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Impact of low molecular weight organic acids on soil phosphorus fractions and its content in wheat (*Triticum aestivum*) and soybean (*Glycine max*)

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ABSTRACT

A greenhouse experiment was conducted at ICAR-IARI, New Delhi during 2017-18 to study the effect of low molecular weight organic acids on phosphorus fractions and its content in wheat and soybean. The results revealed that the applied LMWOAs showed significant increase in the plant available P fraction over control and oxalic acid application out rated the citric acid. The applied citric acid was found to be significantly superior in decreasing the Al and Fe bound P, whereas, oxalic acid was superior to citric acid in reducing the Ca bound P. Application of 20 mg LMWOAs kg^{-1} was found to be better dose in enhancing the available P and reducing the Al, Fe and Ca bound P. The applied 100% recommended dose of phosphorus showed significant increase in available, calcium, aluminium and reductant P fractions over other levels of applied P. The application of LMWOAs significantly enhanced the P content in both wheat and soybean crops. However, both oxalic and citric acid were found at par with each other in enhancing the P content in the crops. Overall, 15 mg LMWOAs kg^{-1} was found to be economic level in enhancing the P content in the crops and 100% RDP application showed significant enhancement in grain, straw and root P content over other levels of applied P. The LMWOAs are actually the organic compounds, so there will be no adverse effect of these products application to the soil compared to the inorganic chemicals and fertilizers.

Key words: Low molecular weight organic acids, wheat, soybean andphosphorus fractions

INTRODUCTION

Wheat **(***Triticum aestivum***)** is one of the important security food crops globally. India accounts for about 14% of total world's wheat production. On the other hand, soybean *(Glycine max)* is one of the major legume crops of the world. Wheat–soybean is one of the most dominant cropping systems mainly in the Vertisols of central India. Phosphorus (P), being an essential nutrient, directly or indirectly affects all plant physiological processes. Phosphorus plays a major role in energy metabolism and its concentration in the chloroplast determines the transport of phosphorylated sugars and synthesis of starch in the plant. Wheat being an exhaustive feeder of plant nutrients (Panwar *et al.*2019), requires a higher amount of available phosphorus for its proper growth and development. On the other hand, soybean crop, a legume need adequate amount of available phosphorus, mainly for the *Rhizobium*-nodulation process. The soil phosphorus content need not be necessarily low, but a high proportion of this P is stored in plant unavailable forms like organic P (Bunemann *et al.* 2011), or depending on soil pH it is bound to aluminum, iron oxides, or calcium minerals. Usually at >6.5 pH, the inorganic P is immobilised predominantly as calcium phosphate minerals, whereas at lower pH, P is bound/adsorbed by Fe, Mn and/or Al (Schaller *et al.* 2019). The soil P content is divided into soluble and loosely bound P, Al-bound P, Fe-bound P, Ca-bound P and reductant P fractions. In which only soluble and loosely bound P is only the available fraction for plant uptake and the remaining fractions are unavailable. The mechanisms by which the LMWOAs enhance the available P in soils are, through dissolution of sparingly soluble P minerals, shifting the soil pH and forming complexes with Fe, Al and Ca (Wang *et al.* 2015).There are several evidences of enhancing the available P content in the soil through LMWOAs application (Hou *et al.* 2018). Both di-carboxylic (oxalic acid) and tri-carboxylic acids (citric acid) were very effective in complexing metal ions in phosphate rock and increased plant-available P than the mono-carboxylic acids (Kpomblekou-A and Tabatabai 2003). The citric

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acid is most effective in enhancing the plant available P through the chelation of Al and Fe in acidic soils, whereas oxalic acid dominates in less acidic or calcareous soils. (Wang *et al.* 2016).The phosphorus constitutes about 0.1 to 0.5% of total dry matter of plant (Tisdale *et al.* 2007), which is essential for respiration, photosynthesis, cellular function, seed setting and seed yield (Pattanayak *et al.* 2009). There are reports of significantly enhanced phosphorus uptake by the crops (Oral and Uygur, 2018). However, the study of impact of extraneous application of LMWOAs on soil P fractions and crops P content and in relation to crop cycles is rare. So the present work carried on the effect of LMWOAs application on P fractionation in the post-harvest soil of wheat-soybean crop cycle and on phosphorus content in grain, straw and root of above crops.

