Original Research Article

The Effects of Organic Acid Application in Soil on Extractable P and Eggplant (*Solanum melongena*) Yield

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ABSTRACT

Phosphorus (P) is a vital nutrient for crop production systems and is a non-renewable resource. Increased global crop yields have been attributed to increased P applications, but it has come at the expense of phosphate rock (PR) depletion. The PR mining is estimated to peak by 2050. On the other hand, soils frequently contain enough native P (3.75 tonnes ha^{-1}) for crop production yet it is unavailable due to P bonds with Ca, Al or Fe; depending on pH. Low-molecular-weight organic acids (LMWOA) are largely responsible for natural release of P from its fixed form, thus making it plant available in soil solution. This study investigated two LMWOAs, 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. Soil tests of the Houston Black soil 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.

1. INTRODUCTION

Low phosphorus (P) availability is a major cause of low yields in global crop production [1]. Less than optimum levels of P can reduce yields by 5%-15% [2]. Agricultural applications of P 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 [4]. 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 [3, 5].

P 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] and 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 the importance of P to agricultural production and global food security, it is necessary to address inefficiencies in P use 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.

34   Phosphate rock (PR) does not release plant available P in soils with pH > 5.5-6.0, and even
35   when conditions are optimal plant yields are lower when PR is used than from soluble
36   phosphate use [10].   The common P fertilizer in use now is triple superphosphate
37     $[Ca(H_2PO_4)_2]$ (TSP), however this also is inefficient [8] because in most soils P is quickly
38   bonded with Ca, Fe or Al, depending on pH, and little of the added P enters the soil solution
39   [11].   To avoid P bonding with soil metals current fertilizer amendments of P fertilizer are
40   placed as close to the root zone as possible when the crop is planted [12].   Even under
41   adequate P fertilization, only 20% or less is removed by the first year's growth [1].   Over
42   time, up to 90% of applied P remains fixed in the soil.

43   Interestingly, most soils have enough native P for crop production [13, 14] originating from
44   parent material and biologic cycling [15].   There is an estimated average P of 3.75 tonnes
45   ha^{-1} in the top 50cm of soils, depending on parent material contribution, but it is largely
46   insoluble [15].   Specifically, mesic regions with slightly acid soils (pH 6.5) have the most
47   available P [11] with less availability in arid regions with slightly acid to alkaline soils [16].
48   Alkaline and calcareous soils are widespread in drier climates and the richness of free
49     $CaCO_3$ tends to fix P as tricalcium phosphate $[Ca_3(PO_4)_2]$ [17].   However, metal bonded P
50   can be released in the presence of organic acids (OA) [18] and increase plant P availability
51   in solution [19].

52   OA can be released by microbes during organic matter decomposition or exuded by plant
53   roots into the rhizosphere.   In most agricultural soils P availability is greater in the upper
54   horizons and the root zone, where deposition from decay, organic matter content, microbial
55   activity, and pH are more conducive [20].   Rhizosphere research has focused on plant
56   mechanisms and OA exudation to increase P availability from the surrounding environment
57   [14, 21, 22].   The best known of the plant-produced OAs are citric, succinic, malic, oxalic,
58   and tartaric [41] and the exudation of these OAs causes significant P availability and
59   changes in the rhizosphere pH (13, 17, 33].

60   Numerous OAs have been investigated in soil P studies, including oxalic, citric acids [14].
61   The effectiveness of individual OAs to increase P availability depends on the number of
62   carboxyl groups they possess and increases in order of monocarboxylic, dicarboxylic, and
63   tricarboxylic acid.   The higher negative charge increases the potential bonding with metal
64   cations in solution, thus making the bonded P anions available [18].   In calcareous soils,
65   oxalate and citrate have been directly linked to P availability through Ca^{2+} complexation and
66   acidification mechanisms using distinct ionic forms of OAs [22].   Citrate also increases the
67   availability of P in calcareous soils by chelating and solubilizing Ca salts, thus lowering Ca^{2+}
68   concentrations [21].   The action of numerous OAs in many soils has been repeatedly tested
69   and provides evidence they increase P availability in solution when applied at various
70   concentrations and times [5, 13, 23, 24, 25, 26].

