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

8 9 10 **ABSTRACT**

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. 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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12 Keywords: calcareous soils, extractable phosphorus, non-renewable resources, organic 13 acids, peak phosphorus.

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17 **1. INTRODUCTION**

18 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 19 20 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). 21 Before PR mining, P was naturally supplied to soils from manure, crushed animal bones, city 22 waste and ash [4]. Over the last half of the 20th Century, the Green Revolution abandoned 23 24 these methods completely for PR-based fertilizers, only to generate the present-day P 25 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 P importance in agricultural production and global food security, it is necessary to address P inefficiencient 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 [Ca(H₂PO₄)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 39 placed as close to the root zone as possible when the crop is planted [12]. Even under 40 adequate P fertilization, only 20% or less is removed by the first year's growth [1]. Over 41 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 42 cycling [15] for crop production [13, 14]. There is an estimated average 3.75 tonnes ha¹ of 43 44 P in the top 50cm of soils, depending on parent material contribution, but it is largely 45 insoluble [16]. Specifically, mesic regions with slightly acid soils (pH 6.5) have the most 46 available P [11] with less availability in arid regions with slightly acid to alkaline soils [17]. 47 Alkaline and calcareous soils are widespread in drier climates and the richness of free 48 CaCO₃ tends to fix P as tricalcium phosphate $[Ca_3(PO_4)_2]$ [18]. However, metal bonded P 49 can be released in the presence of organic acids (OA) [19] and increase plant P availability 50 in solution [20].

51 Organic acids can be released by microbes during organic matter decomposition or exuded 52 by plant roots into the rhizosphere. In most agricultural soils P availability is greater in the 53 upper horizons and the root zone, where deposition from decay, organic matter content, 54 microbial activity, and pH are more conducive [21]. Rhizosphere research has focused on 55 plant mechanisms and OA exudation to increase P availability from the surrounding 56 environment [14, 22, 23]. The best known plant-produced OAs are citric, succinic, malic, oxalic, and tartaric [42] and exudation of these OAs causes significant P availability and 57 58 changes in the rhizosphere pH (13, 18, 34].

59 Numerous OAs have been investigated in soil P studies, including oxalic and citric acids [14]. The effectiveness of individual OAs to increase P availability depends on the number 60 61 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 62 cations in solution, thus making the bonded P anions available [19]. In calcareous soils, 63 oxalate and citrate have been directly linked to P availability through Ca²⁺ complexation and 64 acidification mechanisms using distinct ionic forms of OAs [23]. Citrate also increases the 65 66 availability of P in calcareous soils by chelating and solubilizing Ca salts, thus lowering Ca²⁺ 67 concentrations [22]. The action of numerous OAs in many soils has been repeatedly tested 68 and provides evidence they increase P availability in solution when applied at various 69 concentrations and times [5, 13, 24, 25, 26, 27].

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

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80 2. MATERIAL AND METHODS

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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 [28]. 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 of a Houston Black (HvB) series is defined as a fine, montmorillonitic, thermic Udic Pellusert [28]. This Vertisol was collected (15 cm) from the Blackland Prairie region just east of the plateau (29°46'55.7")

N, 9758'14.8" 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 [29]. 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.

99 A three-week greenhouse pilot study was conducted to measure the effect of citric acid 100 $(C_6H_8O_7 H_2O)$ and oxalic acid $(C_2H_2O_4)$ at different concentrations on extractable P and to 101 determine the rate used in the study. The two soil types were used, HvB and TaB, and five 102 pots of each soil type were saturated with 0.1 mM, 1 mM, 10 mM, or 100 mM of citric or 103 oxalic acid. Soils in 0.5 L pots received an initial treatment of selected OA concentration to 104 saturation, while control pots received deionized water. The volume of OA needed for 105 saturation was determined by the porosity of each soil. OA saturations were allowed to fully 106 drain, two consecutive rainwater applications were applied to mimic natural precipitation and 107 flush excess Ca²⁺ from the soil. The first rainwater application was on day 8 and the second 108 rainwater application on day 12. OA saturations were applied a second time on day 16, 109 followed by saturation with rainwater similar to the previous application.

High P application rates are required for maximum yields in vegetable production [30] therefore eggplant was chosen due to its relatively high P fertilizer demand (200 kg/ha) and for its fruit uniformity in commercial production [31]. Plugs were started in 288 trays 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 plugs were transplanted to 19 L grow bags on April 24, 2014 and moved to an outdoor setting.

