



Effects of Organic Acids Application on Olsen- extractable P and Eggplant (*Solanum melongena*) Yield

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Authors' contributions

This work was carried out in collaboration between both authors. Author DO designed the study, wrote the protocol and wrote the first draft of the manuscript as part of a Masters thesis. Author KM initiated the topic of research, guided the development of the project, managed the revisions and submission. Both authors read and approved the final manuscript.

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ABSTRACT

This study investigated two low molecular weight organic acids (LMWOA), oxalic and citric acid, ability to mineralize fixed P in soils and the effects on production of eggplant when compared to conventional triple superphosphate fertilizer (TSP). Two calcareous soils were used: An alkaline (pH 7.6-7.8) Vertisol in the Houston Black soil series and a slightly acidic (pH 6.5-6.8) Mollisol in the Tarpley soil series. The Houston Black soil test indicated no significant difference in extractable P when comparing treatments of oxalic, citric acid or applied triple superphosphate (TSP) fertilizer ($P > 0.05$). Similarly, eggplant yields indicated no significant difference ($P > 0.05$) between treatments for this soils series. In the Tarpley series, LMWOA treatments produced significantly less extractable P and eggplant yield ($P < 0.05$) when compared to applied TSP fertilizer.

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1. INTRODUCTION

Low phosphorus (P) availability is a major cause of low yields in global crop production [1]. Less than optimum P levels can reduce yields by 5%-15% [2]. Agricultural P applications played a significant role in providing sufficient harvest to meet global food demands in the past, but industrial agriculture has altered the P cycle by mining phosphate rock (PR). Before PR mining, P was naturally supplied to soils from manure, crushed animal bones, city waste and ash [3]. Over the last half of the 20th Century, the Green Revolution abandoned these methods completely for PR-based fertilizers, only to generate the present-day P scarcity concerns [4,5].

Phosphorus fertilizer use increased four-fold between 1960 and 2000's and is estimated to increase further by 20 million metric tons (Mt) per year by 2030 [1]. Also global production increased from 60 Mt in 1960 to 191 Mt in 2011 [6,7]. The peak production curve is estimated to occur around 2050 [1]. Due to P importance in agricultural production and global food security, it is necessary to address P inefficient uses and develop farming systems which aim to reduce P fertilizer inputs [8]. The current global demand for P fertilizers and dependence on the non-renewable PR resource [9] imperils PR-dependent agriculture.

Phosphate rock (PR) does not release plant available P in soils with pH > 5.5-6.0, and even when conditions are optimal plant yields are lower when PR is used than from soluble phosphate use [10]. The common P fertilizer in use now is triple superphosphate $[\text{Ca}(\text{H}_2\text{PO}_4)_2]$ (TSP), however this also is inefficient [8] because in most soils P is quickly bonded with Ca, Fe or Al, depending on pH, and little of the added P enters the soil solution [11]. To avoid P bonding with soil metals current fertilizer amendments of P fertilizer are placed as close to the root zone as possible when the crop is planted [12]. Even under adequate P fertilization, only 20% or less is removed by the first year's growth [1]. Over time, up to 90% of applied P remains fixed in the soil.

Interestingly, most soils have enough native P originating from parent material and biologic cycling [13] for crop production [14,15]. There is

an estimated average 3.75 tonnes ha⁻¹ of P in the top 50 cm of soils, depending on parent material contribution, but it is largely insoluble [16]. Specifically, mesic regions with slightly acid soils (pH 6.5) have the most available P [11] with less availability in arid regions with slightly acid to alkaline soils [17]. Alkaline and calcareous soils are widespread in drier climates and the richness of free CaCO_3 tends to fix P as tricalcium phosphate $[\text{Ca}_3(\text{PO}_4)_2]$ [18]. However, metal bonded P can be released in the presence of organic acids (OA) [19] and increase plant P availability in solution [20].

Organic acids can be released by microbes during organic matter decomposition or exuded by plant roots into the rhizosphere. In most agricultural soils P availability is greater in the upper horizons and the root zone, where deposition from decay, organic matter content, microbial activity, and pH are more conducive [21]. Rhizosphere research has focused on plant mechanisms and OA exudation to increase P availability from the surrounding environment [14,22,23]. The best known plant-produced OAs are citric, succinic, malic, oxalic, and tartaric [24] and exudation of these OAs causes significant P availability and changes in the rhizosphere pH [25].