71   P is an important global resource with diminishing availability and many studies indicate OAs
72   increase P availability, but little has been done on the potential of OA use to release native P
73   as a substitute for applied P.   As the non-renewable PR resources continue to decline more
74   research is necessary to provide methods to reduce depletion of global PR resources [1, 6].
75   Therefore use of OAs as a way to release naturally occurring P needs further investigation to
76   reduce pressure on mined PR.   This study's purpose was to determine the ability of two OAs
77   to increase native P availability in two distinct soils of Texas and the impact on the yield of a
78   high P-demanding crop, eggplant (*Solanum melongena*), when compared to traditional
79   eggplant production using TSP.

80
81   **2. MATERIAL AND METHODS**
82
83   In order to test effects on yield eggplant was used as a signal crop and a fruit yield was
84   measured to determine effects of OA applications compared to conventional fertilizer.
85   Additionally, extractable P was measured to determine differences in extractable P in soil
86   based on treatments.   Two different soil types and orders were used as well.   Further,
87   conventional applications of P fertilizer were used as control because the purpose of the
88   study was to test for differences in production based on conventional production with P
89   fertilizer and OA substitution for P fertilizer.

90   Soils were collected in Hays County, Texas. The A1 horizon (15 cm) of a Tarpley (TaB)
91   series is defined as a montmorillonitic, thermic Lithic Vertic Arguistoll [27]. This Mollisol was
92   collected from the edge of the Edwards Plateau (29°56'18.5" N, 98°00'38.3" W.). This soil is
93   weathered $CaCO_3$ with limited Ca^{2+} (Table 1). Likewise, the Ap horizon of a Houston Black
94   (HvB) series is defined as a fine, montmorillonitic, thermic Udic Pellusert [27]. This Vertisol
95   was collected (15 cm) from the Blackland Prairie region just east of the plateau (29°46'55.7"
96   N, 97°58'14.8" W). These soils contain excess Ca^{2+} and are characterized by an abundance
97   of swelling clays intimately bound to highly polymerized humus and by alternating wet and
98   dry phases [28]. Both soils were allowed to air dry and then screened for foreign materials
99   (plant biomass, stones, insects, etc.) using a 4 mm sieve before transferring to grow bags for
100 experiment. Prior to treatment, soils were analyzed several parameters. The $CaCO_3$
101 content of HvB soils was considerably higher at 8295 mg kg^{-1} compared to TaB at 3200 mg
102 kg^{-1}. Each soil had very low extractable P content: HvB with 4 mg kg^{-1} and TaB with 3 mg
103 kg^{-1}. The N and K analysis for HvB was 8 mg kg^{-1} and 324 mg kg^{-1} and TaB was 9 mg kg^{-1}
104 and 389 mg kg^{-1}.

105 In order to determine the potential of each OA to change available P, a three-week
106 greenhouse pilot study was conducted to measure the effect of citric acid ($C_6H_8O_7 \cdot H_2O$) and
107 oxalic acid ($C_2H_2O_4$) at different concentrations on extractable P. The two soil types were
108 used, HvB and TaB, and five pots of each soil type were saturated with 0.1 mM, 1 mM, 10
109 mM, or 100 mM of citric or oxalic acid. Soils in 0.5 L pots received an initial treatment of
110 selected OA concentration to saturation, while control pots received deionized water. The
111 volume of OA needed for saturation was determined by porosity of each soil. OA saturations
112 were allowed to fully drain, two consecutive rainwater applications were applied to mimic
113 natural precipitation and flush excess Ca^{2+} from the soil. The first rainwater application was
114 on day 8 and the second rainwater application on day 12. OA saturations were applied a
115 second time on day 16, followed by saturation with rainwater similar to the previous
116 application. The extractable P as a response to the OA treatments prompted the
117 assignment of lowest (0.1mM) and highest (100mM) OA concentrations the eggplant
118 production.

119 High application rates of P are required in vegetable production for maximum yields [29]
120 therefore eggplant was chosen due to its relatively high P fertilizer demand (200 kg/ha) and
121 for its fruit uniformity in commercial production [30]. Plugs were started in 288 trays in the
122 greenhouse, fertilized once per week with KNO_3 (15-0-15) starter solution (188 ppm) and
123 applied by bottom-watering method to maintain optimum growth after true leaf emergence.
124 Nine week old plugs were transplanted to 19 L grow bags on April 24, 2014 and moved to an
125 outdoor setting.