MATERIALS AND METHODS

The *Inceptisol* was collected from research farm ICAR-IARI, New Delhi and was air dried in shade, later it was sieved and passed through 2-mm sieve. A pot experiment was conducted with wheat crop in the winter followed by soybean crop during 2017-18. The LMWOAs and fertilizer phosphorus (diammonium phosphate) with different concentrations were applied as per treatments to representative pots containing five kg of soil each and allowed to settle for a month to create the natural micro and macro pores distribution. The treatments comprised three levels of phosphorus i.e. control, 50% and 100% RDP (recommended dose of P fertilizer i.e. 0, 10, 20 mg P kg^{-1} for wheat and 0, 09, 18 mg P kg^{-1} for soybean) and five levels of oxalic acid (0, 5, 10, 15 and 20 mg OA kg^{-1}) and citric acid (0, 5, 10, 15 and 20 mg CA kg^{-1}). The experiment was arranged in factorial completely randomized design with three replications. The soil was neutral in reaction, low in organic carbon, $KMnO_4$ nitrogen, Olsen-phosphorus and medium in available potassium. The DTPA extractable micronutrients were in adequate amounts. Recommended dosage of nitrogen and potassium were applied through urea and muriate of potash, respectively. Five seeds of wheat (*Var. HD 3086*) were placed in each pot and after harvest of the wheat crop, five soybean seeds (*Var. Pusa9712*) pretreated with *Bradyrhizobium* bacteria were sowed in each pot. The irrigations were done as per requirement of crops. After the completion of wheat-soybean crop cycle the post-harvest soil was analyzed for the P fractionation for some selected treatments (i.e. 0, 10 and 20 mg kg^{-1} of oxalic and citric acid). Phosphorus fractionation scheme proposed by Chang and Jackson (1957) as modified by Kuo (1996) was followed to measure soil P content in different fractions i.e. soluble and loosely bound P, Al-bound P, Fe-bound P, Ca-bound P and Reductant P. Phosphomolybdoblue colour method was followed to determine soil P content and the total P content in grain, straw and root of wheat and soybean crops were estimated by yellow color method. Statistical analyses were done for generated data from the wheat pot experiment according to Gomez and Gomez (1984). The SPSS (Statistical Package for the Social Sciences) software was used for measuring significance of data at 5% level.

RESULTS AND DISCUSSION

Soil phosphorus fraction

Soluble and loosely bound phosphorus (SLP)/available phosphorus

It was observed that different treatments significantly affected the SLP content in the post-harvest soil after one crop cycle of wheat and soybean (Table 1). The data revealed that the oxalic acid was significantly superior to the applied citric acid in enhancing the SLP. In which the oxalic acid showed 9% significantly higher SLP over citric acid. Both the levels of LMWOAs showed significantly higher SLP over nil level of LMWOAs (5.55 mg SLP kg^{-1}). About 17.4 and 31.7% significantly higher SLP was noticed under applied of 10 and 20 mg LMWOAs kg^{-1}, respectively over the control. Oxalic acid proved to be the most efficient P releasing LMWOAs which had a desorption percentages varying from 32.7 to 35.5% (Nascimento *et al.* 2021). Similar findings were reported by Mendes *et al.* (2020).All the levels of applied P fertilizer showed significantly higher SLP over control (3.44 mg SLP kg^{-1}). The 50 and 100% RDP showed 87.7 and 175.2% higher SLP over nil P application, respectively and the applied 100% RDP had 46.5% significantly higher SLP than 50% RDP. There was significant increase in soil available phosphorus in the plots which received P fertilizers (Zhang *et al.* 2020).