Numerous OAs have been investigated in soil P studies, including oxalic and citric acids [15]. The effectiveness of individual OAs to increase P availability depends on the number of carboxyl groups they possess and increases in order of monocarboxylic, dicarboxylic, and tricarboxylic acid. The higher negative charge increases the potential bonding with metal cations in solution, thus making the bonded P anions available [19]. In calcareous soils, oxalate and citrate have been directly linked to P availability through Ca^{2+} complexation and acidification mechanisms using distinct ionic forms of OAs [23]. Citrate also increases the availability of P in calcareous soils by chelating and solubilizing Ca salts, thus lowering Ca^{2+} concentrations [22]. The action of numerous OAs in many soils has been repeatedly tested and provides evidence they increase P availability in solution when applied at various concentrations and times [5,13,26-29].

Phosphorus is an important global resource with diminishing availability and many studies indicate OAs increase P availability, but little has been

done on OA potential to release native P as a substitute for applied P. As non-renewable PR resources continue to decline more research is necessary to provide methods to reduce depletion of global PR resources [1,6]. Therefore use of OAs as a way to release naturally occurring P needs further investigation to reduce pressure on mined PR. This study's purpose was to determine the ability of two OAs to increase native P availability in two distinct soils of Texas and the impact on yield of a high P-demanding crop, eggplant (*Solanum melongena*), when compared to traditional eggplant production using TSP.

2. MATERIALS AND METHODS

Eggplant was used as a model crop to test effects of OA applications compared to conventional fertilizer on eggplant yield. Additionally, extractable P was measured to determine differences in extractable P based on treatments. Two different soil types and orders were used as well. Conventional applications of P fertilizer were used as control because the purpose of the study was to test for differences in production based on conventional production with P fertilizer and OA substitution for P fertilizer.

Soils were collected in Hays County, Texas. The A1 horizon (15 cm) of a Tarpley (TaB) series is defined as a montmorillonitic, thermic Lithic Vertic Arguistoll [30]. This Mollisol was collected from the edge of the Edwards Plateau (29°56'18.5" N, 98°00'38.3" W.). This soil is weathered CaCO₃ with limited Ca²⁺ (Table 1). Likewise, the Ap horizon (15 cm) of a Houston Black (HvB) series is defined as a fine, montmorillonitic, thermic Udic Pellusert [30]. This Vertisol was collected from the Blackland Prairie region just east of the plateau (29°46'55.7" N, 97°58'14.8" W). These soils contain excess Ca²⁺ and are characterized by an abundance of swelling clays intimately bound to highly polymerized humus and by alternating wet and dry phases [31]. Both soils were allowed to air dry and then screened for foreign materials (plant biomass, stones, insects, etc.) using a 4 mm sieve before transferring to grow bags for experiment.

A three-week greenhouse pilot study was conducted to measure the effect of citric acid (C₆H₈O₇·H₂O) and oxalic acid (C₂H₂O₄) at different concentrations on extractable P and to determine the rate used in the study. The two soil

types were used, HvB and TaB, and five pots of each soil type were saturated with 0.1 mM, 1 mM, 10 mM, or 100 mM of citric or oxalic acid. Soils in 0.5 L pots received an initial treatment of selected OA concentration to saturation, while control pots received deionized water. The volume of OA needed for saturation was determined by the porosity of each soil. OA saturations were allowed to fully drain, two consecutive rainwater applications were applied to mimic natural precipitation and flush excess Ca²⁺ from the soil. The first rainwater application was on day 8 and the second rainwater application on day 12. OA saturations were applied a second time on day 16, followed by saturation with rainwater similar to the previous application.

High P application rates are required for maximum yields in vegetable production [32] therefore eggplant was chosen due to its relatively high P fertilizer demand (200 kg/ha) and for its fruit uniformity in commercial production [33]. Plants were started in the greenhouse, fertilized once per week with KNO₃ (15-0-15) starter solution (188 ppm) and applied by bottom-watering method to maintain optimum growth after true leaf emergence. Nine week old plants were transplanted to 19 L grow bags on April 24, 2014 and moved to an outdoor setting.

