



Oxalic-acid-treated low-grade rock phosphate can supplement conventional phosphorus fertilizer to grow wheat in Alfisol

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Abstract

Phosphorus (P) fertilizers are produced from high-grade rock phosphates (HGRPs). The reserve of HGRP is finite and non-renewable. We are rapidly shrinking this reserve, by producing P fertilizers. This fact establishes the need of utilizing low-grade rock phosphates (LGRPs) for growing crops. In this experiment, LGRP was treated with 0.5 M oxalic acid solution. The oxalic-acid-treated LGRP was used to supplement chemical P fertilizer in incubation as well as pot experiments in Alfisol. During incubation experiment, oxalic-acid-treated LGRP treatment (T₃) significantly increased soil available P as compared to control (T₁) and untreated LGRP (T₂) treatments. Pot study revealed that under DAP+ oxalic-acid-treated LGRP treatment (T₄), oxalic-acid-treated LGRP (@60 mg P₂O₅ kg⁻¹) supplemented 50% DAP without hampering wheat yield and P uptake, at the same time maintained significantly higher total P, inorganic P, organic-P, Ca-P, saloid P and statistically at par available P, dehydrogenase, acid and alkaline phosphatase activities as compared to DAP treatment (T₃) at all the growth stages of wheat. So, it can be inferred that 0.5 M oxalic acid solution treatment can be useful to utilize LGRP as supplemental P source to grow wheat. Oxalic-acid-treated LGRP (@60 mg P₂O₅ kg⁻¹) can successfully supplement 50% of the chemical P fertilizer for growing wheat. It also improves soil P fertility and sustains dehydrogenase, acid phosphatase and alkaline phosphatase activities in Alfisol.

Keywords Wheat · Phosphorus · Low-grade rock phosphate · Oxalic acid · Phosphorus fractions · Soil enzymes

1 Introduction

Phosphorus (P) is the second most important nutrient for plants, just next to nitrogen (N), and makes up (0.05–0.5%) dry weight of them (Malhotra et al. 2018). Though P is the 11th most abundant element of earth crust (Lian et al. 2019), 43% of the world arable land is P deficient (Liu et al. 2012). Due to very high reactivity, P gets fixed in soil. Only 0.1% of the total P in soil is available for plants (Rawat et al. 2021), which very often limits plant growth (Malhotra et al.

2018). Application of P fertilizer is inevitable for modern-day intensive cultivation (Maharana et al. 2021; Roy et al. 2015). The P fertilizers are produced from high-grade rock phosphates (HGRPs, contains > 30% P₂O₅) (Ditta et al. 2018; Sarkar et al. 2018). That is a finite and non-renewable source with ~ 60,000 million tonnes (Mt) world reserve (Kauwenbergh 2010). The predicted life time of this reserve is 75–175 years (Roy et al. 2018). Very soon we will suffer from scarcity of cheap and readily available good-quality P-fertilizers (Sarkar et al. 2020). In the absence of good-quality P-fertilizers intensive cultivation to feed the ever-increasing world population will be a superhuman task (Roy et al. 2018). Utilization of less expensive low-grade rock phosphate (LGRP) as P source to grow crops can reduce cost of cultivation, at the same time P fertilizer consumption may also reduce; as a result, life span of HGRP may increase. The LGRP (contains <20 % P₂O₅) is not used by the P fertilizer industries. Their low P₂O₅ and high CaCO₃ content is not suitable for economic production of P fertilizers (Kumari and Phogat 2008). India has ~200 Mt of RP reserve, which is mostly LGRP (Basak 2019). The country annually

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imports ~7.5 Mt HGRP for meeting its P fertilizer demand (Sujata 2014), 95% of that is consumed by agricultural sector (Kumari and Phogat 2008). Imported HGRP increases the cost of P fertilizer that eventually declines P fertilizer consumption and results into imbalance fertilization (FAI 2014). Utilization of LGRP as replacement or supplement to the costly chemical P fertilizers can reduce the problems to a great extent. Rock phosphates (RP) as such are quite inert to be applied directly to grow crops. Those need to be modified or activated for their better utilization as P source to crops (Basak 2019). Organic acid treatment can increase P availability from LGRP (Roy et al. 2018). Oxalic acid performs comprehensively to solubilize P from RP (Basak 2019). Mendes et al. (2020) found oxalic acid even more efficient than sulfuric acid to solubilize P from RP. Thus, in search of a lucid method to utilize indigenous LGRP as a cheaper alternate to the costly chemical fertilizers, Udaipur RP (LGRP) was treated with oxalic acid and used as supplemental P source to grow wheat. Different forms of soil P are in complex dynamic equilibrium among them. When P is taken up from available P pool by the growing crops, other soil P pools replenish that (Roy et al. 2018). Thus for clear understanding the P-supplying capacity of the soil under different treatments, short-term P dynamics among different soil P fractions and soil enzyme activities were also studied at different growth stages of wheat. It was hypothesized that oxalic acid treatment may increase P solubility from LGRP; also, oxalic acid may be absorbed or adhered by the LGRP and persist for long time in soil after application. Later, this adhered oxalic acid can mimic the P-solubilizing mechanism of phosphorus-solubilizing microorganisms and solubilize P from LGRP as well as from fixed P pools of soil. The present experiment aimed i) to study the effect of oxalic-acid-treated LGRP on soil available P status by an incubation experiment; ii) to evaluate the effectiveness of oxalic-acid-treated LGRP as supplemental P source to grow wheat; iii) to study the effect of oxalic-acid-treated LGRP on short-term P dynamics among different soil P fractions (i.e., total P, inorganic P, organic P, microbial biomass P, available P, saloid P, Al-P, Fe-P, reductant soluble P, Ca-P) at different growth stages of wheat, and iv) to study the enzyme activities (dehydrogenase, acid phosphatase and alkaline phosphatase) at different growth stages of wheat in Alfisol.

