



# Selection of an Extraction Method Suitable for Estimating Potentially Available Phosphorus Under the Organic Production System of New Alluvial Zone of the Lower Gangetic Plain of India

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Received: 3 September 2022 / Accepted: 19 February 2023  
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## Abstract

The reliability of the methods used in conventional farming for determining immediately available phosphorus (P) might not be suitable for organic farming, where mineralization of labile organic pools is the prime P source for plant availability. A conventional procedure that involves P extraction downplays the significance of organic pools. This necessitates the development of a suitable soil testing procedure for P availability under organic farming. A field experiment was conducted following a 3-year rotation of scented rice-French bean-okra cultivation with farmyard manure (FYM), vermicompost (VC), mustard oil cake (MOC), and poultry manure (PM) along with their combinations. Chemically fertilized plots were appointed as a check. P was evaluated using four extractants, viz, BEDTA (Basic EDTA/0.5 M NaOH+0.5 M EDTA), K<sub>2</sub>CO<sub>3</sub> (1% potassium carbonate), CA (1% citric acid), and 2-KGA (0.5 M 2-ketogluconic acid). In addition, Olsen (0.5 M NaHCO<sub>3</sub>) method was used to compare these methods. CA extracted P (CA-P) showed the strongest association with mineralized P ( $R^2=0.854^{**}$ ), followed by Olsen P ( $R^2=0.789^{**}$ ). Furthermore, the CA-P demonstrated a significant correlation with P% in pod ( $r=0.732^{**}$ ), pod yield ( $r=0.742^{**}$ ), dry matter yield (DMY,  $r=0.754^{**}$ ), and P uptake ( $r=0.765^{**}$ ). Likewise, the Olsen method was strongly related to those plant factors ( $r=0.713^{**}$ ,  $r=0.634^{**}$ ,  $r=0.666^{**}$ , and  $r=0.693^*$ ). Additionally, CA and Olsen methods are significantly related to soil characteristics (pH and organic carbon). However, the principal component analysis demonstrated that the CA was the most reliable. Based on CA method, organic certifying agencies, soil scientists, and laboratory personnel will be able to give better advice to the organic farmers on P management.

**Keywords** Organic production system · P extractants · P estimation methods · Citric acid extractable P

## 1 Introduction

Organic farming aims to produce more ecologically friendly and healthy food, using internal nutrient cycling that minimizes losses compared to chemical fertilizer treated in

conventional farming (Meemken and Qaim 2018; Stockdale et al. 2001; Watson et al. 2002), which increases the demand for organic foods. With the increasing demand for organically produced foods, more farmers choose organic agriculture over conventional farming practices (Das et al.

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2020; Nelson and Janke 2007). However, among 73 million ha of global organic farmland, India accounts for only 2.3 million ha, contributing 30% of the world's organic producers (FiBL survey 2021). According to the Ministry of Commerce & Industry survey report, 2020–2021, organic product exports by India increased by 50.94% from USD 689 million in 2019–2020 to USD 1040 million in 2020–2021. In addition to economical benefits, organic farming practices also help to increase soil organic matter and biological diversity that affect P cycling, which is one of the most crucial nutrients for plant growth (Cooper et al. 2018; Nelson and Janke 2007). P helps in various plant metabolic processes by forming chemical molecules such as adenosine diphosphate and adenosine triphosphate, which act in energy storage and transfer mechanism (Stigter and Plaxton 2015; Tate 1984). But farmers mainly apply organic nutrients to meet crop nitrogen (N) requirements, making organic production systems have unbalanced P (Mikkelsen 2000).

Furthermore, unlike N, it is non-renewable as it cannot be obtained through biological acquisition (Nelson and Mikkelsen 2008). Due to the variation in nutrient sources, the P dynamics in organically fertilized soils are much different from that of chemically fertilized soils (Cooper et al. 2018). An increase in organic matter is accompanied by an increase in the organic P pool, as most of the soil's total P under the organic system is present in organic form (mostly phytic acid). Mineralization of labial organic P pools, which in turn influence the biological availability of P, plays a crucial role in crop growth under the organic production system (Condron et al. 2005). In addition, microbial immobilization of active P pools takes place simultaneously to synthesize building block molecules such as nucleic acids and phospholipids (Nesme et al. 2014).