The outdoor pot study was population based, each plant was an individual in a separate pot in a complete randomized block and a 2x5 full factorial design; two soils and five treatments, equaling 140 individual pots with one plant and conducted over one growing season. An a priori analysis for statistical power, size of difference between treatment mean values, significance level and experimental error determined the sample size (G*Power 3.1 Software) [34]. Input parameters for a priori analysis included a 0.3 effect size f, 0.05 α error probability and 0.8 power (1- β error probability) with ten groups, including controls. This resulted in the sample population for each soil type and treatment of fourteen plants ($n=14$). Spacing was arranged 30 cm between plants in rows and 60 cm between rows. Extractable P based on OA treatments was measured at week 6, week 10 and week 14 after the transplant date.

After transplanting, each OA group received an assigned treatment of OA or TSP fertilizer. Oxalic acid and citric acid were each used at two concentrations: 0.1 mM, 100 mM. The pH of OAs in solution were: citric 0.1 mM, 3.4 pH; citric 100

mM, 1.9 pH; oxalic 0.1 mM, 5.5 pH and oxalic 100 mM, 1.2 pH. Each pot was drenched to saturation with their respective acid treatment. The control, granular TSP fertilizer, represented conventional production. Granular TSP control applications, based on soil analysis recommendations, were 0.80 g P per plant (grow-bag) for HvB and 0.84 g P per plant for TaB. Meanwhile, all plants (HvB and TaB) were equally treated with 0.32 g N of granular urea [CO(NH₂)₂] as a readily available N source. TSP and urea treatments mixed thoroughly with the top 6-7 cm of bulk soil to simulate a broadcast top-dress, till-down application method. Plants were watered weekly with collected rainwater or natural precipitation.

Eggplant response, by treatment, was compared using total fruit yield and soil response, by treatment, using extractable P. Yields were based on quality standards according U.S. Standard Grades of Eggplant [35]. First harvest of fruit and soils samples occurred on June 29, 2014, 9 weeks from transplant and subsequent harvests on week 11 and 13. Fruit was harvested by hand followed by immediate weighing. Response variables included extractable P (mg kg⁻¹) and fruit yield (g). MANOVA was used in IBM SPSS 22.0 software to determine mean differences and significance levels set at P < 0.05. Soil tests for extractable P (mg kg⁻¹) were analyzed using the Olsen P extraction method and Palintest® Spectrophotometer.

3. RESULTS AND DISCUSSION

3.1 Results

The results of the pilot study indicated the extractable P response to the oxalic and citric acids treatments was lowest for 0.1 mM

concentrations and highest for 100mM concentrations. Prior to treatment, soils were analyzed for several parameters (Table 1).

MANOVA results for differences in fruit yield indicate interactions between harvest, harvest*soil class, harvest*treatment and harvest*soil class*treatment were significant (Table 2). MANOVA results indicate that soil test, soil test*soil class, soil test*treatment and interaction between soil test*soil class*treatment were significantly different over time (Table 3).

Significant interactions were evident in the MANOVA tests; therefore a post-hoc pairwise comparison was used to identify specific OA treatments for significantly different eggplant yields. Eggplant yield for plants grown in HvB soils was remarkably similar (Table 4 and Fig. 1). The exception was for Harvest III where eggplant yield was significantly less for treatments of oxalic 100 mM compared to citric 0.1 mM. Eggplant yields in TaB soil produced the greatest mean yield with the conventional TSP fertilizer for Harvests I and II and total yield (Table 5 and Fig. 2). Pairwise comparison of eggplant yield in TaB soils with treatments of oxalic acid 0.1 mM, citric acid 0.1 mM, citric acid 100 mM, were significantly greater compared with oxalic acid 100 mM treatment during Harvest I (Table 5 and Fig. 2). Harvest II yields in TaB soil with TSP treatment were significantly greater than all other treatments (Table 5 and Fig. 2). Subsequently, Harvest III in TaB soil showed significantly less yields with citric acid 0.1 mM than with oxalic acid 100 mM and TSP treatment, while yields in soil treated with oxalic acid 0.1 mM were significantly less than soils treated with oxalic acid 100 mM and TSP treatment (Table 5 and Fig. 2).

