>bc</sup> | 0.360 <sup>bc</sup>  | 12.70 <sup>abe</sup> |  |  |
|             | 10             | 10.07 <sup>bg</sup> | 0.048 <sup>ac</sup> | 0.359 <sup>bc</sup>  | 14.29 <sup>abg</sup> |  |  |
|             | 20             | 10.86 <sup>bf</sup> | 0.046 <sup>c</sup>  | 0.356 <sup>b</sup>   | 15.39 <sup>abf</sup> |  |  |
|             | 40             | 12.12 <sup>bd</sup> | 0.039 <sup>cd</sup> | 0.353 <sup>ab</sup>  | 17.15 <sup>abd</sup> |  |  |
|             | 60             | 13.54 <sup>bc</sup> | 0.035 <sup>ce</sup> | 0.349 <sup>bde</sup> | 19.14 <sup>abc</sup> |  |  |
|             | 80             | 15.03 <sup>b</sup>  | 0.034 <sup>cf</sup> | 0.348 <sup>bd</sup>  | 21.23 <sup>ab</sup>  |  |  |
|             | 100            | 16.43 <sup>ab</sup> | 0.031 <sup>cg</sup> | 0.341 <sup>be</sup>  | 23.16 <sup>ab</sup>  |  |  |
| Acetic acid | 0              | 11.17 <sup>ce</sup> | 0.050 <sup>ab</sup> | 0.362 <sup>c</sup>   | 12.57 <sup>bce</sup> |  |  |
|             | 10             | 9.90 <sup>cg</sup>  | 0.049 <sup>a</sup>  | 0.360 <sup>c</sup>   | 14.04 <sup>bcg</sup> |  |  |
|             | 20             | 10.68 <sup>ct</sup> | 0.048 <sup>ac</sup> | 0.358 <sup>bc</sup>  | 15.14 <sup>bct</sup> |  |  |
|             | 40             | 11.94 <sup>cd</sup> | 0.040 <sup>ad</sup> | 0.354 <sup>ac</sup>  | 16.90 <sup>bcd</sup> |  |  |
|             | 60             | 13.36 <sup>°</sup>  | 0.037 <sup>ae</sup> | 0.352 <sup>cde</sup> | 18.89 <sup>bc</sup>  |  |  |
|             | 80             | 14.85 <sup>bc</sup> | 0.036 <sup>af</sup> | 0.349 <sup>cd</sup>  | 20.98 <sup>bc</sup>  |  |  |
|             | 100            | 16.25 <sup>ac</sup> | 0.032 <sup>ag</sup> | 0.347 <sup>ce</sup>  | 22.91 <sup>abc</sup> |  |  |
| Oxalic acid | 0              | 11.34 <sup>de</sup> | 0.048 <sup>bd</sup> | 0.359 <sup>ac</sup>  | 13.55 <sup>ae</sup>  |  |  |
|             | 10             | 10.15 <sup>dg</sup> | 0.046 <sup>ad</sup> | 0.357 <sup>ac</sup>  | 14.40 <sup>ag</sup>  |  |  |
|             | 20             | 10.94 <sup>df</sup> | 0.044 <sup>cd</sup> | 0.356 <sup>ab</sup>  | 15.50 <sup>af</sup>  |  |  |
|             | 40             | 12.20 <sup>d</sup>  | 0.038 <sup>d</sup>  | 0.353 <sup>a</sup>   | 17.26 <sup>ad</sup>  |  |  |