For estimation of immediately available soil P under conventional farming practice, many extraction methods are available, such as Olsen method (Olsen et al. 1954), Bray and Kurtz II (Bray and Kurtz 1945), calcium-acetate-lactate (Schüller 1969), calcium chloride (Houba et al. 2000), Mehlich 3 (Mehlich 1984), and hydrochloric acid (HCl) (Kuo 1996). But none of these extractants can estimate biologically available organic P pools, which is most important under any organic system (Hayes et al. 2000; Turner et al. 2005). So, the applicability of these methods for determining the actual availability of P under the organic farming system is questionable as these P extraction methods neglect the importance of the organic P pools. To assess the organic P present in soils, many scientists, namely Anderson (1960), Bowman (1989), Bowman and Cole (1978), Bowman and Moir (1993); Mehta et al. (1954), and Thomas and Bowman (1966), have identified organic P extraction methods. Some organic acids in plant rhizospheres, such as citric acid, oxalic acid, lactic acid, are excellent at mobilizing labile organic P pools (Dey et al. 2019; Richardson et al. 2022;

Wei et al. 2010). Different studies have also reported that citric acid, malic acid, succinic acid, 2-ketogluconic acid, and oxalic acid are essential low-molecular organic acids excreted from the root rhizosphere of legume crops (Jemo et al. 2006; Moghimi 1977). However, though these methods can extract organic P from the soil, none of these methods has been studied under pure organically managed soil. Recently, Mukherjee et al. (2021) concluded that methods used to determine available N under the conventional system failed to give satisfactory results in organic production. In this background, we hypothesized that the reserve but potentially available nutrient fractions that accounted for balanced plant nutrition under organic farming soils could not be explained by the dynamic conventional soil testing protocol. Improper understanding of organic P dynamic and soil-biogeochemical cycle leads to unbalanced P nutrition in crops and low crop yields under organic agriculture (Saha and Mandal 2011). Therefore, any organic production system must thoroughly understand the labile and refractive pools and their respective contributions to plant nutrition (Möller 2018; Mukherjee 2021) to address this difficulty and form a suitable soil testing method to estimate the amount of P, depending on the size of possibly contributing P pools and P release capacity (Darch et al. 2016).

However, due to a lack of proper research on the organic nutrient pools and associated test procedures, little or no progress has been made in soil testing-based nutrient management in organic farming (Saha and Mandal 2011; Zou et al. 1992). A few workers have initiated a modest approach to developing soil testing protocols for nutrient determination dedicated to organic farming (Chakraborty and Saha 2017; Dey et al. 2019; Mukherjee et al. 2021). Yet, no comprehensive protocol for P testing focusing on the organic production system has been developed. In this recent state-of-the-art, an attempt was made to determine the appropriate extractant/s suitable for estimating P availability under an organic production system and identify the best method/s that establishes a good relationship with the plant parameters and soil characteristics due to applying different organic sources.

## 2 Materials and Methods

### 2.1 Description of the Experimental Site

For carrying out the investigation, a field trial was performed in the Central Research Farm of Bidhan Chandra Krishi Viswavidyalaya, Gayeshpur (88°29' east, 22°58' north, and 9.75 m above mean sea surface) in West Bengal, India, from 2015–2016 to 2018–2019. The experimental site is a sub-tropical humid region in the new alluvial zone with an annual rainfall of roughly 1500 mm, and yearly minimum and maximum temperatures of 12.5° C and 36.3° C

respectively. The experimental soil is a hyperthermic Aeric Haplaquept with a silty loam texture (Soil Survey Staff 2003). Soil pH, organic carbon, accessible N, P, and potassium (K) concentrations were 6.82, 6.3 g kg<sup>-1</sup>, 211 kg N ha<sup>-1</sup>, 26 kg P ha<sup>-1</sup>, and 171 kg K ha<sup>-1</sup> respectively at 0 to 0.15 m depth (Mukherjee et al. 2021).

The study was conducted under the New Alluvial Zone (land having alluvial soils) because it makes up India's largest cultivated land area (more than 40%) with the highest productivity (Dwevedi et al. 2017). New alluvial soils are formed by surface water deposition in the floodplain areas along the river. The new alluvial zone comes under the Lower Gangetic plains, i.e., one of the fifteen agroclimatic regions of India (Planning Commission of India 1989). This experiment shows that a specific method frequently provides a trustworthy evaluation for the amount of P available under an organic system, especially in new alluvial soils.

## 2.2 Experiment Details and Layout

The field trial was conducted in a randomized block design (RBD) with a cropping system of scented rice (Gobindohog), French bean, and okra. The study was carried out on a field that had been organically fertilized for 3 years before the experiment started, assuming that the soil would reach a condition of stabilization concerning the nutrient cycle during that time. Under this circumstance, the tested techniques will more accurately quantify the typical P fractions that predominate in stabilized soil (Mukherjee et al. 2021). Therefore, farmyard manure (FYM), vermicompost (VC), mustard oil cake (MOC), and poultry manure (PM), as well as various combinations of these four organic materials, were used as sources of P for the field experiment (Table 1). In addition, a chemically fertilized plot in which the recommended

dose of fertilizer was administered (RDF; 50 kg N ha<sup>-1</sup>, 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 40 kg K<sub>2</sub>O ha<sup>-1</sup> according to the 2007–2008 AICRP–STCR Biannual Report). Along with it, a control plot (without fertilizer) was maintained. Following the RBD design, all treatments were split into three replications (plot size: 8 m × 6 m). For each Mg of FYM, VC, MOC, and PM, the corresponding P concentration was 3.1 kg, 3.8 kg, 19.3 kg, and 14.8 kg respectively.