Table 1. Soil analysis from Servi-Tech Laboratories 2014

| Test | Houston black (HvB) | Tarpley (TaB) |
|--|-------------------------|-----------------------------|
| pH | 7.8 | 6.6 |
| NO ₃ -N mg/kg | 8 | 9 |
| OM (%) | 5.2 | 6.2 |
| Phosphorus mg/kg | 4 | 3 |
| Potassium mg/kg | 324 | 389 |
| Calcium mg/kg | 8295 | 3200 |
| Soluble salts (EC) mmho/cm | 0.35 | 0.19 |
| Calcium carbonate (CaCO ₃) | extremely high (excess) | low (within suitable range) |
| CEC meq/100 g | 44 | 19 |

Table 2. MANOVA for yield shows a significant relationship between factors in both soil types

| Effect | Value | F | df | Error df | P-value |
|----------------------------|-------|---------|----|----------|---------|
| Harvest | 0.324 | 135.340 | 2 | 129 | 0.000 |
| Harvest x Soil | 0.555 | 51.800 | 2 | 129 | 0.000 |
| Harvest x Treatment | 0.826 | 3.244 | 8 | 258 | 0.002 |
| Harvest x Soil x Treatment | 0.800 | 3.795 | 8 | 258 | 0.000 |

P < 0.05; based on LMWOAs (citric 0.1, 100 mM, oxalic 0.1, 100 mM) and TSP treatment; n=14

Table 3. MANOVA for phosphate (P) soil test (mg kg⁻¹) shows a significant relationship between factors in both soil types

| Effect | Value | F | df | Error df | P-value |
|---------------------------|-------|--------|----|----------|---------|
| P-test | 0.834 | 12.838 | 2 | 129 | 0.000 |
| P-test x Soil | 0.846 | 11.753 | 2 | 129 | 0.000 |
| P-test x Treatment | 0.735 | 5.368 | 8 | 258 | 0.000 |
| P-test x Soil x Treatment | 0.626 | 8.507 | 8 | 258 | 0.000 |

P < 0.05; based on LMWOAs (citric 0.1, 100 mM, oxalic 0.1, 100 mM) and TSP treatment; n=14

Table 4. Contrast comparisons of acid treatments with significant differences (P-value) in eggplant yields in HvB soils

| Harvest I | citric 0.1 mM | citric 100 mM | oxalic 0.1 mM | oxalic 100 mM |
|----------------------|---------------|---------------|---------------|---------------|
| citric 100 mM | 0.354 | - | - | - |
| oxalic 0.1 mM | 0.905 | 0.419 | - | - |
| oxalic 100 mM | 0.262 | 0.844 | 0.315 | - |
| TSP | 0.963 | 0.331 | 0.869 | 0.243 |
| Harvest II | | | | |
| citric 100 mM | 0.920 | - | - | - |
| oxalic 0.1 mM | 0.872 | 0.794 | - | - |
| oxalic 100 mM | 0.453 | 0.515 | 0.362 | - |
| TSP | 0.857 | 0.936 | 0.733 | 0.568 |
| Harvest III | | | | |
| citric 100 mM | 0.549 | - | - | - |
| oxalic 0.1 mM | 0.214 | 0.518 | - | - |
| oxalic 100 mM | 0.013 | 0.058 | 0.208 | - |
| TSP | 0.111 | 0.317 | 0.721 | 0.366 |
| Total harvest | | | | |
| citric 100 mM | 0.634 | - | - | - |
| oxalic 0.1 mM | 0.403 | 0.191 | - | - |
| oxalic 100 mM | 0.899 | 0.547 | 0.478 | - |
| TSP | 0.388 | 0.153 | 0.903 | 0.405 |

Soil tests for extractable P of each treatment revealed that TSP treatment provided the most extractable P (mg kg⁻¹) in HvB soils (Table 6 and Fig. 3) as well as in TaB soils (Table 7 and Fig. 4), but only significantly so for TaB soils. Pairwise comparisons between HvB soil treatments in test I indicate that extractable P with TSP treatment significantly differed from all other treatments except oxalic 100 mM, while oxalic acid 100 mM also significantly differed from lower OA concentrations (Table 6 and Fig. 3). The third test showed TSP treatment as

significantly different from other treatments, while citric 0.1 mM and oxalic 100 mM were significantly different from oxalic 0.1 mM (Table 6 and Fig. 3). The differences in extractable P for these periods could be a result of sampling bias as the total extractable P across all sampling periods showed no significant differences. Meanwhile, pairwise comparisons by treatment in TaB showed pots treated with TSP fertilizer were significantly higher in extractable P than all other treatments and all testing periods (Table 7 and Fig. 4).