|             | 60             | 13.62 <sup>cd</sup> | 0.034 <sup>de</sup> | 0.350 <sup>ade</sup> | 19.25 <sup>ac</sup>  |  |  |
|             | 80             | 15.11 <sup>bd</sup> | 0.032 <sup>df</sup> | 0.349 <sup>cd</sup>  | 21.34 <sup>ab</sup>  |  |  |
|             | 100            | 16.50 <sup>ad</sup> | 0.030 <sup>dg</sup> | 0.334 <sup>ce</sup>  | 23.27 <sup>a</sup>   |  |  |
| Glycine     | 0              | 11.15 <sup>et</sup> | 0.051 <sup>ab</sup> | 0.364 <sup>c</sup>   | 12.46 <sup>ce</sup>  |  |  |
|             | 10             | 9.80f <sup>g</sup>  | 0.050 <sup>a</sup>  | 0.362 <sup>c</sup>   | 13.90 <sup>cg</sup>  |  |  |
|             | 20             | 9.80 <sup>f</sup>   | 0.049 <sup>ac</sup> | 0.359 <sup>bc</sup>  | 14.70 <sup>cf</sup>  |  |  |
|             | 40             | 11.85 <sup>dt</sup> | 0.041 <sup>ad</sup> | 0.357 <sup>ac</sup>  | 16.77 <sup>cd</sup>  |  |  |
|             | 60             | 13.26 <sup>cf</sup> | 0.038 <sup>ae</sup> | 0.355 <sup>cde</sup> | 18.76 <sup>c</sup>   |  |  |
|             | 80             | 14.76 <sup>bf</sup> | 0.037 <sup>af</sup> | 0.352 <sup>cd</sup>  | 20.85 <sup>bc</sup>  |  |  |
|             | 100            | 16.15 <sup>df</sup> | 0.033 <sup>ag</sup> | 0.349 <sup>ce</sup>  | 22.78 <sup>ac</sup>  |  |  |
| Lysine      | 0              | 11.10 <sup>ce</sup> | 0.057 <sup>bc</sup> | 0.366 <sup>cd</sup>  | 11.49 <sup>ce</sup>  |  |  |
| -           | 10             | 9.75 <sup>eg</sup>  | 0.053 <sup>ae</sup> | 0.363 <sup>cd</sup>  | 13.83 <sup>cg</sup>  |  |  |
|             | 20             | 9.54 <sup>et</sup>  | 0.051 <sup>ce</sup> | 0.360 <sup>bd</sup>  | 14.53 <sup>ct</sup>  |  |  |
|             | 40             | 11.80 <sup>de</sup> | 0.050 <sup>de</sup> | 0.358 <sup>ad</sup>  | 16.70 <sup>cd</sup>  |  |  |
|             | 60             | 13.21 <sup>ce</sup> | 0.038 <sup>e</sup>  | 0.356 <sup>de</sup>  | 18.69 <sup>c</sup>   |  |  |
|             | 80             | 14.70 <sup>be</sup> | 0.038 <sup>ef</sup> | 0.353 <sup>d</sup>   | 20.78 <sup>bc</sup>  |  |  |
|             | 100            | 16.10 <sup>ae</sup> | 0.034 <sup>eg</sup> | 0.352 <sup>de</sup>  | 22.71 <sup>ac</sup>  |  |  |