In order to make the soil friable for growing French beans, it was tilled for three times and then planked. French bean seeds were sown at a rate of 100 kg seed ha<sup>-1</sup> during November, with a spacing of 40 cm × 10 cm and a depth of 0.05 to 0.07 m. Seven days prior to seeding (pre-sowing stage), complete applications of all organic nutrient sources were made. However, urea, di-ammonium phosphate (DAP), and muriate of potash (MOP) were used as fertilizer sources in the chemically fertilized plot. Half-dose of N and full-doses of P and K were applied as basal dose, and the remaining half-dose of N was laid before the flowering stage.

## 2.3 Soil and Plant Sampling and Analysis

Most likely, prolonged submergence in rice farming nullifies minute temporal variations of nutrient dynamics in the system (Mukherjee et al. 2021). Therefore, the crop in the rotation following rice, namely the French bean, should be tested to study the influence of applied organics on P availability. After completing three cropping cycles, soil sampling was started during French bean cultivation. Surface soil (0–15 cm) samples were taken from various growth stages of French bean, such as pre-sowing (the day of fertilizer/organics application, the 0th day), initial stage (14 days after fertilizer application), full growth stage (35 days after fertilizer application), flowering stage (49 days after

**Table 1** Details of the sources of P used in the experiment

| The sources of P and their doses of application  | Notations |
|--|-----------|
| Without any P sources (Control)  | CK        |
| Inorganic fertilizers (IF) for N, P, K   | IF        |
| Farmyard manure (FYM) at 20 Mg ha <sup>-1</sup>  | FYM       |
| Vermicompost (VC) at 10 Mg ha <sup>-1</sup>  | VC        |
| Mustard oil cake (MOC) at 2.5 Mg ha <sup>-1</sup>  | MOC       |
| Poultry manure at 5 Mg ha <sup>-1</sup>  | PM        |
| Farmyard manure at 10 Mg ha <sup>-1</sup> + vermicompost at 5 Mg ha <sup>-1</sup>        | FYM+VC    |
| Farmyard manure at 10 Mg ha <sup>-1</sup> + mustard oil cake at 1.25 Mg ha <sup>-1</sup> | FYM+MOC   |
| Farmyard manure at 10 Mg ha <sup>-1</sup> + poultry manure at 2.5 Mg ha <sup>-1</sup>    | FYM+PM    |
| Vermicompost at 5 Mg ha <sup>-1</sup> + mustard oil cake at 1.25 Mg ha <sup>-1</sup>     | VC+MOC    |
| Vermicompost at 5 Mg ha <sup>-1</sup> + poultry manure at 2.5 Mg ha <sup>-1</sup>        | VC+PM     |
| Mustard oil cake at 1.25 Mg ha <sup>-1</sup> + poultry manure at 2.5 Mg ha <sup>-1</sup> | MOC+PM    |

P for phosphorus, FYM for farmyard manure, VC for vermicompost, MOC for mustard oil cake, and PM for poultry manure. In the case of CK (Control), no fertilizers were used, whereas IF for inorganic fertilizers where urea, di-ammonium phosphate (DAP), and muriate of potash (MOP) were applied for nitrogen (N), phosphorus (P), and potassium (K) respectively

fertilizer application), and harvesting stage (63 days after fertilizer application). P limitation during the early stages of crop growth might restrict crop growth from which the plant cannot recover, even if P supply increases later (Grant et al. 2001). So, P was present in the initial stage of French bean growth (14th day after fertilization), considered the main P fraction for studying our experiment. The soil samples were collected, dried in the shade, sieved through a 2-mm sieve, and then analyzed for P estimate. However, the harvested crop (pod and remaining plant part) was dried in a hot air oven at 65°C until no further change in weight and DMY (dry matter yield) was recorded. Then, the plant samples were ground to a fine powder using a grinder for further analysis.

## 2.4 Analytical Methods for P Estimation

To address our study, two inorganic extractants, basic ethylene diamine tetra-acetic acid (BEDTA) and potassium carbonate ( $K_2CO_3$ ), along with two organic extractants, i.e., citric acid (CA) and 2-ketogluconic acid (2-KGA), were selected to estimate plant utilizable P (Table 2). The reason for choosing these extractants is their ability to extract molecules resembling soils using ligand exchange, chelation with Fe, Al, Ca, or mobilization techniques. Any method's suitability must be based on how it correlates with crop response. The most widely used conventional method, like Olsen (0.5 M  $NaHCO_3$ ), was used to compare different estimated P. BEDTA and  $K_2CO_3$  extractable organic P was measured by subtracting the inorganic P from the total P. Methods proposed by Bowman and Moir (1993), Das (1930), and Hockensmith et al. (1933) were used to determine BEDTA P and  $K_2CO_3$  P respectively (Table 2). CA P was determined

by the method proposed by Dyer (1894), whereas 2-KGA P was extracted by a modified technique of Korndorfer et al. (1995) (Table 2).