2 Material and methods

2.1 Collection and processing of soil

On the basis of prior soil testing, P-deficient bulk soil (0–15 cm) of Alfisol order was collected from the research farm of Palli Siksha Bhavana (Institute of Agriculture), Visva-Bharti University (Central University), Sriniketan, Birbhum,

West Bengal, India. It was air-dried under shade and sieved through a 2-mm sieve and used for incubation and pot experiments conducted at Division of Soil Science and Agricultural Chemistry, Indian Council of Agricultural Research-Indian Agricultural research Institutes (ICAR-IARI), New Delhi. The soil was loamy sand in texture with 81.2% sand, 8.9% silt and 9.6% clay (Bouyoucos 1962), salinity-free ($EC_{(1:2.5 \text{ soil: water})}$ 0.10), acidic in reaction ($pH_{(1:2.5 \text{ soil: water})}$ 5.9) and having 6.7 $cmol(p^+) kg^{-1}$ cation exchange capacity (Jackson 1973). The soil had organic C 0.37% (Walkley and Black 1934), mineralizable nitrogen 59 $mg kg^{-1}$ (Subbiah and Asija 1956), plant-available phosphorus 2.09 $mg kg^{-1}$ (Bray and Kurtz 1945) and available potassium 30.1 $mg kg^{-1}$ (Hanway and Heidel 1952). The initial soil contained 134 $mg kg^{-1}$ total P, out of that 102 $mg kg^{-1}$ was inorganic P and 32 $mg kg^{-1}$ organic P (Page et al. 1982). Inorganic P had 1.57 $mg kg^{-1}$ saloid P, 13.8 $mg kg^{-1}$ Al-P, 33.9 $mg kg^{-1}$ Fe-P, 26.9 $mg kg^{-1}$ Ca-P, 25.7 $mg kg^{-1}$ Red-P (Kuo 1996). Dehydrogenase activity (DHA) of the initial soil was 7.1 μg TPF g^{-1} soil h^{-1} (Klein et al. 1971), alkaline phosphatase activity (Alkaline PA) was 39.5 μg PNP g^{-1} soil h^{-1} and acid phosphatase activity (Acid PA) was 97.3 μg PNP g^{-1} soil h^{-1} (Tabatabai and Bremner 1969).

2.2 Rock phosphate (RP)

Udaipur rock phosphate was used in this experiment. The LGRP (100 mesh size) was collected from Udaipur district of Rajasthan, India, and it had 8.8% total P, 7.24% citrate-insoluble P, 1.57% citrate-soluble P and 0.003% water-soluble P (Roy et al. 2018).

2.3 Preparation of oxalic-acid-treated rock phosphate

To prepare oxalic-acid-treated RP, 1 kg LGRP was mixed with 1 L of 0.5 M oxalic acid solution. The mixture was kept for four days at room temperature to react. It was stirred for 5–10 minutes, twice a day. Then, the oxalic-acid-loaded LGRP was dried properly at 85°C, in a hot air oven. The oxalic-acid-treated LGRP was used in incubation and pot experiments after drying.

2.4 Incubation study

The incubation experiment was conducted in a completely randomized design (CRD) with three treatments and three replications. The treatment details are (T₁) control (No P was added), (T₂) 120 $mg P_2O_5 kg^{-1}$ soil from LGRP and (T₃) 120 $mg P_2O_5 kg^{-1}$ soil from oxalic-acid-treated LGRP. In all the treatments, 100 $mg N kg^{-1}$ soil (from urea) and 60 $mg K_2O kg^{-1}$ soil (from potassium chloride) were applied. After treating as per the treatment details, soil samples (2-mm

sieved, 50 g) were incubated in screw-capped plastic bottles at $30 \pm 1^\circ\text{C}$ with moisture content around field capacity (FC). Five sets of samples were kept for five sampling dates. Incubated soils were analysed for Bray and Kurtz No. 1 solution ($0.03\text{ N NH}_4\text{F}$ and 0.025 N HCl) extractable P (Bray and Kurtz 1945) at 15, 30, 45, 60 and 90 days after incubation (DAI).

2.5 Pot experiment

During winter (*Rabi*) season of 2017–2018, the pot experiment was conducted taking wheat (*Triticum aestivum*) as the test crop. The experiment was conducted in CRD design, with four treatments and three replications. The treatment details are (T_1) control (no P was applied); (T_2) LGRP (120 mg $\text{P}_2\text{O}_5\text{ kg}^{-1}$ soil); (T_3) Di-ammonium phosphate (DAP) (60 mg $\text{P}_2\text{O}_5\text{ kg}^{-1}$ soil) and (T_4) DAP+ oxalic-acid-treated LGRP (30 mg $\text{P}_2\text{O}_5\text{ kg}^{-1}$ soil from DAP+ 60 mg $\text{P}_2\text{O}_5\text{ kg}^{-1}$ soil from oxalic-acid-treated LGRP). Pots were filled with five kg air-dried soil (4.75 mm sieved) after treating as per the treatment details. In each pot, 100 mg N kg^{-1} soil (from urea) and 60 mg $\text{K}_2\text{O}\text{ kg}^{-1}$ soil (from potassium chloride) were added. In DAP-treated pots, N added through DAP was calculated and N addition from urea was adjusted. Five plants were grown in each pot. Three separate sets of pots with same treatment combinations were maintained for collecting soil and plant samples at three different growth stages of wheat.

2.6 Soil and plant sampling and analysis

At maximum tillering (40 days after sowing), flowering (90 days after sowing) and maturity stage of wheat soil and plant samples were collected. Total soil from each pot was taken on a clean plastic sheet and mixed well. Nearly 500 g root-free soil sample was collected from each pot of first second and third set of pots at maximum tillering flowering and maturity stages, respectively. Collected soil was air-dried and sieved through 2-mm sieve. Different soil P fractions and enzyme activities were analysed in the soil samples at all the three different growth stages. At each growth stage, total plant sample from each pot was collected, washed and air-dried in shed. The air-dried plant samples were finally dried in hot air oven at $65 \pm 2^\circ\text{C}$ for 72 h. Dry shoot biomass was recorded at maximum tillering and flowering stage of wheat. Straw and grain biomasses were recorded at maturity stage of wheat. The dried plant samples were grinded in Wiley mill followed by digestion with di-acid mixture ($\text{HNO}_3/\text{HClO}_4 = 9:4$). Total P in the digested plant sample was analysed spectrophotometrically by vanadomolybdo-phosphate yellow colour method (Jackson 1973).

2.7 Soil P fraction analysis

The total and inorganic P in soil were analysed by following the procedure outlined by Walker and Adams (1958). Inorganic P was subtracted from total P to get the estimate of organic P. Soil inorganic P was fractionated into saloid P (sal-P), aluminium P (Al-P), iron P (Fe-P), reductant soluble P (Reds-P) and calcium P (Ca-P). Different fractions of inorganic P were extracted following the sequential extraction method outlined by Kuo (1996). The sal-P, Al-P, Fe-P, and Ca-P contents in the aliquots were determined by ascorbic acid blue colour method (Watanabe and Olsen 1965). For determination of Reds-P, isobutyl alcohol and ammonium molybdate were added in a separatory funnel along with aliquot. The intensity of blue colour developed by the solution mixture in the separatory funnel was measured by spectrophotometer at 660 nm (Page et al. 1982). Plant-available P was extracted from soil by using Bray and Kurtz No. 1 solution (Bray and Kurtz, 1945), and P in the extract was determined by ascorbic acid blue colour method (Watanabe and Olsen 1965). The microbial biomass P (MBP) was estimated by following the procedure outlined by Wu et al. (2000).