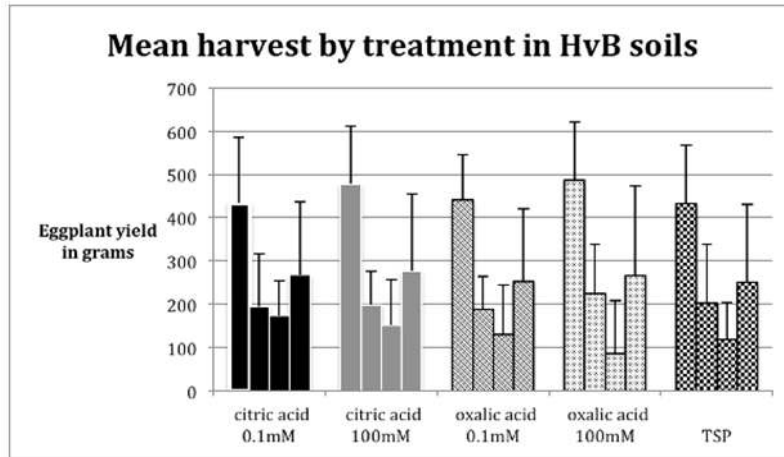


Fig. 1. Each set of columns are in order from left to right: I, II, III mean yield of consecutive harvests, the last column in each set is the total mean harvest. Error bars are standard deviation. TSP harvests were essentially statistically no different from OA treatments (Table 4)

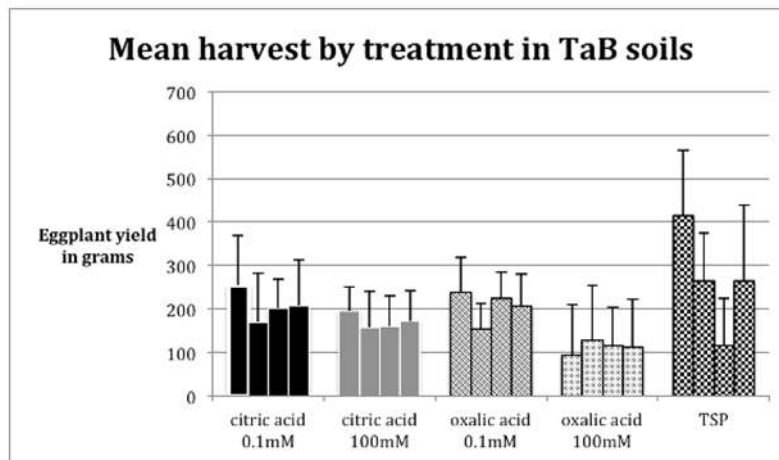


Fig. 2. Each set of columns are in order from left to right: I, II, III mean yield of consecutive harvests, the last column in each set is the total mean harvest. Error bars are standard deviation. Acid treatments showed significantly lower yields in all harvests except harvest III (see Table 5)

3.2 Discussion

The application of OAs in HvB soils appears to be as effective at providing P in soil solution as TSP application but not as effective in TaB soil. Eggplant yields in HvB soils were not significantly different based on treatment. The OA treatments likely released Ca-bonded P into solution in concentration similar to conventional TSP fertilizer. In fact, analysis of harvest for eggplant planted in HvB soils demonstrates that all OA treatments yielded slightly higher than TSP treatment by the study's end (Fig. 1).

Phosphorus loading is not a likely influence since this soil was collected from a site that is native grassland. Precipitation of these OAs in soil or biodegradation did not appear to have an impact on the applied OAs as repeated tests indicate over a course of a few weeks the extractable P in the OA treated soils maintained a fairly consistent level (Fig. 3). Other observations are similar where extractable P was reduced due to biodegradation of citric acid in a high pH calcareous soil, but less so for oxalic acid to [25]. This may simply be due to differences in soil orders used here and citric acid applied to a

calcareous Mollisol (7.58 pH) [34]. Similar to others, the citric acid applications to HvB soils showed a significant increase in extractable P, with effects remaining persistent more than 100 days after the initial application (Fig. 3) [26].

Decreasing soil pH may result in a stronger retention and decreased mobility of P due to

increased positive charges and larger protonation of Fe or Al-oxides at low pH [36]. Soil acidity may decrease the rate of P diffusion while raising the soil pH toward neutral is inversely related to rate of diffusion by increasing the ratio of H_2PO_4^- to HPO_4^{2-} ions available for plant uptake [37]. These scenarios may be coupled with a possible negative reaction of OA