To screen out the best extraction method for estimating P availability under the organic production system, firstly, assessing the relationship of P estimated by various methods with the mineralized P (Min P), assuming that the best-fitted extractants could predict the transformations of P in soils. Min P was estimated by the difference between organic P at pre-sowing stage (0th day) and organic P at the harvesting stage (63rd day). Secondly, we studied the relationship between P derived by different methods with plant parameters (Wünscher et al. 2013), such as P% in pod, pod yield, DMY, and P uptake of the French bean. Thirdly, we monitored the relationship between different method-estimated P and the most critical soil parameters (Wünscher et al. 2013) like soil pH and organic carbon (OC), which are very sensitive to the changes in the soil system due to the alteration in management practices. Therefore, a nutrient extraction approach that can accurately forecast these changes and maintain a good relationship with plant parameters may be considered the most suitable. Fourthly, we calculated a ratio between the highest and lowest extracted P values by different methods for the treatments (excluding CK) to understand the sensitivity of any procedure toward the changes in inputs. It was expected that by determining varying amounts of P from diverse nutrient sources, a potential extraction method should be well sensitive to the variations of organic inputs.

## 2.5 Statistical Procedures

One-way analysis of variance (ANOVA) was carried out to understand the variations in the data that were acquired.

**Table 2** Details of the extraction methods used in the experiment

| Methods                           | Composition  | Soil:extractant | pH  | Extraction process  | Reference   |
|-----------------------------------|--|-----------------|-----|---|---|
| Basic EDTA (BEDTA)                | 0.5 M sodium hydroxide (NaOH) + 0.5 M ethylenediamine tetra-acetic acid (EDTA) | 01:25           | >8  | Manual shaking for 5 min and then refluxing for 2h and then centrifuge for 60 min at 1500 rpm | Bowman and Moir (1993) (modified by Chakraborty and Saha 2017 and Sahoo 2017) |
| Potassium Carbonate ( $K_2CO_3$ ) | 1% $K_2CO_3$   | 01:100          | >8  | Heating for 1 h at a temperature 85°C   | Das 1930 and Hockensmith et al. (1933) (modified by Whitney and Gardner 1936) |
| Citric acid (CA)                  | 1% CA  | 01:100          | 4.5 | Shaking for 2 h then incubated for 12h at room temperature, and then aging shaking for 30 min | Dyer 1894 (modified Thompson 1995)  |
| 2-Ketogluconic acid (2-KGA)       | 0.5 M 2-KGA  | 01:100          | 4.5 | Shaking for 2 h then incubated for 12h at room temperature, and then aging shaking for 30 min | Modification of the method used by Korndorfer et al. (1995)                   |
| Olsen method                      | 0.5 M sodium bicarbonate ( $NaHCO_3$ )   | 01:20           | 8.5 | Shaking for 30 min with activated charcoal  | Olsen et al. (1954)   |

In addition, Duncan's multiple range test (DMRT) was performed to analyze significant differences among the P extracted by various methods at 5% probability levels ( $p \leq 0.05$ ) (Gomez and Gomez 1984). Finally, Pearson's correlation matrix and regression equations were generated using SPSS version 20.0 software (Statistical Package for Social Science) at 1% and 5% probability levels to understand the relation between different methods estimated P with the plant and soil parameters. Furthermore, a biplot analysis was performed (using XLSTAT software) to evaluate the variance of regression factor scoring and contribution of different extractants in plant P nutrition and their prediction of changes in soil characteristics (pH and OC) in order to obtain the final rank for different extractants (Batabyal et al. 2017; Mukherjee et al. 2021).

### 3 Results

#### 3.1 P Derived by Different Extraction Methods

There were considerable discrepancies between the methods employed to extract P from soils. Compared to the  $K_2CO_3$  extractant, the BEDTA estimated a substantially more enormous amount of organic P. On the other hand, CA was capable of extracting more P among the organic acids experienced. In comparison to the traditional Olsen approach,

CA has retrieved more P (Table 3). On average, BEDTA extracted ( $291.50 \text{ kg P ha}^{-1}$ ) a 23% higher organic P than the  $K_2CO_3$  estimated P ( $223.92 \text{ Kg P ha}^{-1}$ ). CA-derived P ( $71.15 \text{ Kg P ha}^{-1}$ ) was 9% and 21% higher than Olsen, and 2-KGA extracted P (average  $64.57 \text{ Kg P ha}^{-1}$  and  $55.65 \text{ Kg P ha}^{-1}$  were estimated by Olsen and 2-KGA respectively) (Table 3). Results also revealed that organically treated soils had more significant P content than inorganically fertilized soil (IF) except for Olsen and 2-KGA extraction methods, which raises doubt on the acceptance of these two methods for the organic system. Regardless of extraction methods, control plots had the least P content.

#### 3.2 Min P and Its Relationship with the Plant Parameters and Different Method-Derived P

After 63 days of the field investigation of the French bean, Min P was measured (Fig. 1). As expected, chemical fertilizers were more readily mineralized compared to organic sources. In contrast, the control field showed the least mineralization potential (Fig. 1).