117 The outdoor pot study was population based, leach plant was an individual in a separate pot 118 in a complete randomized block and a 2x5 full factorial design; two soils and five treatments, 119 equaling 140 individual pots with one plant and conducted over one growing season. An a 120 priori analysis for statistical power, size of difference between treatment mean values, 121 significance level and experimental error determined the sample size (G*Power 3.1 122 Software) [32]. Input parameters for a priori analysis included a 0.3 effect size f, 0.05 α error 123 probability and 0.8 power (1- β error probability) with ten groups, including controls. This 124 resulted in the sample population for each soil type and treatment of fourteen plants (n=14). 125 Spacing was arranged 30 cm between plants in rows and 60 cm between rows. Extractable 126 P base on OA treatments was measured at week 6, week 10 and week 14 after the 127 transplant date.

128 After transplanting, each OA group received an assigned treatment of OA or TSP fertilizer. 129 Oxalic acid and citric acid were each used at two concentrations: 0.1 mM, 100 mM. The pH 130 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 131 and oxalic 100 mM, 1.2 pH. Each pot was drenched to saturation with their respective acid 132 treatment. The control of granular TSP fertilizer represents conventional production. 133 Granular TSP control applications, based on soil analysis recommendations, were 0.80 g P 134 per plant (grow-bag) for HvB and 0.84 g P per plant for TaB. Meanwhile, all plants (HvB and 135 TaB) were equally treated with 0.32 g N of granular urea $[CO(NH_2)_2]$ as a readily available N 136 source. TSP and urea treatments mixed thoroughly with the top 6-7 cm of bulk soil to 137 simulate a broadcast top-dress, till-down application method. Plants were watered weekly 138 with collected rainwater or natural precipitation.

139 Eggplant response, by treatment, was compared using total fruit yield and soil response, by 140 treatment, using extractable P. Yields were based on guality standards according U.S. 141 Standard Grades of Eggplant [33]. First harvest of fruit and soils samples occurred on June 142 29, 2014, 9 weeks from transplant and subsequent harvests on week 11 and 13. Fruit was 143 harvested by hand followed by immediate weighing. Response variables included 144 extractable P (mg kg⁻¹) and fruit yield (g). MANOVA was used in IBM SPSS 22.0 software to 145 determine mean differences and significance levels set at P < 0.05. Soil tests for extractable 146 P (mg kg⁻¹) were analyzed using the Olsen P extraction method and Palintest® 147 Spectrophotometer.

149 3. RESULTS AND DISCUSSION

150 3.1 Results

- 151 The results of the pilot study indicated the extractable P response to the oxalic and citric
- 152 acids treatments was lowest for 0.1mM concentrations and highest for 100mM

153 concentrations. Prior to treatment, soils were analyzed for several parameters (Table 1).

154 Table 1. Soil analysis from Servi-Tech Laboratories 2014.

Test	Houston Black (HvB)	Tarpley (TaB
рН	7.8	6.6
NO ₃ -N	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)	0.35	0.19
Calcium carbonate (CaCO ₃)	extremely high (excess)	low (within suitable range)
CEC meq/100g	44	19

MANOVA results for differences in fruit yield indicate interactions between harvest, harvest*soil class, harvest*treatment and harvest*soil class*treatment were significant (Table
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).

Table 2. MANOVA for yield shows a significant relationship between factors in both soil types, p< 0.05; based on LMWOAs (citric 0.1, 100 mM, oxalic 0.1, 100 mM) and TSP treatment; n=14.

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

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Table 3. MANOVA for phosphate (P) soil test (mg kg⁻¹) shows a significant relationship between factors in both soil types, p< 0.05; based on LMWOAs (citric 0.1, 100 mM, oxalic 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

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170 Significant interactions were evident in the MANOVA tests; therefore a post-hoc pairwise 171 comparison was used to identify specific OA treatments for significantly different eggplant 172 yields. Eggplant yield for plants grown in HvB soils was remarkably similar (Table 4 and 173 Figure 1). The exception was for Harvest III where eggplant yield was significantly less for 174 treatments of oxalic 100 mM compared to citric 0.1 mM. Eggplant yields in TaB soil 175 produced the greatest mean yield with the conventional TSP fertilizer for Harvests I and II 176 and total yield (Table 5 and Figure 2). Pairwise comparison of eggplant yield in TaB soils 177 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 Figure 2). Harvest II yields in TaB soil with TSP treatment were significantly greater than all other treatments (Table 5 and Figure 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, 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 Figure 2).