\*The numerical values for all the acids of different concentrations in a column followed by dissimilar letter in the superscript are significantly different at (P < 0.05) by Least significant difference (LSD)

| Sources of  | Concentrations | Simple elovich       |                     | Power function       |                       |  |
|-------------|----------------|----------------------|---------------------|----------------------|-----------------------|--|
| acids       | of acids (mM)  | α                    | β                   | b                    | <b>q</b> <sub>0</sub> |  |
| Citric acid | 0              | 7.569 <sup>at</sup>  | 0.061 <sup>ae</sup> | 0.360 <sup>at</sup>  | 10.80 <sup>at</sup>   |  |
|             | 10             | 8.851 <sup>ae</sup>  | 0.052 <sup>be</sup> | 0.359 <sup>ae</sup>  | 12.05 <sup>ae</sup>   |  |
|             | 20             | 8.919 <sup>ae</sup>  | 0.049 <sup>ce</sup> | 0.359 <sup>ae</sup>  | 12.30 <sup>ae</sup>   |  |
|             | 40             | 9.777 <sup>ad</sup>  | 0.045 <sup>de</sup> | 0.358 <sup>ad</sup>  | 13.59 <sup>ad</sup>   |  |
|             | 60             | 11.18 <sup>ac</sup>  | 0.039 <sup>e</sup>  | 0.356 <sup>ac</sup>  | 15.58 <sup>ac</sup>   |  |
|             | 80             | 12.67 <sup>ab</sup>  | 0.035 <sup>ef</sup> | 0.354 <sup>ab</sup>  | 17.68 <sup>ab</sup>   |  |
|             | 100            | 15.59 <sup>a</sup>   | 0.030 <sup>eg</sup> | 0.350 <sup>a</sup>   | 21.34 <sup>a</sup>    |  |
| Malic acid  | 0              | 7.532 <sup>bf</sup>  | 0.063 <sup>ad</sup> | 0.363 <sup>abf</sup> | 10.54 <sup>bt</sup>   |  |
|             | 10             | 8.493 <sup>be</sup>  | 0.059 <sup>bd</sup> | 0.362 <sup>abe</sup> | 11.55 <sup>be</sup>   |  |
|             | 20             | 8.563 <sup>be</sup>  | 0.054 <sup>cd</sup> | 0.361 <sup>abe</sup> | 11.60 <sup>be</sup>   |  |
|             | 40             | 9.421 <sup>bd</sup>  | 0.048 <sup>d</sup>  | 0.360 <sup>abd</sup> | 13.09 <sup>bd</sup>   |  |
|             | 60             | 10.83 <sup>bc</sup>  | 0.042 <sup>de</sup> | 0.358 <sup>abc</sup> | 15.08 <sup>bc</sup>   |  |
|             | 80             | 12.31 <sup>b</sup>   | 0.036 <sup>df</sup> | 0.356 <sup>ab</sup>  | 17.18 <sup>b</sup>    |  |
|             | 100            | 15.13 <sup>ab</sup>  | 0.035 <sup>dg</sup> | 0.353 <sup>ab</sup>  | 19.64 <sup>ab</sup>   |  |
| Acetic acid | 0              | 7.463 <sup>def</sup> | 0.064 <sup>ac</sup> | 0.364 <sup>bcf</sup> | 10.10 <sup>cf</sup>   |  |
|             | 10             | 8.031 <sup>de</sup>  | 0.059 <sup>bc</sup> | 0.363 <sup>bce</sup> | 11.03 <sup>ce</sup>   |  |
|             | 20             | 8.435 <sup>de</sup>  | 0.055 <sup>°</sup>  | 0.362 <sup>bce</sup> | 11.22 <sup>ce</sup>   |  |
|             | 40             | 8.890 <sup>de</sup>  | 0.049 <sup>cd</sup> | 0.361 <sup>bcd</sup> | 12.49 <sup>cd</sup>   |  |
|             | 60             | 10.31 <sup>cde</sup> | 0.043 <sup>ce</sup> | 0.359 <sup>bc</sup>  | 14.49 <sup>c</sup>    |  |
|             | 80             | 11.79 <sup>bde</sup> | 0.037 <sup>ct</sup> | 0.357 <sup>bc</sup>  | 16.59 <sup>bc</sup>   |  |
|             | 100            | 14.81 <sup>ade</sup> | 0.036 <sup>cg</sup> | 0.354 <sup>abc</sup> | 19.34 <sup>ca</sup>   |  |
| Oxalic acid | 0              | 7.543 <sup>bct</sup> | 0.062 <sup>ac</sup> | 0.362 <sup>ab</sup>  | 10.72 <sup>bt</sup>   |  |
|             | 10             | 8.696 <sup>bce</sup> | 0.058 <sup>bc</sup> | 0.360 <sup>abe</sup> | 11.63 <sup>be</sup>   |  |
|             | 20             | 8.892 <sup>bce</sup> | 0.053 <sup>°</sup>  | 0.359 <sup>abe</sup> | 11.75 <sup>be</sup>   |  |
|             | 40             | 9.620 <sup>bcd</sup> | 0.047 <sup>cd</sup> | 0.359 <sup>abd</sup> | 13.58 <sup>bd</sup>   |  |