Regarding the relationship between Min P and plant parameters, it was discovered that they had an excellent association ( $R^2=0.753^{**}$ ,  $R^2=0.649^*$ ,  $R^2=0.654^{**}$ , and  $R^2=0.694^{**}$  for Min P with P % in the pod, pod yield, DMY, and P uptake respectively) (Fig. 2a, b, c, d). A connection between Min P and plant characteristics supports the role

**Table 3** P estimated ( $\text{kg ha}^{-1}$ ) by different methods from soils receiving different organic sources

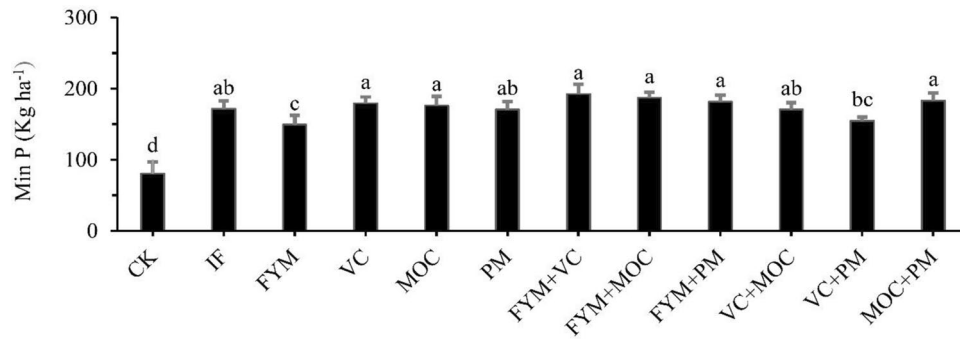
| Source  | Different methods derived P |                       |                      |                       |                        |
|---------|-----------------------------|-----------------------|----------------------|-----------------------|------------------------|
|         | BEDTA P                     | $K_2CO_3$ P           | CA P                 | 2-KGA P               | Olsen P                |
| CK      | 184.72 $\pm$ 15.45 g        | 114.57 $\pm$ 6.44 f   | 34.33 $\pm$ 5.91 f   | 25.11 $\pm$ 7.00 d    | 27.35 $\pm$ 9.11 e     |
| IF      | 252.50 $\pm$ 8.58 f         | 188.98 $\pm$ 12.87 e  | 58.30 $\pm$ 3.83 e   | 54.30 $\pm$ 8.68 bc   | 55.39 $\pm$ 12.04 cd   |
| FYM     | 313.30 $\pm$ 10.62 bc       | 257.50 $\pm$ 10.89 a  | 67.15 $\pm$ 3.23 cde | 50.21 $\pm$ 9.85 c    | 54.35 $\pm$ 9.71 d     |
| VC      | 327.03 $\pm$ 8.78 ab        | 235.68 $\pm$ 7.23 bcd | 80.08 $\pm$ 10.05 ab | 62.86 $\pm$ 8.73 abc  | 75.93 $\pm$ 5.79 ab    |
| MOC     | 307.70 $\pm$ 6.37 bc        | 250.65 $\pm$ 15.08 ab | 71.20 $\pm$ 3.98 bcd | 53.66 $\pm$ 12.57 bc  | 62.48 $\pm$ 8.93 abcd  |
| PM      | 326.53 $\pm$ 11.60 ab       | 262.92 $\pm$ 11.44 a  | 75.76 $\pm$ 8.82 bc  | 69.49 $\pm$ 9.38 ab   | 70.01 $\pm$ 9.35 abcd  |
| FYM+VC  | 308.03 $\pm$ 11.97 bc       | 236.65 $\pm$ 9.08 bc  | 87.59 $\pm$ 6.57 a   | 56.23 $\pm$ 9.38 bc   | 79.92 $\pm$ 11.83 a    |
| FYM+MOC | 294.89 $\pm$ 9.20 cd        | 223.73 $\pm$ 10.88 cd | 80.60 $\pm$ 2.55 ab  | 63.15 $\pm$ 10.73 abc | 75.79 $\pm$ 8.20 ab    |
| FYM+PM  | 267.93 $\pm$ 11.94 ef       | 243.62 $\pm$ 11.38 ab | 80.04 $\pm$ 4.85 ab  | 55.79 $\pm$ 8.06 bc   | 74.16 $\pm$ 5.27 ab    |
| VC+MOC  | 278.00 $\pm$ 11.33 de       | 217.59 $\pm$ 10.71 d  | 75.44 $\pm$ 4.50 bc  | 53.32 $\pm$ 6.31 bc   | 60.05 $\pm$ 7.66 bcd   |
| VC+PM   | 335.04 $\pm$ 9.03 a         | 255.85 $\pm$ 7.70 a   | 62.56 $\pm$ 6.32 de  | 74.07 $\pm$ 9.36 a    | 72.24 $\pm$ 7.95 abc   |
| MOC+PM  | 302.36 $\pm$ 8.53 c         | 199.26 $\pm$ 7.60 e   | 80.79 $\pm$ 4.11 ab  | 49.66 $\pm$ 9.22 c    | 67.12 $\pm$ 10.73 abcd |
| Mean    | 291.50                      | 223.92                | 71.15                | 55.65                 | 64.57                  |