126 The outdoor pot study was population based, as each plant was an individual in a separate
127 pot in a complete randomized block and a 2x5 full factorial design; two soils and five
128 treatments, equaling 140 individual pots with one plant and conducted over one growing
129 season. An a priori analysis for statistical power, size of difference between treatment mean
130 values, significance level and experimental error determined the sample size (G*Power 3.1
131 Software) [31]. Input parameters for a priori analysis included a 0.3 effect size f, 0.05 α error
132 probability and 0.8 power (1-β error probability) with ten groups, including controls. This
133 resulted in the sample population for each soil type and treatment of fourteen plants (n=14).
134 Spacing was arranged 30 cm between plants in rows and 60 cm between rows. Extractable
135 P base on OA treatments was measured at week 6, week 10 and week 14 after the
136 transplant date.

137 After transplanting, each OA group received an assigned treatment of OA or TSP fertilizer.
138 Oxalic acid and citric acid were each used at two concentrations: 0.1 mM, 100 mM. The pH
139 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
140 and oxalic 100 mM, 1.2 pH. Each pot was drenched to saturation with their respective acid
141 treatment. The control of granular TSP fertilizer represents conventional production.
142 Granular TSP control applications, based on soil analysis recommendations, were 0.80 g P
143 per plant (grow-bag) for HvB and 0.84 g P per plant for TaB. Meanwhile, all plants (HvB and
144 TaB) were equally treated with 0.32 g N of granular urea [$CO(NH_2)_2$] as a readily available N
145 source. TSP and urea treatments mixed thoroughly with the top 6-7 cm of bulk soil to

146 simulate a broadcast top-dress, till-down application method. Plants were watered weekly
147 with collected rainwater or natural precipitation.

148 Eggplant response, by treatment, was compared using total fruit yield and soil response, by
149 treatment, using extractable P. Yields were based on quality standards according U.S.
150 Standard Grades of Eggplant [32]. First harvest of fruit and soils samples occurred on June
151 29, 2014, 9 weeks from transplant and subsequent harvests on week 11 and 13. Fruit was
152 harvested by hand followed by immediate weighing.   Response variables included
153 extractable P (mg kg^{-1}) and fruit yield (g). MANOVA was used in IBM SPSS 22.0 software to
154 determine mean differences and significance levels set at P < 0.05. Soil tests for extractable
155 P (mg kg^{-1}) were analyzed using the Olsen P extraction method and Palintest®
156 Spectrophotometer.

157
158 3. RESULTS AND DISCUSSION
159
160 MANOVA results for differences in fruit yield indicate interactions between harvest,
161 harvest*soil class, harvest*treatment and harvest*soil class*treatment were significant (Table
162 1).   MANOVA results indicate that soil test, soil test*soil class, soil test*treatment and
163 interaction between soil test*soil class*treatment were significantly different over time (Table
164 2).

165 Table 1. MANOVA for yield shows a significant relationship between factors in both soil
166 types, p< 0.05; based on LMWOAs (citric 0.1, 100 mM, oxalic 0.1, 100 mM) and TSP
167 treatment; n=14.

Effect	Value	F	df	Error df	P-value
Harvest	0.323	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

168

169

170 Table 2. MANOVA for phosphate (P) soil test (mg kg^{-1}) shows a significant relationship
171 between factors in both soil types, p< 0.05; based on LMWOAs (citric 0.1, 100 mM, oxalic
172 0.1, 100 mM) and TSP treatment; sample size of 14 plants per treatment.

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

173

174 Significant interactions were evident in the MANOVA tests; therefore a post-hoc pairwise
175 comparison was used to identify specific OA treatments for significantly different eggplant
176 yields. Eggplant yield for plants grown in HvB soils was remarkably similar (Table 3 and
177 Figure 1). The exception was for Harvest III where eggplant yield was significantly less for
178 treatments of oxalic 100 mM compared to citric 0.1 mM. Eggplant yields in TaB soil
179 produced the greatest mean yield with the conventional TSP fertilizer for Harvests I and II
180 and total yield (Table 4 and Figure 2). Pairwise comparison of eggplant yield in TaB soils
181 with treatments of oxalic acid 0.1 mM, citric acid 0.1 mM, citric acid 100 mM, were
182 significantly greater compared with oxalic acid 100 mM treatment during Harvest I (Table 4
183 and Figure 2). Harvest II yields in TaB soil with TSP treatment were significantly greater
184 than all other treatments (Table 4 and Figure 2). Subsequently, Harvest III in TaB soil
185 showed significantly less yields with citric acid 0.1 mM than with oxalic acid 100 mM and
186 TSP treatment, while yields in soil treated with oxalic acid 0.1 mM were significantly less
187 than soils treated with oxalic acid 100 mM and TSP treatment (Table 4 and Figure 2).