2.8 Soil enzyme activities

Soil dehydrogenase activity was estimated by monitoring the production rate of triphenyl formazone (TPF) from triphenyl tetrazolium chloride (TTC) as per the standard protocol of Klein et al. (1971). The acid PA and alkaline PA were monitored as per the standard procedure described by Tabatabai and Bremner (1969).

2.9 Statistical analysis

Analysis of variance (ANOVA) of the data obtained from incubation and pot experiments was carried out with SAS version 9.3 software. At $P \leq 0.05$, the least significance difference (LSD) between the treatment means was calculated (Gomez and Gomez 1984).

3 Result

3.1 Phosphorus release pattern from RP and oxalic-acid-treated RP

Outcome of incubation experiment confirmed the superiority of oxalic-acid-treated LGRP over untreated LGRP (Figure 1). Oxalic-acid-treated LGRP treatment (T_3) maintained ~101, 83.9, 83.7, 83.9 and 84.1% higher available P over untreated LGRP treatment (T_2) at 15, 30, 45, 60 and 90 DAI, respectively. That indicates, application of oxalic-acid-treated LGRP can supply more P to the crops as compared

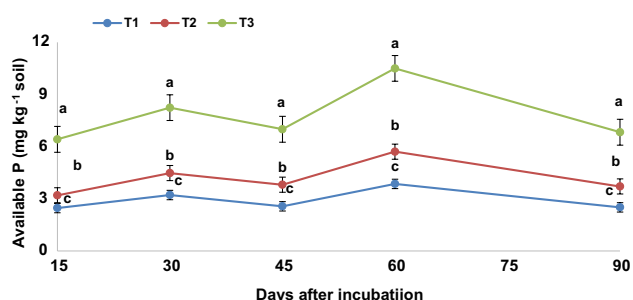


Figure 1 Soil-available P during 90-day incubation period. T₁= No P was added (control), T₂=120 mg P₂O₅ kg⁻¹ soil from untreated low-grade rock phosphate (LGRP); and T₃= 120 mg P₂O₅ kg⁻¹ soil from oxalic-acid-treated LGRP. Treatment means with at least one common letter among them are statistically at par as per Duncan multiple range test at LSD (P < 0.05). Error bars indicate SD.

to inert raw LGRP. During the incubation period, P release pattern showed alternate trough and crest.

3.2 Wheat yield and P uptake

P-untreated control (T₁) and untreated LGRP (T₂) treatments produced at par wheat yield and P uptake at all the crop growth stages. Wheat grain and straw yields under DAP+ oxalic-acid-treated LGRP treatment (T₄) were significantly higher as compared to control (T₁) and untreated LGRP (T₂) treatments (Table 1). Grain and straw yields from DAP+ oxalic-acid-treated LGRP treatment (T₄) were ~541 and

Table 1 Yield and P uptake at different growth stages of wheat during pot experiment

Treatment	MTS	FS	MAS	
			Grain	Straw
Yield (g pot⁻¹)				
T ₁	0.51±0.03c	1.84±0.09c	0.38±0.02b	2.36±0.11c
T ₂	0.90±0.04c	2.13±0.11c	1.35±0.07b	2.07±0.10c
T ₃	6.83±0.24a	23.15±0.82a	9.67±0.48a	17.2±0.67a
T ₄	5.19±0.18b	16.19±0.57b	8.65±0.44a	16.5±0.62a
P uptake (mg pot⁻¹)				
T ₁	1.43±0.07b	2.56±0.13b	1.31±0.64b	1.47±0.07b
T ₂	1.93±0.10b	2.25±0.11b	2.43±0.12b	1.16±0.06b
T ₃	13.3±0.47a	18.4±0.65a	18.8±0.77a	10.5±0.37a
T ₄	13.6±0.48a	17.7±0.62a	17.2±0.71a	10.7±0.38a

T₁= No P was added (control), T₂=120 mg P₂O₅ kg⁻¹ soil from untreated low-grade rock phosphate (LGRP); T₃= 60 mg P₂O₅ kg⁻¹ soil from di-ammonium phosphate (DAP) and T₄= 30 mg P₂O₅ kg⁻¹ soil from DAP+ 60 mg P₂O₅ kg⁻¹ soil from oxalic-acid-treated LGRP. Treatment means with at least one common letter among them are statistically at par as per Duncan multiple range test at LSD (P < 0.05). MTS= Maximum tillering stage, FS= flowering stage and MAS= maturity stage

697% higher, as compared to those from untreated LGRP treatment (T₂). Similarly, grain and straw P uptakes under DAP+ oxalic-acid-treated LGRP treatment (T₄) were ~608 and 822% higher, respectively, over those from untreated LGRP treatment (T₂). In DAP+ oxalic-acid-treated LGRP treatment (T₄), 50% less fertilizer P was added as compared to DAP treatment (T₃), and an attempt was made to supplement it by oxalic-acid-treated LGRP. Nevertheless, DAP+ oxalic-acid-treated LGRP treatment (T₄) and DAP treatment (T₃) maintained statistically at par grain and straw yields and P uptakes throughout the crop growth stages. Thus, wheat yield and P uptake data proved that oxalic-acid-treated LGRP successfully supplemented 50% DAP.

3.3 Different forms of soil P

Soil total P and inorganic P were found significantly higher (Table 2) under untreated LGRP (T₂) and DAP+ oxalic-acid-treated LGRP (T₄) treatments, as compared to DAP treatment (T₃). The DAP+ oxalic-acid-treated LGRP treatment (T₄) maintained ~18, 19 and 20% higher total P, 21, 20 and 22% higher inorganic P as compared to DAP treatment (T₃), at maximum tillering, flowering and maturity stages, respectively. Total P as well as inorganic P was maximum at maximum tillering stage and minimum at maturity stage of wheat. The DAP+ oxalic-acid-treated LGRP treatment

Table 2 Total, inorganic and available P status of soil at different growth stages of wheat during pot experiment

Treatment	MTS	FS	MAS
Total P (mg kg⁻¹)			
T ₁	133±6.4d	131±6.3d	131±6.1d
T ₂	253±13a	251±12a	250±11a
T ₃	181±6.6c	173±6.1c	164±5.8c
T ₄	213±10b	206±9.6b	197±7.0b
Inorganic P (mg kg⁻¹)			
T ₁	99.3±4.9d	99.4±4.9d	99.6±4.8d
T ₂	217±9.9a	217±10a	216±9.8a
T ₃	143±5.1c	138±5.1c	130±4.8c
T ₄	173±6.1b	166±6.0b	158±5.6b
Available P (mg kg⁻¹)			
T ₁	1.63±0.07b	1.51±0.06b	1.53±0.07b
T ₂	1.82±0.08b	1.68±0.07b	1.70±0.08b
T ₃	4.67±0.18a	3.36±0.14a	3.61±0.18a
T ₄	4.99±0.19a	3.99±0.16a	4.36±0.21a