Table 5. Contrast comparisons of acid treatments with significant differences (P-value) in eggplant yields in TaB soils

| Harvest I | citric 0.1 mM | citric 100 mM | oxalic 0.1 mM | oxalic 100 mM |
|----------------------|---------------|---------------|---------------|---------------|
| citric 100 mM | 0.208 | - | - | - |
| oxalic 0.1 mM | 0.715 | 0.369 | - | - |
| oxalic 100 mM | 0.001 | 0.026 | 0.002 | - |
| TSP | 0.001 | 0.000 | 0.000 | 0.000 |
| Harvest II | | | | |
| citric 100 mM | 0.770 | - | - | - |
| oxalic 0.1 mM | 0.696 | 0.922 | - | - |
| oxalic 100 mM | 0.295 | 0.449 | 0.510 | - |
| TSP | 0.019 | 0.009 | 0.007 | 0.001 |
| Harvest III | | | | |
| citric 100 mM | 0.247 | - | - | - |
| oxalic 0.1 mM | 0.502 | 0.068 | - | - |
| oxalic 100 mM | 0.015 | 0.199 | 0.002 | - |
| TSP | 0.017 | 0.208 | 0.002 | 0.979 |
| Total harvest | | | | |
| citric 100 mM | 0.041 | - | - | - |
| oxalic 0.1 mM | 0.872 | 0.059 | - | - |
| oxalic 100 mM | 0.000 | 0.001 | 0.000 | - |
| TSP | 0.002 | 0.000 | 0.001 | 0.000 |

Table 6. Contrast comparisons of acid treatments with significant differences (P-value) in P (mg kg^{-1}) availability in HvB soils

| Test I | citric 0.1mM | citric 100mM | oxalic 0.1mM | oxalic 100mM |
|-----------------|--------------|--------------|--------------|--------------|
| citric 100 mM | 0.000 | - | - | - |
| oxalic 0.1 mM | 0.000 | 0.129 | - | - |
| oxalic 100 mM | 0.000 | 0.504 | 0.030 | - |
| TSP | 0.000 | 0.010 | 0.000 | 0.056 |
| Test II | | | | |
| citric 100 mM | 0.324 | - | - | - |
| oxalic 0.1 mM | 0.256 | 0.880 | - | - |
| oxalic 100 mM | 0.142 | 0.626 | 0.737 | - |
| TSP | 0.261 | 0.890 | 0.990 | 0.727 |
| Test III | | | | |
| citric 100 mM | 0.496 | - | - | - |
| oxalic 0.1 mM | 0.042 | 0.173 | - | - |
| oxalic 100 mM | 0.945 | 0.541 | 0.050 | - |
| TSP | 0.002 | 0.000 | 0.000 | 0.001 |
| Total P | | | | |
| citric 100 mM | 0.634 | - | - | - |
| oxalic 0.1 mM | 0.403 | 0.191 | - | - |
| oxalic 100 mM | 0.899 | 0.547 | 0.478 | - |
| TSP | 0.338 | 0.153 | 0.903 | 0.405 |

treatments leading to excess Fe uptake by plants, due to a combination of readily abundant cations in solution from soil acidification effects during treatment. This probability is reminiscent of other outcomes [19], in which they recorded the mobilization efficiency of citric acid totaling about a 56% release of Ca plus a 10% release of Fe into solution for several soils. Based on other research it is possible a different OA (e.g. sodium citrate, sodium oxalate, potassium citrate, potassium oxalate) may have also resulted in better yields and more extractable P. Some have found potassium citrate was more rapidly biodegraded than the protonated form of citrate while oxalate forms had little to no effect on P availability in a calcareous soil [23]. For two acid soils (3.8 and 6.0 pH), others found [38] an increase in P due to citrate (20 mM), malate (15 mM), and oxalate (2.5 mM) mixed with KOH and likely due to the exchange of OH⁻ ions for H₂PO₄⁻ in addition to chelating mechanisms.

The difference in OA effects between soils was most obvious through consecutive P nutrient soil testing (Figs. 3 and 4). Ultimately, extractable P with OAs treatments were most similar to TSP treatment in HvB soil during Test II analysis, at which time no significant differences were present in extractable P for all treatments (Table 6 and Fig. 3). On the other hand, tests for extractable P in TaB soil showed less than expected success with OA treatments after

numerous spectrophotometer readings found undetectable amounts of P. In fact the extractable P in the TaB soil was less than the original soil test of 3 mg kg⁻¹ indicating the OA applications actually depressed the extractable P, especially when compared to the TSP applications, which show extractable P was elevated above the initial soil test. This is most likely after reviewing eggplant yields in both soils and indicating the TSP applications resulted in similar yields for both soils.