188 Table 3. Contrasts comparison of treatments with significant differences (P-value) in
189 eggplant yields in HvB soils.
190

Harvest I	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.354	-	-	-
oxalic 0.1mM	0.905	0.419	-	-
oxalic 100mM	0.262	0.844	0.315	-
TSP	0.963	0.331	0.869	0.243

Harvest II	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.920	-	-	-
oxalic 0.1mM	0.872	0.794	-	-
oxalic 100mM	0.453	0.515	0.362	-
TSP	0.857	0.936	0.733	0.568

Harvest III	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.549	-	-	-
oxalic 0.1mM	0.214	0.518	-	-
oxalic 100mM	0.013	0.058	0.208	-
TSP	0.111	0.317	0.721	0.366

Total Harvest	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.634	-	-	-
oxalic 0.1mM	0.403	0.191	-	-
oxalic 100mM	0.899	0.547	0.478	-
TSP	0.388	0.153	0.903	0.405

191
192 Table 4. Contrasts comparison of treatments with significant differences (P-value) in
193 eggplant yields in TaB soils.
194

Harvest I	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.208	-	-	-
oxalic 0.1mM	0.715	0.369	-	-
oxalic 100mM	0.001	0.026	0.002	-
TSP	0.001	0.000	0.000	0.000

Harvest II	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.770	-	-	-
oxalic 0.1mM	0.696	0.922	-	-
oxalic 100mM	0.295	0.449	0.510	-
TSP	0.019	0.009	0.007	0.001

Harvest III	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.247	-	-	-
oxalic 0.1mM	0.502	0.068	-	-
oxalic 100mM	0.015	0.199	0.002	-
TSP	0.017	0.208	0.002	0.979

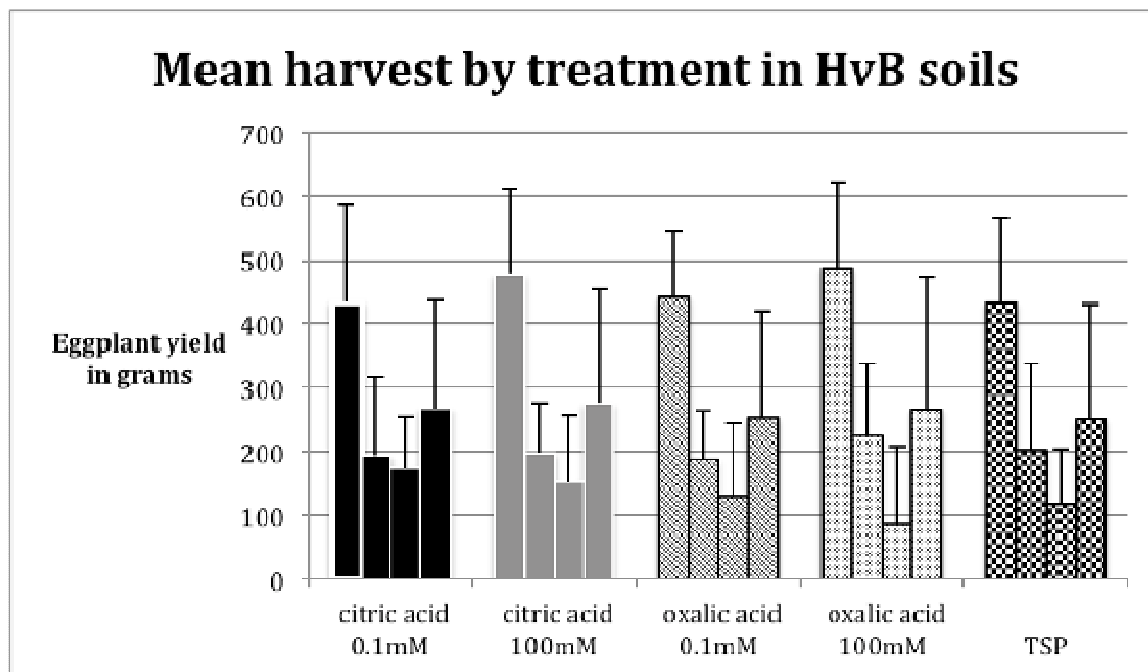| Total Harvest	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.041	-	-	-
oxalic 0.1mM	0.872	0.059	-	-
oxalic	0.000	0.001	0.000	-