T₁= No P was added (Control), T₂=120 mg P₂O₅ kg⁻¹ soil from untreated low-grade rock phosphate (LGRP); T₃= 60 mg P₂O₅ kg⁻¹ soil from di-ammonium phosphate (DAP) and T₄= 30 mg P₂O₅ kg⁻¹ soil from DAP+ 60 mg P₂O₅ kg⁻¹ soil from oxalic-acid-treated LGRP. Treatment means with at least one common letter among them are statistically at par as per Duncan multiple range test at LSD (P < 0.05). MTS= Maximum tillering stage, FS= flowering stage and MAS= maturity stage

(T₄) provided ~174, 138 and 157% higher available P over LGRP treatment (T₂) at maximum tillering, flowering and maturity stages, respectively (Table 2). Available P content under DAP+ oxalic-acid-treated LGRP (T₄) and DAP (T₃) treatments was statistically at par at all the crop growth stages. For all the treatments, available P was maximum at maximum tillering stage, minimum at flowering stage; interestingly, at maturity stage soil-available P content increased to higher level as compared to flowering stage of wheat.

To clearly understand the short-term P dynamics in Alfisol, different fractions of inorganic P were studied at three different growth stages of wheat (Table 3). Under control treatment, Fe-P contributed most to the inorganic soil P followed by Ca-P, Reds-P, Al-P and Sal-P. But LGRP treatments increased the content of Ca-P fraction of the soil even above Fe-P. The Sal-P and Ca-P contents significantly increased under DAP+ oxalic-acid-treated LGRP treatment (T₄) as compared to DAP treatment (T₃).

Under DAP+ oxalic-acid-treated LGRP treatment (T₄), Sal-P was ~20, 28 and 28% higher and Ca-P was ~21, 20 and 18 % higher over those under DAP treatment (T₃) at maximum tillering, flowering and maturity stages, respectively, whereas Al-P and Fe-P build up in soil were more from DAP treatment (T₃) as compared to DAP+ oxalic-acid-treated LGRP (T₄) and untreated LGRP treatments (T₂).

Organic P content was maximum at maximum tillering stage and minimum at flowering stage of wheat (Table 4). Maximum organic P was observed under DAP+ oxalic-acid-treated LGRP treatment (T₄). Organic P from DAP+ oxalic-acid-treated LGRP treatment (T₄) was ~7, 10 and 14% higher over those from DAP treatment (T₃) at maximum tillering, flowering and maturity stages, respectively. Microbial biomass phosphorus (MBP) was maximum at flowering stage and minimum at maturity stage of the crop (Table 4). DAP+ oxalic-acid-treated LGRP treatment (T₄) and DAP

Table 3 Inorganic P fractions of soil at different growth stages of wheat during pot experiment

Treatment	Sal-P (mg kg ⁻¹)	Al-P (mg kg ⁻¹)	Fe-P (mg kg ⁻¹)	Reds-P (mg kg ⁻¹)	Ca-P (mg kg ⁻¹)
Maximum tillering stage					
T ₁	1.21±0.06c	11.1±0.44b	29.1±1.2c	23.8±0.95c	26.2±1.1d
T ₂	1.15±0.04c	10.9±0.43b	28.5±1.1c	38.2±1.5a	57.8±2.3a
T ₃	3.03±0.11b	15.8±0.73a	38.8±1.5a	31.2±1.2b	38.1±1.5c
T ₄	3.64±0.14a	14.6±0.68a	35.2±1.3b	32.1±1.3b	46.0±1.8b
Flowering stage					
T ₁	1.03±0.05c	9.17±0.37c	28.0±1.1b	22.4±0.89c	25.1±1.0d
T ₂	0.98±0.04c	7.22±0.29d	25.4±1.0c	36.0±1.4a	53.8±2.2a
T ₃	2.28±0.10b	12.3±0.49a	39.7±1.6a	29.4±1.1b	35.3±1.4c
T ₄	2.91±0.12a	10.8±0.438b	37.7±1.5a	30.3±1.2b	42.3±1.7b
Maturity stage					
T ₁	1.24±0.06c	11.0±0.51c	28.9±1.1b	23.6±0.94c	25.1±1.0d
T ₂	1.18±0.05c	12.1±0.53c	26.2±1.0c	37.0±1.5a	52.2±2.1a
T ₃	2.73±0.11b	17.2±0.67a	44.3±1.8a	30.0±1.3b	34.4±1.4c
T ₄	3.50±0.14a	15.8±0.61b	42.6±1.7a	30.9±1.2b	40.6±1.6b

T₁= No P was added (control), T₂=120 mg P₂O₅ kg⁻¹ soil from untreated low-grade rock phosphate (LGRP); T₃= 60 mg P₂O₅ kg⁻¹ soil from di-ammonium phosphate (DAP) and T₄= 30 mg P₂O₅ kg⁻¹ soil from DAP+ 60 mg P₂O₅ kg⁻¹ soil from oxalic-acid-treated LGRP. Treatment means with at least one common letter among them are statistically at par as per Duncan multiple range test at LSD (P < 0.05).

Table 4 Organic P and MBP of soil at different growth stages of wheat during pot experiment

Treatment	Organic P (mg kg ⁻¹)			MBP (mg kg ⁻¹)		
	MTS	FS	MAS	MTS	FS	MAS
T ₁	33.2±1.2c	31.1±1.1c	31.5±1.4c	0.37±0.012b	0.39±0.014b	0.39±0.010b
T ₂	35.6±1.3bc	34.1±1.3b	34.2±1.3b	0.38±0.011ab	0.41±0.013ab	0.40±0.011ab
T ₃	37.4±1.2b	35.4±1.2b	34.5±1.2b	0.40±0.013a	0.43±0.016a	0.42±0.013a
T ₄	40.1±1.3a	38.9±1.4a	39.2±1.6a	0.40±0.014a	0.43±0.015a	0.42±0.012a

T₁= No P was added (Control), T₂=120 mg P₂O₅ kg⁻¹ soil from untreated low-grade rock phosphate (LGRP); T₃= 60 mg P₂O₅ kg⁻¹ soil from di-ammonium phosphate (DAP) and T₄= 30 mg P₂O₅ kg⁻¹ soil from DAP+ 60 mg P₂O₅ kg⁻¹ soil from oxalic-acid-treated LGRP. Treatment means with at least one common letter among them are statistically at par as per Duncan multiple range test at LSD (P < 0.05). MBP= Microbial biomass P, MTS= maximum tillering stage, FS= flowering stage and MAS= maturity stage

treatment (T_3) maintained at par MBP throughout the crop growth stages.