Calcium-P

The application of oxalic and citric acid recorded 199.43 and 200.75 mg Ca-P kg^{-1}, respectively. However, both the LMWOAs did not show significant difference between them in reducing the Ca-P (Table 1). All the levels of applied LMWOAs showed significantly lower Ca-P over nil LMWOAs treatment and applied 10 and 20 mg LMWOAs kg^{-1} recorded 2.5 and 4% significantly lower Ca-P, respectively over nil LMWOAs (204.63 mg Ca-P kg^{-1}). Application of 20 mg LMWOAs kg^{-1} showed significantly lowers Ca-P over 10 mg LMWOAs kg^{-1}, which recorded 1.52% lower Ca-P than 10 mg LMWOAs kg^{-1}. The oxalic acid mainly enhances the inorganic P solubility from the HCl-extractable P pool (Teng *et al.* 2020). With increase in the applied P, the Ca-P content also significantly enhanced. Applied 100% RDP recorded 203.00 mg kg^{-1} and 50% RDP recoded 199.80 mg kg^{-1}. The 100% RDP recorded 2.72% significantly higher Ca-P over nil P application treatment (197.47 mg kg^{-1}). In neutral soils, the part of the applied P fertilizer forms Ca and Mg phosphate minerals (Saleem *et al.* 2017). At non acidic soil pH, the applied phosphorus fertilizers will get precipitated as calcium phosphate (Mohammadi and Shariatmadari, 2020).

Table 1: Effect of levels of P and low molecular organic acids (LMWOAs) on soil phosphorus fractions (mg kg^{-1}) after completion of one crop cycle of wheat and soybean

Treatment	SLP	Ca- P	Al- P	Fe- P	Red- P	Total- P
			LMWOAs			
Oxalic acid	6.74	199.43	28.02	20.24	76.29	543.56
Citric acid	6.18	200.75	27.16	19.48	76.50	543.80
SEm(±)	0.02	0.65	0.10	0.06	0.30	2.24
CD (5%)	0.074	N/A	0.290	0.194	N/A	N/A
			Levels of LMWOAs (mg kg^{-1})			
0	5.55	204.63	30.98	21.28	76.080	544.26
10	6.52	199.32	26.62	19.70	76.30	544.42
20	7.31	196.32	25.16	18.60	76.81	542.36
SEm(±)	0.03	0.80	0.12	0.08	0.37	2.74
CD (5%)	0.09	2.31	0.35	0.23	N/A	N/A
			Levels of P (mg kg^{-1})			
0% RDP	3.44	197.47	27.11	18.21	75.37	541.13
50% RDP	6.46	199.80	27.61	20.49	76.31	543.99
100% RDP	9.47	203.00	28.04	20.87	77.50	545.91
SEm(±)	0.03	0.80	0.12	0.08	0.37	2.74
CD (5%)	0.09	2.31	0.35	0.23	1.06	N/A

SLP: soluble and loosely bound P; Al-P: Aluminium bound P; Fe-P: Iron bound P; Ca-P: Calcium bound P; Red-P: Reductant soluble P; Tot-P: Total P; CA: Citric acid; OA: Oxalic acid.

Aluminium-P

The applied citric acid showed significant decrease in Al-P compared to oxalic acid by recording 27.16 mg Al-P kg^{-1}. With increase in levels of LMWOAs application, the aluminium bound P significantly decreased from 30.98 mg Al-P kg^{-1} in nil LMWOAs application to 25.16 mg Al-P kg^{-1} with 20 mg LMWOAs kg^{-1} (Table 1). Applied 20 mg LMWOAs kg^{-1} showed 5.8 and 23.1% significantly lower aluminium bound P over 10 and 0 mg LMWOAs kg^{-1}, respectively. Due to LMWOAs, there was a decrease in NaOH-P, which represents P bound to Fe- and Al-oxides (Yan *et al.* 2015; Kunito *et al.* 2018). The aluminium bound P converted into easily soluble phosphorus due to presence of LMWOAs in the treatment (Qian *et al.* 2019). Whereas, increase in the applied rate of P significantly increased the Al-P content in the soil and highest Al-P was recorded with 100% RDP (28.04 mg Al-P kg^{-1}) and the lowest in 0% RDP (27.11 mg Al-P kg^{-1}). The applied P fertilizer are easily and rapidly immobilized to insoluble precipitates by reacting with Al3+ and Fe3+ in the soils and the precipitation induced by aluminum is a major cause of P fixation under an acidic soils (Liu *et al.* 2014).