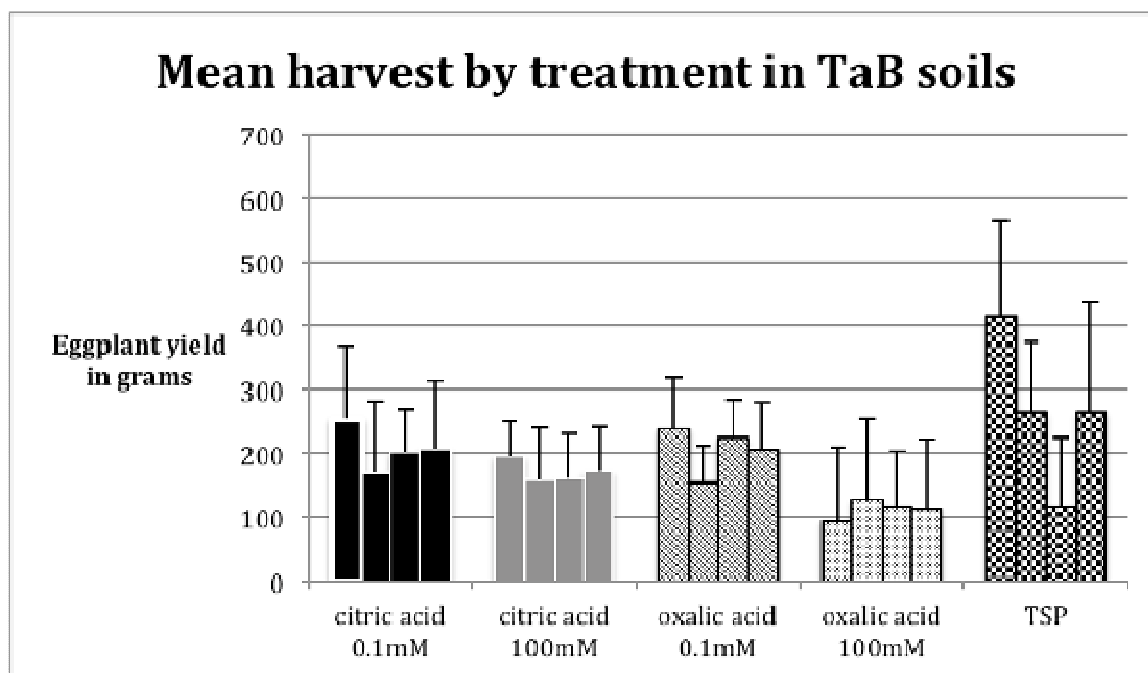| 100mM TSP	0.002	0.000	0.001	0.000

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Figure 1a. 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 (StD). TSP harvests were essentially statistically no different from OA treatments (Table 3).

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202

Figure 1b. 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 (StD). Acid treatments showed significantly lower yields in all harvests except harvest III (see Table 4).

207

208   Soil tests for extractable P of each treatment revealed that TSP treatment provided the most
209   extractable P (mg kg^{-1}) in HvB soils (Table 5 and Figure 3) as well as in TaB soils (Table 6
210   and 5), but only significantly so for Tab soils. Pairwise comparisons between HvB soil
211   treatments in test I indicate that extractable P with TSP treatment significantly differed from
212   all other treatments except oxalic 100 mM, while oxalic acid 100 mM also significantly
213   differed from lower OA concentrations (Table 5 and Figure 3). The third test showed TSP
214   treatment as significantly different from other treatments, while citric 0.1 mM and oxalic 100
215   mM were significantly different from oxalic 0.1 mM (Table 5 and Figure 3). The differences
216   in extractable P for these periods could be a result of sampling bias as the total extractable P
217   across all sampling periods showed no significant differences.   Meanwhile, pairwise
218   comparisons by treatment in TaB showed pots treated with TSP fertilizer were significantly
219   higher in extractable P than all other treatments and all testing periods (Table 6 and 5).