The yield of eggplant using OAs was highly dependent on soil type and treatment throughout. This study demonstrates the ability of OAs to release sufficient P for eggplant production in high pH calcareous soils like HvB Vertisols. The fact that eggplant yield and extractable P in TaB Mollisols treated with OAs showed significantly lower yields compared to TSP treatments indicates OAs may not serve as suitable alternatives for conventional P fertilizers for vegetable production purposes in less calcareous soils (<7.0 pH). However, the success of OAs to compete with TSP fertilizers in a high pH, calcareous soil for a P-demanding crop like eggplant should be investigated further. The potential of OAs as a P fertilizer substitute in calcareous soils is backed by an extensive body of knowledge dedicated to recognizing OAs as indispensable components in rhizosphere processes for P acquisition and plant nutrient uptake.

Table 7. Contrast comparisons of acid treatments with significant differences (P-value) in P (mg kg⁻¹) availability in TaB soils

| Test I | citric 0.1 mM | citric 100 mM | oxalic 0.1 mM | oxalic 100 mM |
|-----------------|----------------------|----------------------|----------------------|----------------------|
| citric 100 mM | 0.701 | - | - | - |
| oxalic 0.1 mM | 0.034 | 0.013 | - | - |
| oxalic 100 mM | 0.850 | 0.567 | 0.054 | - |
| TSP | 0.000 | 0.000 | 0.002 | 0.000 |
| Test II | | | | |
| citric 100 mM | 0.690 | - | - | - |
| oxalic 0.1 mM | 0.825 | 0.536 | - | - |
| oxalic 100 mM | 0.785 | 0.502 | 0.959 | - |
| TSP | 0.000 | 0.000 | 0.000 | 0.000 |
| Test III | | | | |
| citric 100 mM | 0.855 | - | - | - |
| oxalic 0.1 mM | 0.469 | 0.365 | - | - |
| oxalic 100 mM | 0.655 | 0.529 | 0.781 | - |
| TSP | 0.000 | 0.000 | 0.000 | 0.000 |
| Total P | | | | |
| citric 100 mM | 0.661 | - | - | - |
| oxalic 0.1 mM | 0.163 | 0.068 | - | - |
| oxalic 100 mM | 0.691 | 0.404 | 0.317 | - |
| TSP | 0.000 | 0.000 | 0.000 | 0.000 |

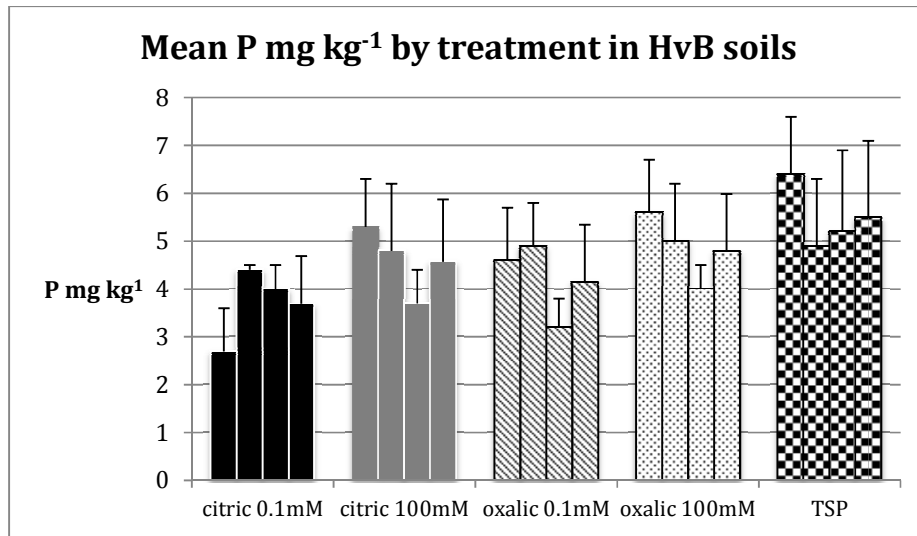


Fig. 3. Each set of columns are in order from left to right: I, II, III analysis in mean P mg kg⁻¹, the last column in each set is the total mean P. error bars are standard deviation