|             | 60             | 11.03 <sup>,∞</sup>  | 0.041 <sup>ce</sup> | 0.357 <sup>abc</sup> | 15.56 <sup>bc</sup>   |  |
|             | 80             | 12.55 <sup>℃</sup>   | 0.036 <sup>ct</sup> | 0.355 <sup>ab</sup>  | 17.61 <sup>°</sup>    |  |
|             | 100            | 15.23 <sup>abc</sup> | 0.034 <sup>cg</sup> | 0.352 <sup>ab</sup>  | 19.84 <sup>ab</sup>   |  |
| Glycine     | 0              | 7.353 <sup>cdf</sup> | 0.065 <sup>ab</sup> | 0.366 <sup>bcf</sup> | 10.09 <sup>ct</sup>   |  |
|             | 10             | 7.960 <sup>cde</sup> | 0.061 <sup>°</sup>  | 0.364 <sup>bce</sup> | 10.87 <sup>ce</sup>   |  |
|             | 20             | 8.235 <sup>cde</sup> | 0.056 <sup>°C</sup> | 0.362 <sup>bce</sup> | 11.14 <sup>ce</sup>   |  |
|             | 40             | 8.628 <sup>cd</sup>  | 0.050 <sup>ba</sup> | 0.363 <sup>bca</sup> | 12.35 <sup>ca</sup>   |  |
|             | 60             | 10.27 <sup>ca</sup>  | 0.044 <sup>be</sup> | 0.360 <sup>bc</sup>  | 14.13 <sup>°</sup>    |  |
|             | 80             | 11.20 <sup>bcd</sup> | 0.038 <sup>br</sup> | 0.358 <sup>bc</sup>  | 16.42 <sup>bc</sup>   |  |
|             | 100            | 14.43 <sup>acd</sup> | 0.037 <sup>bg</sup> | 0.355 <sup>abc</sup> | 19.21 <sup>ac</sup>   |  |
| Lysine      | 0              | 7.104 <sup>er</sup>  | 0.065 <sup>°</sup>  | 0.369 <sup>cr</sup>  | 9.871 <sup>°°</sup>   |  |
|             | 10             | 7.650 <sup>e</sup>   | 0.063 <sup>ab</sup> | 0.367 <sup>ce</sup>  | 10.49 <sup>de</sup>   |  |
|             | 20             | 7.833                | 0.058 <sup>°°</sup> | 0.366                | 10.76 <sup>de</sup>   |  |
|             | 40             | 8.567                | 0.053 <sup>au</sup> | 0.365 ั              | 12.26 <sup>°</sup>    |  |
|             | 60             | 10.17 <sup>ce</sup>  | 0.045 <sup>ae</sup> | 0.362 <sup>°</sup>   | 14.05 <sup>ca</sup>   |  |
|             | 80             | 11.08                | 0.040 <sup>ar</sup> | 0.359 <sup>bc</sup>  | 16.37 <sup>0</sup>    |  |
|             | 100            | 14 12 <sup>ae</sup>  | 0 038 <sup>ay</sup> | 0 356 <sup>ac</sup>  | 19 09 <sup>au</sup>   |  |

Table 5. Kinetic parameters of the selected simple elovich and power function models describing the kinetic release of P after 1440 min from highly calcareous soil (21.5% free CaCO<sub>3</sub>) treated with various acids of different concentrations of organic acids and amino acids

\*The numerical values for all the acids of different concentrations in a column followed by dissimilar letter in the superscript are significantly different at (P < 0.05) by Least significant difference (LSD)

# 4. CONCLUSION

Both amino acids and LMWOAs increased the rate of kinetics P release in highly calcareous soils, but the magnitude of the release rate was greater with LMWOA addition. The P release

was best fitted using the simple elovich model, followed by the power function equation, based on the kinetic equations evaluated. The kinetics of P release rate was significant when 100 mM of citric acid among LMWOA and glycine among amino acids were added; however, it reduced with increasing soil calcareousness. From the result, it was noticed that soil calcareousness slows the rate of P release by all the acids evaluated. The addition of both LMWOA and amino acids enhanced the kinetics of P release rate, but LMWOA was more efficacious in releasing P from highly calcareous soils, as shown by this short-term kinetic experiment. However, glycine, which is also an important component of root exudates, improved the rate of P release from highly calcareous soils, emphasizing the importance of amino acids in nutrient solubilization.

# DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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