220   Table 5. Contrasts comparison of treatments with significant differences (P-value) in P (mg
221   kg^{-1}) availability in HvB soils.

Test I	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.000	-	-	-
oxalic 0.1mM	0.000	0.129	-	-
oxalic 100mM	0.000	0.504	0.030	-
TSP	0.000	0.010	0.000	0.056

Test II	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.324	-	-	-
oxalic 0.1mM	0.256	0.880	-	-
oxalic 100mM	0.142	0.626	0.737	-
TSP	0.261	0.890	0.990	0.727

Test III	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.496	-	-	-
oxalic 0.1mM	0.042	0.173	-	-
oxalic 100mM	0.945	0.541	0.050	-
TSP	0.002	0.000	0.000	0.001

Total P	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.634	-	-	-
oxalic 0.1mM	0.403	0.191	-	-
oxalic 100mM	0.899	0.547	0.478	-
TSP	0.338	0.153	0.903	0.405

222
223   Table 6. Contrasts comparison of treatments with significant differences (P-value) in P (mg
224   kg^{-1}) availability in TaB soils.
225

Test I	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.701	-	-	-
oxalic 0.1mM	0.034	0.013	-	-
oxalic 100mM	0.850	0.567	0.054	-
TSP	0.000	0.000	0.002	0.000

Test II	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.690	-	-	-
oxalic 0.1mM	0.825	0.536	-	-
oxalic 100mM	0.785	0.502	0.959	-
TSP	0.000	0.000	0.000	0.000

Test III	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.855	-	-	-

oxalic 0.1mM	0.469	0.365	-	-
oxalic 100mM	0.655	0.529	0.781	-
TSP	0.000	0.000	0.000	0.000

Total P	citric 0.1mM	citric 100mM	oxalic 0.1mM	oxalic 100mM
citric 100mM	0.661	-	-	-
oxalic 0.1mM	0.163	0.068	-	-
oxalic 100mM	0.691	0.404	0.317	-
TSP	0.000	0.000	0.000	0.000

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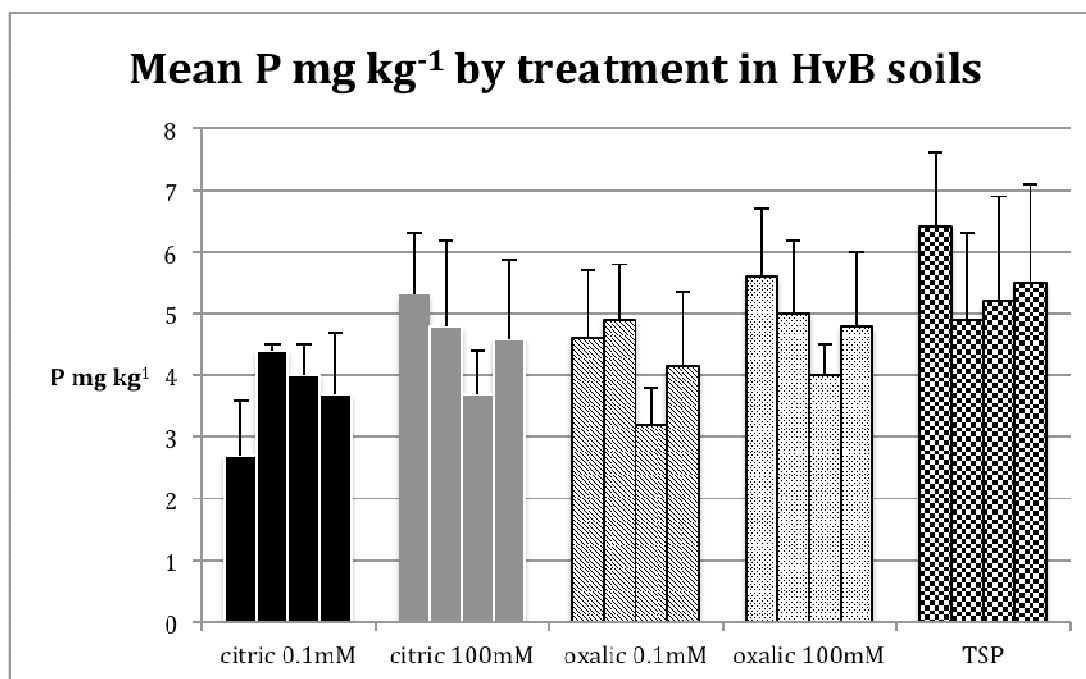
227

228 Figure 2. Each set of columns are in order from left to right: I, II, III analysis in mean P mg
229 kg^{-1}, the last column in each set is the total mean P. Error bars are standard deviation (StD).