Table 4. Contrasts comparison of treatments with significant differences (P-value) in
 eggplant yields in HvB soils.

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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
	i.			
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
	1			
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

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188 Table 5. Contrasts comparison of treatments with significant differences (P-value) in 189 eggplant yields in TaB soils.

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	0.015	0.199	0.002	-

100mM 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				
100mM	0.000	0.001	0.000	-
TSP	0.002	0.000	0.001	0.000



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Figure 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 (StD). TSP harvests were essentially statistically no different from OA treatments (Table 4).



Figure 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 (StD). Acid treatments showed significantly lower yields in all harvests except harvest III (see Table 5).

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204 Soil tests for extractable P of each treatment revealed that TSP treatment provided the most 205 extractable P (mg kg⁻¹) in HvB soils (Table 6 and Figure 3) as well as in TaB soils (Table 7 206 and Figure 4), but only significantly so for TaB soils. Pairwise comparisons between HvB 207 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 208 209 differed from lower OA concentrations (Table 6 and Figure 3). The third test showed TSP treatment as significantly different from other treatments, while citric 0.1 mM and oxalic 100 210 211 mM were significantly different from oxalic 0.1 mM (Table 6 and Figure 3). The differences 212 in extractable P for these periods could be a result of sampling bias as the total extractable P 213 across all sampling periods showed no significant differences. Meanwhile, pairwise 214 comparisons by treatment in TaB showed pots treated with TSP fertilizer were significantly 215 higher in extractable P than all other treatments and all testing periods (Table 7 and Figure 216 4).

Table 6. Contrasts comparison of 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 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
Total P citric 100mM	citric 0.1mM 0.634	citric 100mM	oxalic 0.1mM -	oxalic 100mM -
Total P citric 100mM oxalic 0.1mM	citric 0.1mM 0.634 0.403	citric 100mM - 0.191	oxalic 0.1mM - -	oxalic 100mM - -
Total P citric 100mM oxalic 0.1mM oxalic 100mM	citric 0.1mM 0.634 0.403 0.899	<u>-</u> 0.191 0.547	oxalic 0.1mM - - 0.478	oxalic 100mM - - -

221 Table 7. Contrasts comparison of treatments with significant differences (P-value) in P (mg kg^{-1}) availability in TaB soils.

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
Test III citric 100mM	citric 0.1mM 0.855	citric 100mM -	oxalic 0.1mM -	oxalic 100mM -
Test III citric 100mM oxalic 0.1mM	citric 0.1mM 0.855 0.469	citric 100mM - 0.365	oxalic 0.1mM - -	oxalic 100mM - -
Test III citric 100mM oxalic 0.1mM oxalic 100mM	citric 0.1mM 0.855 0.469 0.655	citric 100mM - 0.365 0.529	oxalic 0.1mM - - 0.781	oxalic 100mM - - -
Test III citric 100mM oxalic 0.1mM oxalic 100mM TSP	citric 0.1mM 0.855 0.469 0.655 0.000	<u>-</u> 0.365 0.529 0.000	oxalic 0.1mM - - 0.781 0.000	oxalic 100mM - - - 0.000
Test III citric 100mM oxalic 0.1mM oxalic 100mM TSP	citric 0.1mM 0.855 0.469 0.655 0.000	citric 100mM - 0.365 0.529 0.000	oxalic 0.1mM - - 0.781 0.000	oxalic 100mM - - - 0.000
Test III citric 100mM oxalic 0.1mM oxalic 100mM TSP Total P	citric 0.1mM 0.855 0.469 0.655 0.000 citric 0.1mM	citric 100mM - 0.365 0.529 0.000 citric 100mM	oxalic 0.1mM - - 0.781 0.000 oxalic 0.1mM	oxalic 100mM - - 0.000 oxalic 100mM
Test III citric 100mM oxalic 0.1mM oxalic 100mM TSP Total P citric 100mM	citric 0.1mM 0.855 0.469 0.655 0.000 citric 0.1mM 0.661	citric 100mM - 0.365 0.529 0.000 citric 100mM -	oxalic 0.1mM - 0.781 0.000 oxalic 0.1mM -	oxalic 100mM - - - 0.000 oxalic 100mM -
Test III citric 100mM oxalic 0.1mM oxalic 100mM TSP Total P citric 100mM oxalic 0.1mM	citric 0.1mM 0.855 0.469 0.655 0.000 citric 0.1mM 0.661 0.163	citric 100mM - 0.365 0.529 0.000 citric 100mM - 0.068	oxalic 0.1mM - - 0.781 0.000 oxalic 0.1mM - -	oxalic 100mM - - - 0.000 oxalic 100mM - -
Test III citric 100mM oxalic 0.1mM oxalic 100mM TSP Total P citric 100mM oxalic 0.1mM oxalic 100mM	citric 0.1mM 0.855 0.469 0.655 0.000 citric 0.1mM 0.661 0.163 0.691	citric 100mM - 0.365 0.529 0.000 citric 100mM - 0.068 0.404	oxalic 0.1mM - 0.781 0.000 oxalic 0.1mM - - 0.317	oxalic 100mM - - 0.000 oxalic 100mM - - - -
Test III citric 100mM oxalic 0.1mM oxalic 100mM TSP Total P citric 100mM oxalic 0.1mM oxalic 100mM TSP	citric 0.1mM 0.855 0.469 0.655 0.000 citric 0.1mM 0.661 0.163 0.691 0.000	citric 100mM - 0.365 0.529 0.000 citric 100mM - 0.068 0.404 0.000	oxalic 0.1mM - 0.781 0.000 oxalic 0.1mM - - 0.317 0.000	oxalic 100mM - - 0.000 oxalic 100mM - - - 0.000