3.4 Soil enzymes

For all the treatments, soil DHA (Figure 2) was maximum at flowering stage and minimum at maximum tillering stage, whereas acid PA (Figure 3) was maximum at flowering stage and minimum at maturity stage of wheat. DAP+ oxalic-acid-treated LGRP treatment (T_4) and DAP treatment (T_3) maintained at par DHA between them. Both the treatments provided significantly higher DHA over control (T_1) and untreated LGRP (T_2) treatments, at all the crop growth stages. For every treatment, alkaline PA (Figure 4) was least at flowering stage and maximum at maturity stage. Control (T_1) and untreated LGRP (T_2) treatments maintained maximum alkaline PA throughout the crop growth stages, which were at par with the alkaline PA under DAP+ oxalic-acid-treated LGRP treatment (T_4). As compared to untreated LGRP treatment (T_2), the DAP+ oxalic-acid-treated LGRP treatment (T_4) produced ~29.2, 37.2 and 29.9% higher acid PA ~8.03, 9.0 and 5.68% lower alkaline PA at maximum tillering, flowering and maturity stages, respectively.

4 Discussion

Oxalic acid (an organic acid) treatment can solubilize P from LGRP (Mendes et al. 2020). Organic acid anions (Jones, 1998) and protons (Calvaruso et al. 2006) associated with organic acids help them to solubilize P from RP as well as from soil. Complexation (Fox et al. 1990) or chelation (Sagoe et al. 1998) of Ca^{2+} from LGRP as well as from soil

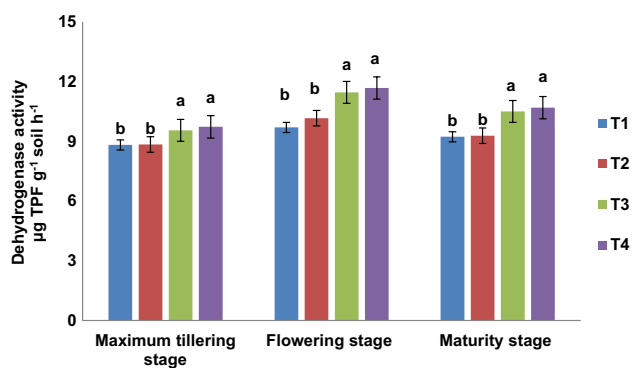


Figure 2 Soil dehydrogenase activity at different growth stages of wheat during pot experiment. T_1 = No P was added (Control), T_2 =120 mg P_2O_5 kg^{-1} soil from untreated low-grade rock phosphate (LGRP); T_3 = 60 mg P_2O_5 kg^{-1} soil from di-ammonium phosphate (DAP) and T_4 = 30 mg P_2O_5 kg^{-1} soil from DAP+ 60 mg P_2O_5 kg^{-1} soil from oxalic-acid-treated LGRP. Treatment means with at least one common letter among them are statistically at par as per Duncan multiple range test at LSD ($P < 0.05$). Error bars indicate SD.

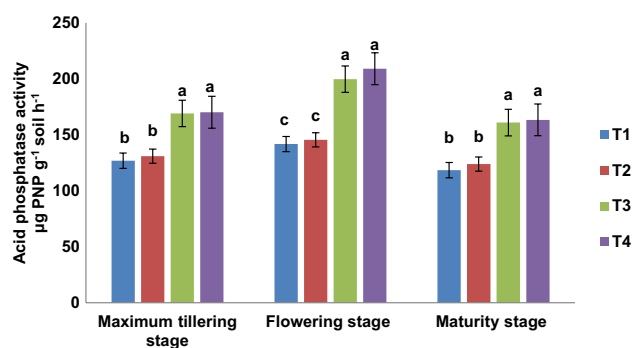


Figure 3 Soil acid phosphatase activity at different growth stage of wheat during pot experiment. T_1 = No P was added (control), T_2 =120 mg P_2O_5 kg^{-1} soil from untreated low-grade rock phosphate (LGRP); T_3 = 60 mg P_2O_5 kg^{-1} soil from di-ammonium phosphate (DAP) and T_4 = 30 mg P_2O_5 kg^{-1} soil from DAP+ 60 mg P_2O_5 kg^{-1} soil from oxalic-acid-treated LGRP. Treatment means with at least one common letter among them are statistically at par as per Duncan multiple range test at LSD ($P < 0.05$). Error bars indicate SD.

by oxalate and formation of calcium oxalate (Welch et al. 2002), proton-promoted P dissolution (Calvaruso et al. 2006, Hammond et al. 1986) are the mechanisms of P solubilization from LGRP by oxalic acid. During incubation experiment, oxalic acid treatment increased P solubilization from LGRP. As a result, oxalic-acid-treated LGRP treatment (T_3) maintained significantly higher soil-available P over LGRP treatment (T_2). During incubation period, P release pattern followed alternate peak and fall. Roy et al. (2018) also reported to have similar unique pattern of P release from LGRP in soil. Difference in P release and uptake by soil microorganisms (Rodríguez and Fraga 1999), P fixation by soil and its subsequent release by ligand exchange of phosphate (Otani et al. 1996) or anion exchange of PO_4^{3-} by

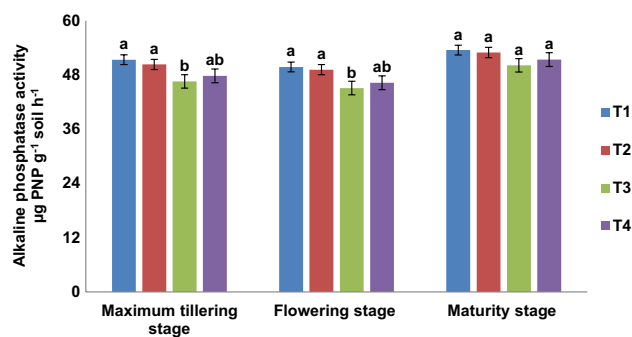


Figure 4 Soil alkaline phosphatase activity at different growth stages of wheat during pot experiment. T_1 = No P was added (Control), T_2 =120 mg P_2O_5 kg^{-1} soil from untreated low-grade rock phosphate (LGRP); T_3 = 60 mg P_2O_5 kg^{-1} soil from di-ammonium phosphate (DAP) and T_4 = 30 mg P_2O_5 kg^{-1} soil from DAP+ 60 mg P_2O_5 kg^{-1} soil from oxalic-acid-treated LGRP. Treatment means with at least one common letter among them are statistically at par as per Duncan multiple range test at LSD ($P < 0.05$). Error bars indicate SD.

oxalate ions, formation of organic P compound by oxalate and their subsequent utilization by soil inherent microbes (Illmer and Schinner 1992) may be the probable reasons behind this unique P release pattern.