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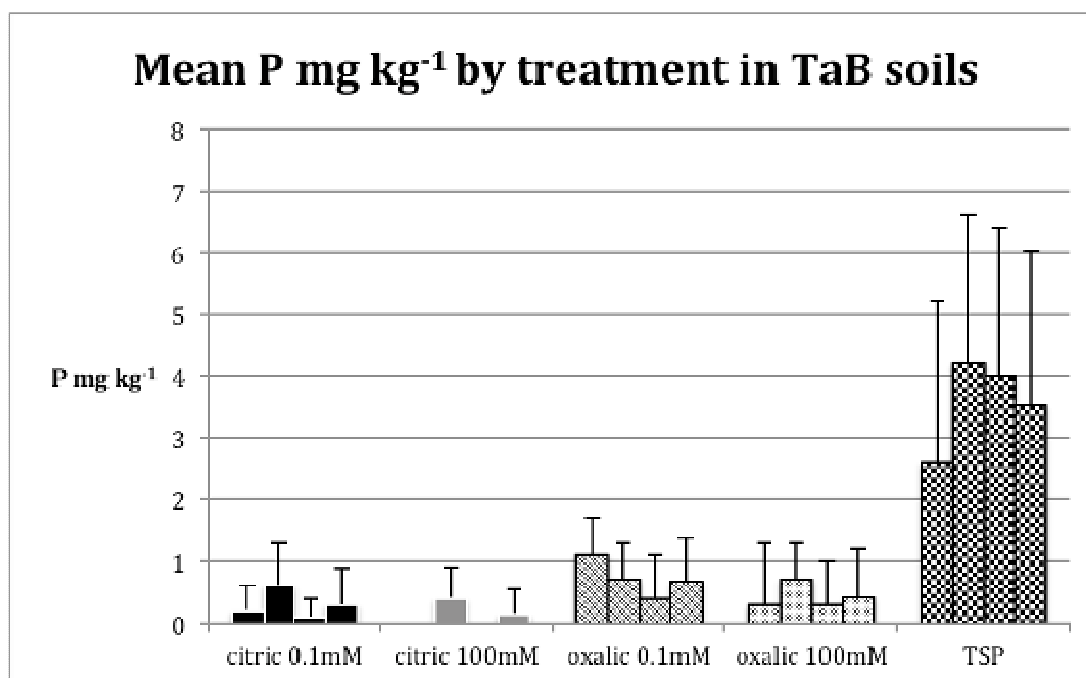
231   Figure 3. Each set of columns are in order from left to right: I, II, III analysis in mean P mg
232   kg^{-1}, the last column in each set is the total mean P. Error bars are standard deviation (StD).

233   **4. CONCLUSION**

234

235   The application of OAs in HvB soils appears to be as effective at providing P in soil solution
236   as TSP application but not as effective in TaB soil. Eggplant yields in HvB soils were not
237   significantly different based on treatment. The OA treatments likely released Ca bonded P
238   into solution in concentration similar to conventional TSP fertilizer. In fact, analysis of
239   harvest for eggplant planted in HvB soils demonstrates that all OA treatments yielded slightly
240   higher than TSP treatment by the study's end (Figure 1). Phosphorus loading is not a likely
241   influence since this soil was collected from a site that is native grassland. Precipitation of
242   these OAs in soil or biodegradation did not appear to have an impact on the applied OAs as
243   repeated tests indicate over a course of a few weeks the extractable P in the OA treated
244   soils maintained a fairly consistent level (Figure 3). Other observations are similar where
245   extractable P was reduced due to biodegradation of citric acid in a high pH calcareous soil,
246   but less so for oxalic acid to *[33]*. This may simply be due to differences in soil orders used
247   here and citric acid applied to a calcareous Mollisol (7.58 pH) [33]. Similar to others, the
248   citric acid applications to HvB soils showed a significant increase in extractable P, with
249   effects remaining persistent more than 100 days after the initial application (Figure 3) [23].