Iron P

Similar to Al-P fraction, application of citric acid showed significantly lower Fe bound P compared to oxalic acid. Citric and oxalic acid recorded 19.48 and 20.24 mg Fe-P kg^{-1}, respectively (Table 1). Application of 20 mg LMWOAs kg^{-1} recorded significantly lower Fe-P over both 10 mg LMWOAs kg^{-1} and control. About 12.5% significantly lower Fe-P was noticed under 20 mg LMWOAs kg^{-1} than control (21.28 mg Fe-P kg^{-1}). Significant decrease in the NaOH-P i.e. iron and aluminium bound P was also noticed by Menezes-Blackburn *et al.* (2021). The citric acid had greater affinity to form complex with Fe^{3+} and Al^{3+} and it releases the P contained in Fe–P and Al–P minerals (Taghipour and Jalali 2013). Increased levels of P application significantly enhanced the Fe bound P fraction. The highest Fe-P was recorded under 100% RDP (20.87 mg Fe-P kg^{-1}) followed by 50% RDP (20.49 mg Fe-P kg^{-1}) and control (18.21 mg Fe-P kg^{-1}). The applied P fertilizers are easily and rapidly immobilized to insoluble precipitates by reacting with Al^{3+} and Fe^{3+} in the soil (Liu *et al.* 2014).

Reductant and Total-P

Application of different LMWOAs or their different levels were found insignificant in decreasing the reductant-P (Table 1). However, applied 100% RDP showed significantly higher reductant-P over control by recording 77.50 mg reductant-P kg^{-1}. Even the applied 50% RDP was found to be at par with that of control (75.37 mg reductant-P kg^{-1}). However, in case of total-P, the different LMWOAs used i.e. oxalic and citric acid, their different levels were at par with each other, revealing that the applied LMWOAs were ineffective in enhancing the total P concentration. However, the applied P levels were also ineffective in enhancing the total–P content.

Phosphorus content in crops

There was no significant difference between applied oxalic and citric acid in enhancing the P content in wheat and soybean grain (Table 2). All the levels of applied LMWOAs were found to be significantly superior over control. Applied 15 mg LMWOAs kg^{-1} recorded 19.94%, 13.30% and 8.60% significantly higher grain P content over control, 05 and 10 mg LMWOAs kg^{-1}, respectively and found at par with 20 mg LMWOAs kg^{-1}. With increase in phosphorus rate, the garin P content of wheat crop increased significantly. Applied 100% RDP showed 23.7% and 13.1% significantly higher grain P in wheat crop over control and 50% RDP respectively. Applied 10 mg LMWOAs kg^{-1} was found to be superior level of LMWOAs in enhancing the soybean grain P, as it was significantly superior over control and found at par with other higher LMWOAs levels. Applied 100% RDP recorded the highest grain P content in soybean and the lowest grain P content was under control (5.035 mg kg^{-1}).

Oxalic and citric acid showed no significant difference between them on wheat straw P content (Table 2). The lower levels of LMWOAs application i.e. 05 and 10 mg LMWOAs kg^{-1} were found to be at par with that of control (0.312 mg kg^{-1}). Applied 15 mg LMWOAs kg^{-1} was found to be superior dose of LMWOAs. The straw P content of wheat ranged from 0.257 to 0.435 mg kg^{-1} with control and 100% RDP, respectively. The applied 100% RDP recorded 69.2% significantly higher straw P content over control. The applied oxalic and citric acid and their different levels were at par in case of straw P content of soybean crop. However, 18.6% and 55.2% significicnatly higher straw P of soybean were recorded by 50% and 100% RDP, respectively over control (Table 2).