Figure 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 (StD).



Figure 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 (StD).

230 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 (Figure 1). Phosphorus loading is not a likely influence since this soil was collected from a site that is native grassland. Precipitation of

238 these OAs in soil or biodegradation did not appear to have an impact on the applied OAs as 239 repeated tests indicate over a course of a few weeks the extractable P in the OA treated 240 soils maintained a fairly consistent level (Figure 3). Other observations are similar where 241 extractable P was reduced due to biodegradation of citric acid in a high pH calcareous soil, 242 but less so for oxalic acid to [34]. This may simply be due to differences in soil orders used 243 here and citric acid applied to a calcareous Mollisol (7.58 pH) [34]. Similar to others, the 244 citric acid applications to HvB soils showed a significant increase in extractable P, with 245 effects remaining persistent more than 100 days after the initial application (Figure 3) [24].

246 Decreasing soil pH may result in a stronger retention and decreased mobility of P due to 247 increased positive charges and larger protonation of Fe or Al-oxides at low pH [35]. Soil acidity may decrease the rate of P diffusion while raising the soil pH toward neutral is 248 249 inversely related to rate of diffusion by increasing the ratio of $H_2PO_4^{-1}$ to HPO_4^{2-1} ions available 250 for plant uptake [36]. These scenarios may be coupled with a possible negative reaction of 251 OA treatments leading to excess Fe uptake by plants, due to a combination of readily 252 abundant cations in solution from soil acidification effects during treatment. This probability 253 is reminiscent other outcomes [19], in which they recorded the mobilization efficiency of citric 254 acid totaling about a 56% release of Ca plus a 10% release of Fe into solution for several 255 soils. Based on other research it is possible a different OA (e.g. sodium citrate, sodium 256 oxalate, potassium citrate, potassium oxalate) may have also resulted in better yields and 257 more extractable P. While some have found potassium citrate was more rapidly 258 biodegraded than the protonated form of citrate while oxalate forms had little to no effect on 259 P availability in a calcareous soil [23]. For two acid soils (3.8 and 6.0 pH), other found [37] 260 found an increase in P due to citrate (20 mM), malate (15 mM), and oxalate (2.5 mM) mixed 261 with KOH and likely due to the exchange of OH ions for H₂PO₄ in addition to chelating 262 mechanisms.

263 The difference in OA effects between soils was most obvious through consecutive P nutrient 264 soil testing (Figures 3 and 4). Ultimately, extractable P with OAs treatments were most 265 similar to TSP treatment in HvB soil during Test II analysis, at which time no significant 266 differences were present in extractable P for all treatments (Table 6 and Figure 3). On the 267 other hand, tests for extractable P in TaB soil showed less than excepted success with OA 268 treatments after numerous spectrophotometer readings found undetectable amounts of P. 269 In fact the extractable P in the TaB soil was less than the original soil test of 3 mg kg 270 indicating the OA applications actually depressed the extractable P, especially when 271 compared to the TSP applications, which show extractable P was elevated above the initial 272 soil test. This is most likely after reviewing eggplant yields in both soils and indicating the 273 TSP applications resulted in similar yields for both soils.