250   Decreasing soil pH may result in a stronger retention and decreased mobility of P due to
251   increased positive charges and larger protonation of Fe or Al-oxides at low pH [34]. Soil
252   acidity may decrease the rate of P diffusion while raising the soil pH toward neutral is
253   inversely related to rate of diffusion by increasing the ratio of $H_2PO_4^-$ to HPO_4^{2-} ions available
254   for plant uptake [35]. These scenarios may be coupled with a possible negative reaction of
255   OA treatments leading to excess Fe uptake by plants, due to a combination of readily
256   abundant cations in solution from soil acidification effects during treatment. This probability
257   is reminiscent other outcomes [18], in which they recorded the mobilization efficiency of citric
258   acid totaling about a 56% release of Ca plus a 10% release of Fe into solution for several
259   soils. Based on other research it is possible a different OA (e.g. sodium citrate, sodium
260   oxalate, potassium citrate, potassium oxalate) may have also resulted in better yields and
261   more extractable P. While some have found potassium citrate was more rapidly
262   biodegraded than the protonated form of citrate while oxalate forms had little to no effect on
263   P availability in a calcareous soil [22]. For two acid soils (3.8 and 6.0 pH), other found [36]
264   found an increase in P due to citrate (20 mM), malate (15 mM), and oxalate (2.5 mM) mixed
265   with KOH and likely due to the exchange of OH^- ions for $H_2PO_4^-$ in addition to chelating
266   mechanisms.

267   The difference in OA effects between soils was most obvious through consecutive P nutrient
268   soil testing (Figures 3 and 4). Ultimately, extractable P with OAs treatments were most
269   similar to TSP treatment in HvB soil during Test II analysis, at which time no significant
270   differences were present in extractable P for all treatments (Table 5). On the other hand,
271   tests for extractable P in TaB soil showed less than excepted success with OA treatments
272   after numerous spectrophotometer readings found undetectable amounts of P. In fact the
273   extractable P in the TaB soil was less than the original soil test of 3 mg kg^{-1} indicating the
274   OA applications actually depressed the extractable P, especially when compared to the TSP
275   applications, which show extractable P was elevated above the initial soil test. This is most
276   likely after reviewing eggplant yields in both soils and indicating the TSP applications
277   resulted in similar yields for both soils.

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

287 dedicated to recognizing OAs as indispensable components in rhizosphere processes for P
288 acquisition and plant nutrient uptake.

289 The use of OAs integrates natural biological cycles produced by plants and microorganisms
290 to increase P availability in soils. Plants like white lupin (*Lupinus albus)* have been shown to
291 exude citric acid from proteoid root zones in response to surrounding P deficiency in
292 calcareous soils [37]. Microorganisms like soil borne fungus (*Penicillin bilaii)* have been
293 found to produce oxalic and citric acids that solubilized $CaHPO_4$ in agar cultures [38].
294 Others used *Enterobacter agglomerans* as a phosphate solubilizing bacteria along with an
295 arbuscular fungus (*Glomus etunicatum*) to increase P uptake in tomato (*Solanum*
296 *lyccopersicum [39].* Together, these cases provide sufficient evidence to continue and
297 further expand research for adopting such improvements using OAs or microbial inoculants
298 as marketable products that are linked with P mobilization capabilities in calcareous soils.
299 OAs like citric, oxalic and gluconic acid are easily prepared through fermentation of glucose
300 or sucrose by fungus (*Aspergillus niger)* and in 1998 the worldwide production of citric acid
301 alone was 879,000 Mt [40]. Other OAs like acetic acid are produced using bacterial strains
302 of *Acetobacter* spp. [41].

303 There is additional evidence synergistic applications of OAs with added P fertilizers that may
304 also enhance crop productivity [26]; [42], yet these methods bypass the conservation efforts
305 of mining limited PR resources. Nevertheless, similar approaches to aid P soil solubilization
306 should not be overlooked, as shown by in which OAs were exclusively incorporated into a
307 compost system through inoculation of P-solubilizing bacteria [43]. Even further, some
308 suggest the use of microorganisms entrapped in gel or polyurethane foam as forms of
309 inoculants, which may also help equip OAs with alternative application modes in the future
310 [42].

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

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