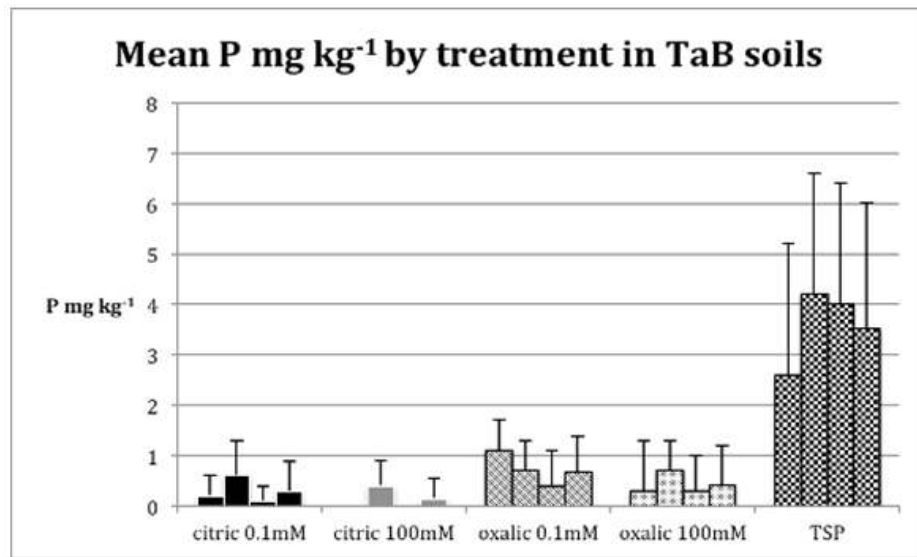


Fig. 4. Each set of columns are in order from left to right: I, II, III analysis in mean P mg kg⁻¹, the last column in each set is the total mean P. Error bars are standard deviation

The use of OAs integrates natural biological cycles produced by plants and microorganisms to increase P availability in soils. Plants like white lupin (*Lupinus albus*) have been shown to exude citric acid from proteoid root zones in response to surrounding P deficiency in calcareous soils [39]. Microorganisms like soil borne fungus (*Penicillin bilaii*) have been found to produce oxalic and citric acids that solubilized CaHPO₄ in agar cultures [40]. Others used *Enterobacter agglomerans* as a phosphate solubilizing bacteria along with an arbuscular

fungus (*Glomus etunicatum*) to increase P uptake in tomato (*Solanum lycopersicum* [41]. Together, these cases provide sufficient evidence to continue and further expand research for adopting such improvements using OAs or microbial inoculants as marketable products that are linked with P mobilization capabilities in calcareous soils. OAs like citric, oxalic and gluconic acid are easily prepared through fermentation of glucose or sucrose by fungus (*Aspergillus niger*) and in 1998 the worldwide production of citric acid alone was

879,000 Mt [42]. Other OAs like acetic acid are produced using bacterial strains of *Acetobacter* spp. [24].

There is additional evidence synergistic applications of OAs with added P fertilizers that may also enhance crop productivity [29,43], yet these methods bypass the conservation efforts of mining limited PR resources. Nevertheless, similar approaches to aid P solubilization should not be overlooked, such as studies that have incorporated P-solubilizing bacteria into a compost system to release OAs [44]. Even further, some suggest the use of microorganisms entrapped in gel or polyurethane foam as forms of inoculants, which may also help equip OAs with alternative application modes in the future [43].

4. CONCLUSION

Outcomes of this investigation strengthen the prospects of adopting OAs for crop production purposes in calcareous soils with pH >7.0. OA action mechanisms serve as an exemplary model for confronting the multiple P obstacles facing agriculture today through simulation of root and microbial rhizosphere processes for facilitating P uptake in plants. It may be fitting to directly employ OA supplements as a potential P fertilizer alternative in order to help diminish PR-based fertilizer applications where soil conditions allow and conventional P fertilizers are inefficient. The problem of providing adequate P nutrition to agricultural soils is not just an application dilemma but also a limitation issue due to the growing concern of PR depletion within the next century. The additional environmental factors associated with P fertilizers in agriculture are immense and it seems antithetical that PR scarcity concerns are accompanied by constant misuse of PR-based fertilizers with resulting problems like continuous eutrophication of water bodies. With an ever-increasing global population expected to reach 9 billion by year 2050, agriculture faces many new challenges within the next few decades including the exponential increase in demand for food, fiber, fodder and biofuels with a limited amount of natural resources like PR. For these reasons, it is only appropriate to consider embracing natural rhizosphere cycles by adopting OA mechanisms that facilitate native P uptake in soils.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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