During pot experiment, untreated inert rock phosphate failed to supply significant amount of P (Basak 2019) to increase wheat yield and P uptake over control. As a result, P-untreated control (T_1) and untreated LGRP treatments (T_2) provided statistically at par yield and P uptake, whereas oxalic-acid-promoted P solubilization from LGRP increased P availability to the growing crop. The solubilized P may have supplemented 50% of the chemical P fertilizers; thus, in spite of having 50% less DAP addition, DAP+ oxalic-acid-treated LGRP treatment (T_4) maintained at par yield and P uptake with DAP treatment (T_3). The outcome of our study is in accordance with Roy et al. (2015); they also reported that oxalic-acid-treated LGRP treatment increased wheat yield and P uptake. The P in LGRP (Udaipur RP) is Ca-bound inorganic P (Roy et al. 2018). Higher amount of P was added through Udaipur RP in untreated LGRP (T_2) and DAP+ oxalic-acid-treated LGRP (T_4) treatments as compared DAP treatment (T_3). Higher addition of Ca-bound inorganic P increased total P, inorganic P and Ca-P content in LGRP-treated soils (i.e., under T_3 and T_4 treatments) over DAP treatment (T_3). P uptake by wheat removed P from soil in significant quantity, which may have decreased total P and inorganic P from maximum tillering to maturity of wheat. Oxalic acid treatment solubilized P from LGRP in substantial amount (Basak 2019, Mendes et al. 2020) and increased soil-available P. Thus, in spite of applying 50% less DAP; DAP+ oxalic-acid-treated LGRP treatment (T_4) maintained at par available P with DAP treatment (T_3) without reducing the yield and P uptake of wheat. P added in soil as chemical fertilizer under DAP treatment (T_3) immediately got fixed by the soil or got converted to initial reaction products (Tiwari 2009). Whereas in DAP+ oxalic-acid-treated LGRP treatment (T_4), oxalic acid solubilized P from LGRP and soil (Basak 2019), it also chelated and complexed phosphate-binding Al^{3+} , Fe^{3+} and Ca^{2+} cations (Mendes et al. 2020). Oxalate anions also competed with phosphate for common bounding sites in soil (Biswas et al. 2021). Thus presence of oxalic acid may have reduced P fixation in soil also. As a result DAP+ oxalic-acid-treated LGRP treatment (T_4) maintained significantly higher Sal-P as compared to DAP treatment (T_3). Maximum P removal occurred during the period of maximum tillering to flowering stage of wheat, which may have brought available P and saloid P level to minimum at flowering stage, and disturbed the equilibrium between soil solution P and fixed P. After maturity of wheat, soil P removal by the crop stopped. The equilibrium between soil-fixed P and solution P was restored with time. As a result, P from other soil P fractions was added to soil-available P and Sal-P and increased their concentration (Biswas et al.

2021; Roy et al. 2018). Phloem transport also translocates back some plant nutrients from shoot to root and root to soil after maturity of the plant (Biswas et al., 2017; Mengel and Kirkby 2012), which can increase soil available P and Sal-P, content after maturity stage as compared to flowering stage of wheat. Biswas et al. (2021) also reported that soil-available P was minimum at 70 days after transplanting rice and that increased after maturity of rice in an Inceptisol. Soil biological parameters like organic P, MBP, DHA, acid PA and alkaline PA were also altered by external application of P and crop growth stages. During the period of maximum tillering stage to flowering stage, shoot and root growth rate of wheat become maximum (Biswas et al. 2017); thereby, at flowering stage amount of root exudates in soil upsurges, which act as food and energy sources for soil microorganism and increase the growth and activity of soil microbes (Biswas et al. 2019); these microbes immobilized soil P in their body and increased MBP to maximum at flowering stage. Soil dehydrogenase is an oxido-reductase enzyme that indicates total oxidative activity by soil micro-flora (Nannipieri et al. 2017). Thus increased microbial activity may have increased soil DHA. Our findings are in agreement with Bera et al. (2017), Biswas et al. (2019), where they found maximum DHA at maximum vegetative growth period of wheat and those were less at maturity stage and earlier growth stage. Along with soil microbes, plant roots also produce acid phosphatase, and alkaline phosphatase is solely produced by soil bacteria (McLachlan 1980), thus, at flowering stage increased root growth and microbial activity increased the level of acid PA to maximum, and at the same time rhizosphere acidification may have reduced the alkaline PA to the minimum (Eivazi and Tabatabai 1977). Alkaline PA was maximum in the P-limited condition, i.e., under control (T_1) and untreated LGRP (T_2) treatments. Cotner and Wetzel (1991) also found higher alkaline PA in P-limited conditions. Under DAP+ oxalic-acid-treated LGRP treatment (T_4), oxalic acid may have acidified soil micro-environment; as a result, acid PA increased and alkaline PA decreased under this treatment as compared to untreated LGRP (T_2) treatment (Kamran et al. 2018). At flowering stage, higher root growth and augmented microbial activity increased acid PA; this subsequently mineralized and broke down soil organic P (Zhu et al. 2017) and decreased its level to minimum.

5 Conclusion

Higher available P under oxalic-acid-treated low-grade rock phosphate (LGRP) treatment over control and untreated rock phosphate treatment, throughout the incubation period, indicates oxalic acid treatment can increase P availability from LGRPs. It also indicates oxalic-acid-treated LGRP can

maintain higher available P in soil for long duration. This statement was supported by the outcome of pot experiment. There di-ammonium phosphate (DAP)+ oxalic-acid-treated LGRP treatment maintained similar crop yield and P uptake as compared to DAP treatment. Though the earlier treatment was having 50% less added P via DAP. That indicates oxalic-acid-treated LGRP can supplement chemical P fertilizer to grow wheat in Alfisol. Application of oxalic-acid-treated LGRP (@60 mg P₂O₅ kg⁻¹ soil) can reduce 50% of the chemical P fertilizer consumption by wheat in Alfisol. Study of different soil P fractions and enzyme activities at different growth stages of wheat has given proper insight into short-term P dynamics and enzyme activities under wheat in Alfisol. Beside supporting wheat yield and P uptake, oxalic-acid-treated rock phosphate treatment can improve the status of soil saloid P, total P, inorganic P and calcium P as compared to chemical fertilizer treatment, those can exert beneficial residual effect on the next crop. Soil-available P, saloid P, organic P and alkaline phosphatase activity reduce to minimum at flowering stage and increase after maturity of wheat. Soil dehydrogenase activity, acid phosphatase activity and microbial biomass phosphorus increase to maximum at flowering and reduce after maturity of wheat. Further, a field experiment on this topic can more consolidate the fact of using oxalic-acid-treated low-grade rock phosphate as supplemental P source to grow wheat in Alfisol.

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Declarations

Disclosure statement There is no potential conflict of interest among the authors.