P for phosphorus, BEDTA P = basic EDTA (0.5 M NaOH + 0.5 M EDTA) estimated P,  $K_2CO_3$  P = 1% potassium carbonate estimated P, CA P = 1% citric acid estimated P, 2-KGA P = 0.5 M 2-ketogluconic acid estimated P, Olsen P = 0.5 M  $NaHCO_3$  estimated P

FYM for farmyard manure, VC for vermicompost, MOC for mustard oil cake, and PM for poultry manure. In the case of CK (Control), no fertilizers were used, whereas IF for inorganic fertilizers where urea, di-ammonium phosphate (DAP), and muriate of potash (MOP) were applied for nitrogen (N), phosphorus (P), and potassium (K) respectively

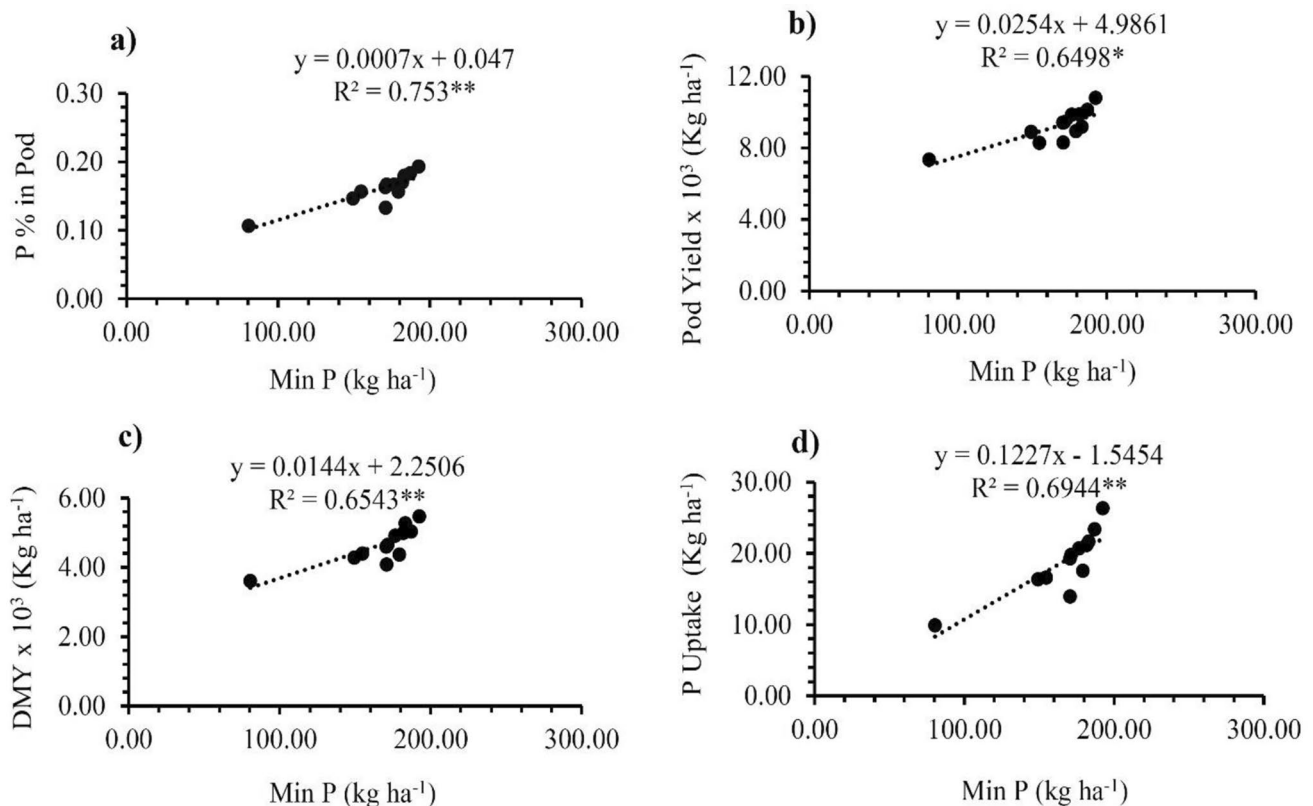
Duncan's multiple range test ( $p = 0.05$ ) showed that values in a column with various lowercase letters are significantly different. The value after  $\pm$  sign denoted the standard deviation of the mean