Applied citric acid was significantly better (0.100 mg kg^{-1}) than applied oxalic acid (0.087 mg kg^{-1}) in enhancing the root P content of wheat crop (Table 2). Applied 20 mg LMWOAs kg^{-1} was found to be significantly superior over other levels of LMWOAs, which recorded 46.6%, 30.9%, 18.2% and 6.7% significantly higher root P content of wheat crop over control, 05, 10 and 15 mg LMWOAs kg^{-1}, respectively. Applied 100% RDP recorded 169.3% significantly higher root P content in wheat over control (0.075 mg kg^{-1}). Whereas 50% RDP recorded 102% significantly higher root P content over the control. Application of higher doses of P reported in higher P content in the straw and roots of soybean crop (Mahanta *et al.* 2014).

Table 2: Effect of LMWOAs on grain, straw and root phosphorus content of wheat and soybean crops

Treatment	Wheat			Soybean		
	Grain P (mg kg^{-1})	Straw P (mg kg^{-1})	Root P (mg kg^{-1})	Grain P (mg kg^{-1})	Straw P (mg kg^{-1})	Root P (mg kg^{-1})
LMWOAs						
Oxalic acid	2.509	0.340	0.087	5.650	1.228	1.046
Citric acid	2.573	0.340	0.100	5.661	1.290	1.041
SEm(±)	0.029	0.005	0.001	0.061	0.025	0.011
CD (5%)	N/A	N/A	0.003	N/A	N/A	N/A
Levels of LMWOAs (mg kg^{-1})						
0	2.223	0.312	0.075	5.308	1.177	0.986
5	2.451	0.328	0.084	5.618	1.262	0.996
10	2.557	0.334	0.093	5.735	1.288	1.066
15	2.777	0.363	0.104	5.917	1.307	1.077
20	2.696	0.362	0.110	5.700	1.261	1.091
SEm(±)	0.046	0.008	0.002	0.096	0.040	0.018
CD (5%)	0.131	0.023	0.005	0.272	N/A	0.051
Levels of P (mg kg^{-1})						
0% RDP	2.289	0.257	0.049	5.035	1.010	0.815
50% RDP	2.502	0.328	0.099	5.795	1.198	1.095
100% RDP	2.832	0.435	0.132	6.137	1.568	1.219
SEm(±)	0.036	0.006	0.001	0.075	0.031	0.014
CD (5%)	0.101	0.018	0.004	0.211	0.087	0.039

(For wheat 50 and 100% RDP are 10 and 20 mg kg^{-1} and for soybean 50 and 100% RDP are 09 and 18 mg kg^{-1})

The root P content of soybean did not show any significant difference between oxalic and citric acid. Among various levels of LMWOAs application, applied 10 mg LMWOAs kg^{-1} was found to be the superior over control (0.986 mg kg^{-1}) and 05 mg LMWOA kg^{-1} (0.996 mg kg^{-1}) and at par with other higher levels(Table 2). Applied 100% RDP was found to be significantly superior over cotrol (0.815 mg kg^{-1}) and 50% RDP (1.095 mg kg^{-1}). Similar results are reported by Sagoe *et al.* (2017), Wang *et al.* (2017) and Shabnam *et al.* (2018). It can be concluded from the results that the calcium, iron and aluminium bound phosphorus fractions showed significant decrease with the LMWOAs application. Between the LMWOAs, oxalic acid significantly mobilized the Ca bound P iron and aluminium bound P by citric acid. As the soil was neutral in reaction, overall, the applied oxalic acid was significantly superior to the citric acid in enhancing the available P content in the soil. The applied LMWOAs also enhanced phosphorus content of grain, straw and root in both the crops studied. Thus the LMWOAs usage can significantly enhances the soil available phosphorus, increases the phosphorus use efficiency and finally leading to better crop growth.

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