References

- Basak BB (2019) Phosphorus release by low molecular weight organic acids from low-grade Indian rock phosphate. *Waste Biomass Valorization* 10:3225–3233. <https://link.springer.com/article/https://doi.org/10.1007/s12649-018-0361-3>. Accessed 14 Nov 2021
- Bera T, Sharma S, Thind HS, Sidhu HS, Jat ML (2017) Soil biochemical changes at different wheat growth stages in response to conservation agriculture practices in a rice-wheat system of north-western India. *Soil Res* 56:91–104. <https://doi.org/10.1071/SR16357>
- Biswas SS, Biswas DR, Purakayastha TJ, Sarkar A, Kumar R, Das TK, Barman M, Pabbi S, Ghosh A, Pal R (2021) Residual effect of rock-phosphate and PSB on rice yield and soil properties. *Indian J Agric Sci* 91:440–444
- Biswas SS, Ghosh A, Singhal SK, Biswas DR, Roy T, Sarkar A, Das D (2019) Phosphorus enriched organic amendments can increase nitrogen use efficiency in wheat. *Commun Soil Sci Plant Anal* 50:1178–1191. <https://doi.org/10.1080/00103624.2019.1604736>
- Biswas SS, Singhal SK, Biswas DR, Singh RD, Roy T, Sarkar A, Ghosh A, Das D (2017) Synchronization of nitrogen supply with demand by wheat using sewage sludge as organic amendment in an Inceptisol. *J Indian Soc Soil Sci* 65:264–273. <https://doi.org/10.5958/0974-0228.2017.00030.5>
- Bouyoucos GJ (1962) Hydrometer method improved for making particle size analysis of soils. *Agron J* 54:464–465. <https://doi.org/10.2134/agronj1962.00021962005400050028x>
- Bray RH, Kurtz LT (1945) Determination of total, organic, and available forms of phosphorus in soils. *Soil Sci* 59:39–46
- Calvaruso C, Turpault MP, Frey-Klett P (2006) Root-associated bacteria contribute to mineral weathering and to mineral nutrition in trees: a budgeting analysis. *Appl Environ Microbiol* 72:1258–1266. <https://journals.asm.org/doi/full/https://doi.org/10.1128/AEM.72.2.1258-1266.2006>. Accessed 14 Nov 2021
- Cotner JB, Wetzel RG (1991) Bacterial phosphatases from different habitats in a small, hardwater lake. In: *Microbial enzymes in aquatic environments*. Springer, New York, pp 187–205. https://link.springer.com/chapter/https://doi.org/10.1007/978-1-4612-3090-8_11. Accessed 14 Nov 2021
- Ditta A, Muhammad J, Imtiaz M, Mehmood S, Qian Z, Tu S (2018) Application of rock phosphate enriched composts increases nodulation, growth and yield of chickpea. *Int J Recycl Org Waste Agric* 7:33–40. <https://link.springer.com/article/https://doi.org/10.1007/s40093-017-0187-1>. Accessed 14 Nov 2021
- Eivazi F, Tabatabai MA (1977) Phosphatases in soils. *Soil Biol Biochem* 9:167–172
- FAI (2014) Fertiliser Statistics 2013–14. The Fertiliser Association of India, New Delhi
- FCO (1985) The Fertiliser (Inorganic, Organic or Mixed) (Control) Order, 1985 (As amended up to February 2019). The Fertiliser Association of India, FAI House, 10 Shaheed Jit Singh Marg, New Delhi, 330p
- Fox TR, Comerford NB, McFee WW (1990) Phosphorus and aluminum release from a spodic horizon mediated by organic acids. *Soil Sci Soc Am J* 54:1763–1767. <https://doi.org/10.2136/sssaj1990.03615995005400060043x>
- Gomez KA, Gomez AA (1984) *Statistical procedures for agricultural research*. 2nd ed. Philippines: John Wiley & Sons, International Rice Research Institute
- Hammond LL, Chien SH, Mokwunye AU (1986) Agronomic value of unacidulated and partially acidulated phosphate rocks indigenous to the tropics. *Adv Agron* 40:89–140. [https://doi.org/10.1016/S0065-2113\(08\)60281-3](https://doi.org/10.1016/S0065-2113(08)60281-3)
- Hanway JJ, Heidel H (1952) Soil analysis methods as used in Iowa State College, Soil Testing Laboratory. *Iowa Agric* 54:1–31
- Illmer P, Schinner F (1992) Solubilization of inorganic phosphates by microorganisms isolated from forest soils. *Soil Biol Biochem* 24:389–395. [https://doi.org/10.1016/0038-0717\(92\)90199-8](https://doi.org/10.1016/0038-0717(92)90199-8)
- Jackson ML (1973) *Soil Chemical Analysis*. Prentice Hall of India Private Limited, New Delhi
- Jones DL (1998) Organic acids in the rhizosphere—a critical review. *Plant Soil* 205:25–44. <https://link.springer.com/article/https://doi.org/10.1023/A:1004356007312>. Accessed 14 Nov 2021
- Kamran MA, Jiang J, Li JY, Shi RY, Mehmood K, Abdulaha-Al Baquy M, Xu RK (2018) Amelioration of soil acidity, Olsen-P, and phosphatase activity by manure-and peat-derived biochars in different acidic soils. *Arab J Geosci* 11:1–15. <https://link.springer.com/article/https://doi.org/10.1007/s12517-018-3616-1>. Accessed 14 Nov 2021
- Kauwenbergh S (2010) International fertiliser development centre. Washington, DC, USA