274 The yield of eggplant using OAs was highly dependent on soil type and treatment 275 throughout. This study demonstrates the ability of OAs to release sufficient P for eggplant 276 production in high pH calcareous soils like HvB Vertisols. The fact that eggplant yield and 277 extractable P in TaB Mollisols treated with OAs showed significantly lower yields compared 278 to TSP treatments indicates OAs may not serve as suitable alternatives for conventional P 279 fertilizers for vegetable production purposes in less calcareous soils (<7.0 pH). However, 280 the success of OAs to compete with TSP fertilizers in a high pH, calcareous soil for a P-281 demanding crop like eggplant should be investigated further. The potential of OAs as a P 282 fertilizer substitute in calcareous soils is backed by an extensive body of knowledge 283 dedicated to recognizing OAs as indispensable components in rhizosphere processes for P 284 acquisition and plant nutrient uptake.

285 The use of OAs integrates natural biological cycles produced by plants and microorganisms 286 to increase P availability in soils. Plants like white lupin (Lupinus albus) have been shown to 287 exude citric acid from proteoid root zones in response to surrounding P deficiency in 288 calcareous soils [38]. Microorganisms like soil borne fungus (Penicillin bilaii) have been 289 found to produce oxalic and citric acids that solubilized CaHPO₄ in agar cultures [39]. 290 Others used Enterobacter agglomerans as a phosphate solubilizing bacteria along with an 291 arbuscular fungus (Glomus etunicatum) to increase P uptake in tomato (Solanum 292 lyccopersicum [40]. Together, these cases provide sufficient evidence to continue and 293 further expand research for adopting such improvements using OAs or microbial inoculants 294 as marketable products that are linked with P mobilization capabilities in calcareous soils.

295 OAs like citric, oxalic and gluconic acid are easily prepared through fermentation of glucose 296 or sucrose by fungus (*Aspergillus niger*) and in 1998 the worldwide production of citric acid 297 alone was 879,000 Mt [41]. Other OAs like acetic acid are produced using bacterial strains 298 of *Acetobacter* spp. [42].

299 There is additional evidence synergistic applications of OAs with added P fertilizers that may 300 also enhance crop productivity [27]; [43], yet these methods bypass the conservation efforts 301 of mining limited PR resources. Nevertheless, similar approaches to aid P soil solubilization 302 should not be overlooked, as shown by in which OAs were exclusively incorporated into a 303 compost system through inoculation of P-solubilizing bacteria [44]. Even further, some 304 suggest the use of microorganisms entrapped in gel or polyurethane foam as forms of 305 inoculants, which may also help equip OAs with alternative application modes in the future 306 [43].

4. CONCLUSION

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309 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 310 311 model for confronting the multiple P obstacles facing agriculture today through simulation of 312 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 313 314 diminish PR-based fertilizer applications where soil conditions allow and conventional P 315 fertilizers are inefficient. The problem of providing adequate P nutrition to agricultural soils is 316 not just an application dilemma but also a limitation issue due to the growing concern of PR 317 depletion within the next century. The additional environmental factors associated with P 318 fertilizers in agriculture are immense and it seems antithetical that PR scarcity concerns are 319 accompanied by constant misuse of PR-based fertilizers with resulting problems like 320 continuous eutrophication of water bodies. With an ever-increasing global population 321 expected to reach 9 billion by year 2050, agriculture faces many new challenges within the 322 next few decades including the exponential increase in demand for food, fiber, fodder and 323 biofuels with a limited amount of natural resources like PR. For these reasons, it is only 324 appropriate to consider embracing natural rhizosphere cycles by adopting OA mechanisms 325 that facilitate native P uptake in soils.

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327 **REFERENCES**

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329 1. Vance CP, Uhde-Stone C, Allan DL. Phosphorus acquisition and use: critical adaptions by
 330 plants for securing a nonrenewable resource. New Phytol. 2003;157:424-447.

331 2. Shenoy VV, Kalagudi GM. Enhancing plant P use efficiency for sustainable cropping.
332 Biotechnol Adv. 2005;24: 501-513.