**Fig. 1** Phosphorus mineralization ( $\text{kg ha}^{-1}$ ) in soils receiving different nutrient sources. P for phosphorus, Min P for mineralized P, FYM for farmyard manure, VC for vermicompost, MOC for mustard oil cake, and PM for poultry manure. In the case of CK (Control), no fertilizers were used, whereas IF for inorganic fertilizers where urea, di-ammonium phosphate (DAP), and muriate of potash (MOP)

were applied for nitrogen (N), phosphorus (P), and potassium (K) respectively. Using Duncan's multiple range test ( $p = 0.05$ ), columns labeled with distinct lowercase letters indicate significantly different amounts of Min P. The error bar showed the standard deviation of mean



**Fig. 2** Relationship of Min P with **a** P % in pod, **b** pod yield, **c** DMY, and **d** P uptake by French bean crop. P for phosphorus, Min P = mineralized P, P % in pod = percentage of P present in pod, DMY = dry

matter yield, P uptake = P uptake by plant.  $R^2$  indicated the coefficient of determination. \* and \*\* indicated significant association at 0.05 and 0.01 levels of confidence respectively

of labile organic pools for crop P availability. On the other hand, while examining the relationship between P estimated by various methods and Min P, the CA-P and the Olsen

P exhibited a significant relation ( $R^2 = 0.854^{**}$  and  $R^2 = 0.789^{**}$ ) with Min P (Table 4). However, the remaining methods extracted P did not significantly relate to Min P.

**Table 4** Relation between Min P with different methods derived P

| P estimated by different methods | Regression equation with Min P                           | $R^2$ value |
|----------------------------------|--|-------------|
| BEDTA P                          | BEDTA P = 0.9351 Min P + 135.87                          | 0.445       |
| K <sub>2</sub> CO <sub>3</sub> P | K <sub>2</sub> CO <sub>3</sub> P = 0.9268 Min P + 69.668 | 0.444       |
| CA P                             | CA P = 0.4448 Min P + 2.8796                             | 0.854**     |
| 2-KGA P                          | 2-KGA P = 0.2754 Min P + 9.8215                          | 0.449       |
| Olsen P                          | Olsen P = 0.429 Min P + 6.8356                           | 0.789**     |

P for phosphors, Min P = mineralized P, BEDTA P = basic EDTA (0.5 M NaOH + 0.5 M EDTA) estimated P, K<sub>2</sub>CO<sub>3</sub> P = 1% potassium carbonate estimated P, CA P = 1% citric acid estimated P, 2-KGA P = 0.5 M 2-ketogluconic acid estimated P, Olsen P = 0.5 M NaHCO<sub>3</sub> estimated P

\* and \*\* indicated significant linear regression relationships at 0.05 and 0.01 levels of confidence respectively, and ns represented non-significant data

### 3.3 Relationship of P Estimated by Different Methods with Plant Parameters

The capacity of different extractants to acquire P and their differential contributions to plant nutrition and yield were investigated using Pearson's correlation analysis (Table 5). Although the degree of the relationship was varied, both CA and Olsen methods demonstrated a significant positive association with P % in the pod, pod yield, DMY, and P uptake of French bean. When compared to Olsen method ( $r = 0.713^{**}$ ,  $r = 0.634^*$ ,  $r = 0.666^*$ , and  $r = 0.693^*$  for P % in pod, pod yield, DMY, and P uptake respectively), CA substantially correlated more favorably with plant parameters ( $r = 0.732^{**}$ ,  $r = 0.742^{**}$ ,  $r = 0.754^{**}$ , and  $r = 0.765^{**}$  for P % in pod, pod yield, DMY, and P uptake respectively). Other potential extractants, however, had no association with plant characteristics.

**Table 5** P estimated by different methods and their relationship with plant parameters

| Different methods derived P      | Plant parameters |  |  |                                 |
|----------------------------------|------------------|--|--|---------------------------------|
|                                  | P % in pod       | Pod yield $\times 10^3$ (kg ha <sup>-1</sup> ) | DMY $\times 10^3$ (kg ha <sup>-1</sup> ) | P uptake (kg ha <sup>-1</sup> ) |
| BEDTA P                          | ns (0.427)       | ns (0.284)                                     | ns (0.334)                               | ns (0.343)                      |
| K <sub>2</sub> CO <sub>3</sub> P | ns (0.389)       | ns (0.390)                                     | ns (0.322)                               | ns (0.352)                      |
| CA P                             | 0.732**          | 0.742**  | 0.754**                                  | 0.765**                         |
| 2-KGA P                          | ns (0.393)       | ns (0.261)                                     | ns (0.261)                               | ns (0.312)                      |
| Olsen P                          | 0.713**          | 0.634*   | 0.666*                                   | 0.693*                          |

P for phosphors, BEDTA P = basic EDTA (0.5 M NaOH + 0.5 M EDTA) estimated P, K<sub>2</sub>CO<sub>3</sub> P = 1% potassium carbonate estimated P, CA P = 1% citric acid estimated P, 2-KGA P = 0.5 M 2-ketogluconic acid estimated P, Olsen P = 0.5 M NaHCO<sub>3</sub> estimated P, whereas P % in pod = percentage of P present in pod, DMY = dry matter yield, P uptake = P uptake by plant