- Klein DA, Loh TC, Goulding RL (1971) A rapid procedure to evaluate the dehydrogenase activity of soils low in organic matter. *Soil Biol Biochem* 3:385–387
- Kumari K, Phogat VK (2008) Rock phosphate: Its availability and solubilization in the soil—A review. *Agric Rev* 29:108–116
- Kuo S (1996) Phosphorus. In *Methods of Soil Analysis. Part III. Chemical Methods*. D.L. Sparks et al. (Eds.), Soil Science Society of America and American Society of Agronomy Madison, WI, pp. 869–920
- Lian T, Ma Q, Shi Q (2019) High aluminum stress drives different rhizosphere soil enzyme activities and bacterial community structure between aluminum-tolerant and aluminum-sensitive soybean genotypes. *Plant Soil* 440:409–425 <https://link.springer.com/article/https://doi.org/10.1007/s11104-019-04089-8>. Accessed 15 Nov 2021
- Liu Y, Feng L, Hu H, Jiang G, Cai Z, Deng YJ (2012) Phosphorus release from low-grade rock phosphates by low molecular weight organic acids. *J Food Agric Environ* 10:1001–1007
- Maharana R, Basu A, Dhal NK, Adak T (2021) Biosolubilization of rock phosphate by *Pleurotus ostreatus* with brewery sludge and its effect on the growth of maize (*Zea mays* L.). *J Plant Nutr* 44:395–410 <https://www.tandfonline.com/doi/full/https://doi.org/10.1080/01904167.2020.1822397>. Accessed 16 Nov 2021
- Malhotra H, Vandana SS, Pandey R (2018) Phosphorus Nutrition: Plant Growth in Response to Deficiency and Excess. *Plant Nutrients and Abiotic Stress Tolerance*. In: Hasanuzzaman M, Fujita M, Oku H, Nahar K y Hawrylak-Nowak B (Eds). Springer, Singapore, 171–190 https://link.springer.com/chapter/https://doi.org/10.1007/978-981-10-9044-8_7. Accessed 17 Nov 2021
- McLachlan KD (1980) Acid phosphatase activity of intact roots and phosphorus nutrition in plants. I. Assay conditions and phosphatase activity. *Aust J Agric Res* 31:429–440
- Mendes GO, Murta HM, Valadares RV, Silveira WB, Silva IR, Costa M (2020) Oxalic acid is more efficient than sulfuric acid for rock phosphate solubilization. *Miner Eng* 155:106458. <https://doi.org/10.1016/j.mineng.2020.106458>
- Mengel K, Kirkby EA (2012) *Principles of plant nutrition*. 5th ed. Springer Science & Business Media
- Nannipieri P, Greco S, Ceccanti B (2017) Ecological significance of the biological activity in soil. *Soil Biochem* 12:293–356. <https://doi.org/10.1201/9780203739389-6>
- Otani T, Ae N, Tanaka H (1996) Phosphorus (P) uptake mechanisms of crops grown in soils with low P status: II. Significance of organic acids in root exudates of pigeonpea. *Soil Sci Plant Nutr* 42:553–560 <https://www.tandfonline.com/doi/abs/https://doi.org/10.1080/00380768.1996.10416324>. Accessed 16 Nov 2021
- Page AL, Miller RH, Keeney DR (1982) *Methods of Soil Analysis. Part 2. Chemical and microbiological properties*, 2nd edn. Agronomy, 9 ASA, SSSA, Madison, WI, p 1159.
- Rawat P, Das S, Shankhdhar D, Shankhdhar SC (2021) Phosphate-solubilizing microorganisms: mechanism and their role in phosphate solubilization and uptake. *J Soil Sci Plant Nutr* 21:49–68 <https://link.springer.com/article/https://doi.org/10.1007/s42729-020-00342-7>. Accessed 16 Nov 2021
- Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv* 17:319–339 [https://doi.org/10.1016/S0734-9750\(99\)00014-2](https://doi.org/10.1016/S0734-9750(99)00014-2)
- Roy T, Biswas DR, Datta SC, Dwivedi BS, Bandyopadhyay KK, Sarkar A, Agarwal BK, Shahi DK (2015) Solubilization of Purulia rock phosphate through organic acid loaded nanoclay polymer composite and phosphate solubilizing bacteria and its effectiveness as P-fertilizer to wheat. *J Indian Soc Soil Sci* 63:327–338. <https://doi.org/10.5958/0974-0228.2015.00043.2>
- Roy T, Biswas DR, Datta SC, Sarkar A, Biswas SS (2018) Citric acid loaded nano clay polymer composite for solubilization of Indian rock phosphates: a step towards sustainable and phosphorus secure future. *Arch Agro Soil Sci* 64:1564–1581. <https://doi.org/10.1080/03650340.2018.1444275>
- Sagoe CI, Ando T, Kouno K, Nagaoka T (1998) Relative importance of protons and solution calcium concentration in phosphate rock dissolution by organic acids. *Soil Sci Plant Nutr* 44:617–625 <https://www.tandfonline.com/doi/abs/https://doi.org/10.1080/00380768.1998.10414485>. Accessed 18 Nov 2021
- Sarkar A, Biswas DR, Datta SC, Roy T, Biswas SS, Ghosh A, Saha M, Moharana PC, Bhattacharyya R (2020) Synthesis of poly (vinyl alcohol) and liquid paraffin-based controlled release nitrogen-phosphorus formulations for improving phosphorus use efficiency in wheat. *Soil Sci Plant Nutr* 20:1770–1784 <https://link.springer.com/article/https://doi.org/10.1007/s42729-020-00249-3>
- Sarkar A, Biswas DR, Datta SC, Roy T, Moharana PC, Biswas SS, Ghosh A (2018) Polymer coated novel controlled release rock phosphate formulations for improving phosphorus use efficiency by wheat in an Inceptisol. *Soil Tillage Res* 180:48–62. <https://doi.org/10.1016/j.still.2018.02.009>
- Subbiah BV, Asija GL (1956) A rapid procedure for the determination of available nitrogen in soils. *Curr Sci* 25:259–260
- Sujata MV (2014) International and national market perspective of “P” fertilisers. *Indian J Fertilisers* 10:72–80
- Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol Biochem* 1:301–307. [https://doi.org/10.1016/0038-0717\(69\)90012-1](https://doi.org/10.1016/0038-0717(69)90012-1)
- Tiwari KN (2009) Phosphorus. In: Goswami NN, Rattan RK, Dev G, Narayanasamy G, Das DK, Sanyal SK, Pal DK, Rao DLN (eds) *Fundamentals of Soil Science*. Indian Society of Soil Science, Pusa, New Delhi, pp 413–430
- Walker TW, Adams AFR (1958) Studies on soil organic matter: I. Influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulfur, and organic phosphorus in grassland soils. *Soil Sci* 85:307–318
- Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci* 37:29–38
- Watanabe FS, Olsen SR (1965) Test of ascorbic acid method for determining phosphorus in water and sodium bicarbonate extracts of soil. *Soil Sci Soc Am Proceed* 29:677–678. <https://doi.org/10.2136/sssaj1965.03615995002900060025x>
- Welch SA, Taunton AE, Banfield JF (2002) Effect of microorganisms and microbial metabolites on apatite dissolution. *Geomicrobiol J* 19:343–367. <https://doi.org/10.1080/01490450290098414>
- Wu J, He ZL, Wei WX, O'donnell AG, Syers JK (2000) Quantifying microbial biomass phosphorus in acid soils. *Biol Fert Soils* 32:500–507 <https://link.springer.com/article/https://doi.org/10.1007/s003740000284>. Accessed 18 Nov 2021
- Zhu J, Qu B, Li M (2017) Phosphorus mobilization in the Yeyahu Wetland: phosphatase enzyme activities and organic phosphorus fractions in the rhizosphere soils. *Int Biodeterior Biodegradation* 124:304–313. <https://doi.org/10.1016/j.ibiod.2017.05.010>

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