333 3. Cordell D, White S. Peak phosphorus: Clarifying the key issues of a vigorous debate 334 about long-term phosphorus security. Sustainability. 2011;3:2027-2049.

4. Van Vuuren DP, Bouwman AF, Beusen AHW. Phosphorus demand for the 1970-2100
period: A scenario analysis of resource depletion. Global Environ Change 2010;20:428-439.

5. Liu Y, Feng L, Hu H, Jiang G, Cai Z, Deng Y. Phosphorus release from low-grade rock
phosphorus by low molecular weight organic acids. J of Food Agr and Environ.
2012;10:1001-1007.

340 6. Déry P, Anderson B. Peak phosphorus. 2007; Accessed 24 February 2015. Available:
341 http://www.resilience.org/stories/2007-08-13/peak-phosphorus.

342 7. USGS. Mineral commodity summaries. U.S. Geological Survey, Virginia, 2015.

343 8. Simpson RJ, Oberson A, Culvenor RA, Ryan MH, Veneklass EJ, Lambers H, et al.
344 Strategies and agronomic interventions to improve the phosphorus-use efficiency of farming
345 systems. Plant and Soil 2011;349:89-120.

346 9. Lehr JR. Phosphate raw materials and fertilizers: Part 1- A look ahead, In: Khasawneh
347 FE, Sample EC, Kamprath EJ, editors. The Role Of Phosphorus In Agriculture. Madison,
348 American Society of Agronomy; 1980.

349 10. Singh H, Reddy SM. Effect of inoculation with phosphate solubilizing fungus on growth
and nutrient uptake of wheat and maize plants fertilized with phosphate rock in alkaline soils.
351 European J of Soil Biol. 2011;47:30-34.

352 11. Plante AF. Soil Biogeochemical cycling of inorganic nutrients and metals, In: Paul EA,
 aditor. Soil Microbiology, Ecology and Chemistry, 3rd ed. Amsterdam, Elsevier Inc., 2007.

12. Barber, SA. Soil-plant interactions in the phosphorus nutrition of plants, In: Khasawneh
FE, Sample EC, Kamprath EJ, editors. The Role Of Phosphorus In Agriculture. Madison,
American Society of Agronomy; 1980.

357 13. Oburger E, Jones DL, Wenzel WW. Phosphorus saturation and pH differentially regulate
358 the efficiency of organic acid anion-mediated P solubilization mechanisms in soil. Plant and
359 Soil 2011;341:363-382.

360 14. Richardson AE. Prospects for using soil microorganisms to improve the acquisition of361 phosphorus by plants. Austral J of Plant Physiol. 2001;28:897-906.

362 15. Anderson G. Assessing organic phosphorus in soils. In: Khasawneh FE, Sample EC,
 363 Kamprath EJ, editors. The Role of Phosphorus In Agriculture. Madison, American Society of
 364 Agronomy; 1980.

365 16. Smil V. Phosphorus in the environment: natural flows and human interferences. Annu.366 Rev of Energy and the Environ. 2000;25:53-88.

367 17. Dregne HE. Soils of arid regions, in Developments in Soil Science. New York, Elsevier.368 1976.

- 369 18. Marschner H. Mineral nutrition of higher plants. 2nd ed. San Diego: Academic Press;
 370 2002.
- 371 19. Jones DL. Organic acids in the rhizosphere-A critical review. Plant and Soil 1998;205:25-372 44.
- 20. Ryan PR, Delhaize E, Jones DL. Function and mechanism of organic anion exudation
 from plant roots. Annu Rev of Plant Physiol and Plant Mol Biol. 2001;52:527-560.
- 21. Lynch JP, Brown KM. Topsoil foraging-Architectural adaptation of plants to low
 phosphorus availability. Plant and Soil 2001;247:225-237.

377 22. Marschner P, Crowley D, Rengel Z. Rhizosphere interactions between microorganisms
378 and plants govern iron and P acquisition along the root axis-model and research methods.
379 Soil Biol and Biochem. 2011; 3:883-894.

23. Khademi Z, Jones DL, Malakouti MJ, Asadi F. Organic acids differ in enhancing P uptake
 by *Triticum aestivum* L. - Effects of rhizosphere concentration and counter ion. Plant and
 Soil. 2010;334:151-159.

383 24. Gerke J. Kinetics of soil phosphate desorption as affected by citric acid. J of Plant Nutr
 384 and Soil Sci. 1994;157:17-22.

- 385 25. Fox TR, Comerford NB. Influence of oxalate loading on phosphorus and aluminum
 386 solubility in spodosols. Soil Sci Soc of Amer J. 1992;56:290-294.
- Kpomblekou-A K, Tabatabai MA. Effect of low-molecular weight organic acids on P
 release and phytoavailability of P in phosphate rocks added to soils. Agr Ecosys and
 Environ. 2003;100:275-284.
- 390 27. Bolan NS, Naidu R, Mahimairaja S, Baskaran S. Influence of low-molecular-weight
 391 organic acids on the solubilization of phosphates. Biol and Fert of Soils. 1994;18:311-319.
- 392 28. USDA. USDA: Soil Conservation Service). Soil Survey of Comal and Hays Counties,
 393 Texas. 1984. Accessed 5 May 2015. http://soils.usda.gov/survey/online_surveys/texas/.
- 394 29. Lozet J, Mathieu C. Dictionary of Soil Science, 2nd. ed. Rotterdam, A. A. Balkema, 1991.
- 30. Lorenz OA, Vittum MT. Phosphorus nutrition of vegetable crops and sugar beets, In:
 Khasawneh FE, Sample EC, Kamprath EJ, editors. The Role Of Phosphorus In Agriculture.
 Madison, American Society of Agronomy; 1980.
- 398 31. Maynard DN, Hochmuth GJ. Knott's Handbook for Vegetable Growers, 5th Ed. Hoboken,
 399 John Wiley & Sons, Inc.; 2007.
- 32. Collins C, Seeney F. Statistical experimental design and interpretation. New York, John
 Wiley and Sons; 1999.
- 402 33. USDA. United States standards for grades of eggplant. 1997. 12 August 2012.
 403 <u>https://ag.purdue.edu/hla/fruitveg/Documents/pdf/veg%20standards/eggplant.pdf</u>.
- 404 34. Ström L, Owen AG, Godbold DL, Jones DL. Organic acid behavior in a calcareous soil 405 implications for rhizosphere nutrient cycling. Soil Biol and Biochem. 2005;37:2046-2054.
- 406 35. Hinsinger P. Bioavailability of soil inorganic P in the rhizosphere as affected by root-407 induced chemical changes: a review. Plant and Soil 2001; 237:173-195.
- 408 36. Gillespie AR, Pope PE. Consequences of rhizosphere acidification on delivery and uptake kinetics of soil phosphorus. Tree Physiol. 1991;8:195-204.
- 410 37. Lopez-Hernandez D, Flores D, Siegert G, Rodriguez JV. The effect of some organic 411 anions on phosphate removal from acid and calcareous soils. Soil Sci. 1979;6:321-326.
- 38. Dinkelaker B, Romheld V, Marschner H. Citric acid excretion and precipitation of calcium
 citrate in the rhizosphere of white lupin (*Lupinus albus* L.). Plant Cell and Environ.
 1989;12:285-292.
- 415 39. Cunningham JE, Kuiack C. Production of citric and oxalic acids and solubilization of 416 calcium phosphate by *Penicillium bilaii*. Appl and Environ Microbio. 1992;58:1451-1458.
- 40. Kim KY, Jordan D, McDonald GA. Effect of phosphate-solubilizing bacteria and
 vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biol and Fert.
 of Soils 1998;26:79-87.
- 420 41. Magnuson JK, Lasure LL. Organic acid production by filamentous fungi. In: Edited by
 421 Lange J. and Lange L, editors. Advances in Fungal Biotechnology for Industry, Agriculture
 422 and Medicine. Kluwer Academic/Plenum Publishers; 2004.
- 423 42.Stevenson FJ. Organic acids in soil, In; Mclaren AD, and Peterson GH, editors. Soil 424 Biochemistry. New York, Marcel Dekker, Inc. 1967.

- 425 43. Vassilev N, Medina A, Azcon R, Vassileva M. Microbial solubilization of phosphate rock
 426 on media containing agro-industrial wastes and effect of the resulting products on plant
 427 growth and P uptake. Plant and Soil 2006;287:77-84.
- 428 44. Singh CP, Amberger A. Organic acids and P solubilization in straw composted with rock 429 phosphate. Bioresource Technol. 1998 ;63:13-16.