\* and \*\* indicated significant correlation at 0.05 and 0.01 levels of confidence respectively, and ns represented non-significant data

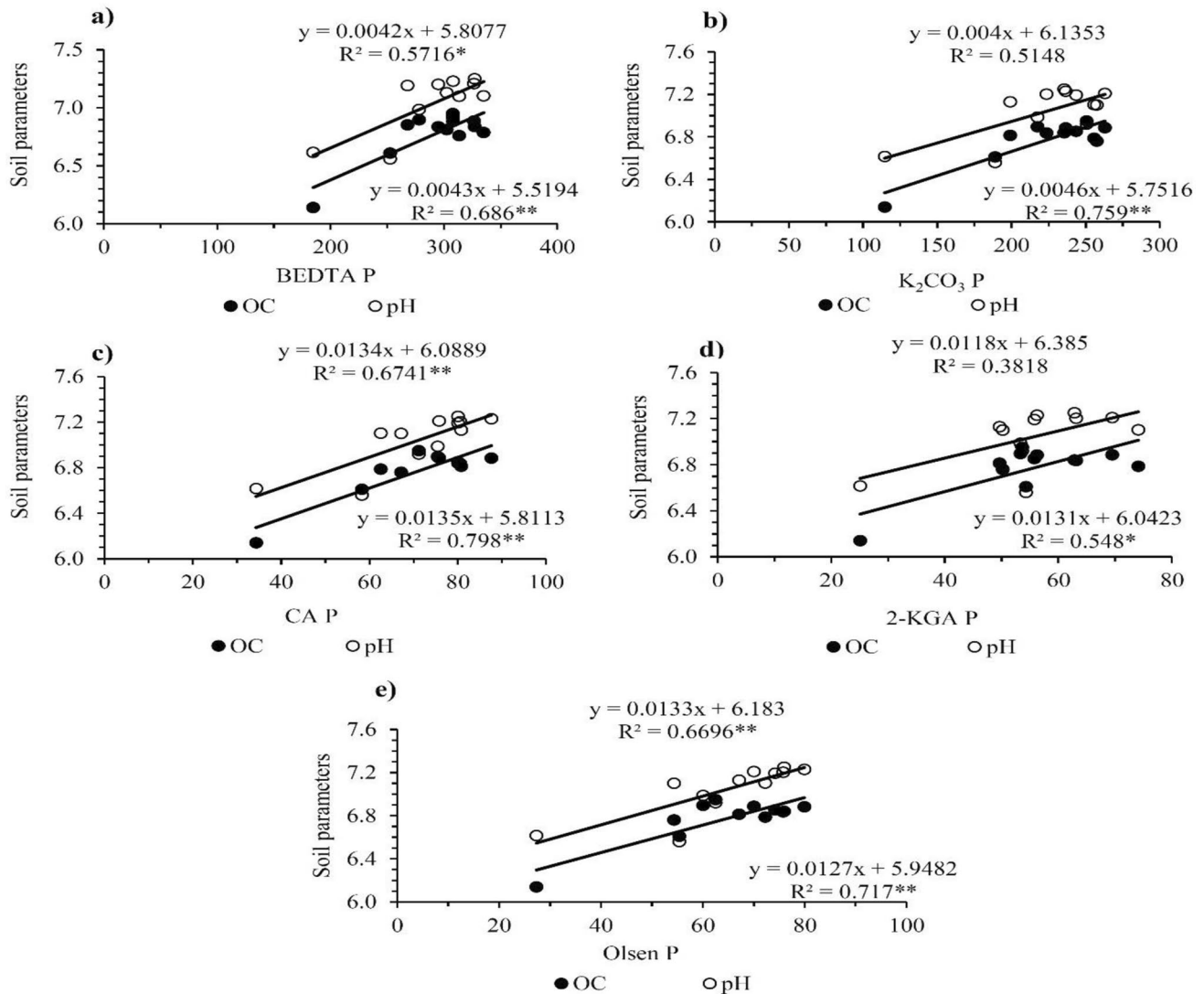
### 3.4 The Relationship Between Essential Soil Characteristics and P Derived from Different Methods

Extracting agents extracted variable levels of P from soils that absorbed a variety of organic substances during the production of French bean. Different fertilizers having distinct chemical composition sources can alter soil physiochemical features. Therefore, a nutrient extraction approach that can accurately forecast these changes while maintaining a significant relationship with plant parameters may be considered a suitable nutrient estimation method. In this scenario, the Olsen and CA extraction procedures had a robust association with soil OC and pH (Fig. 3). The CA extraction method exhibited  $R^2=0.798^{**}$  for OC and  $R^2=0.674^{**}$  for pH, whereas the Olsen method discovered  $R^2=0.717^{**}$  and  $R^2=0.669^{**}$  for OC and soil pH respectively.

### 3.5 Evaluation of the P Extraction Methods Using Principal Component Analysis (PCA) and Rank-Sum Scoring

The effectiveness of numerous extractants toward potentially available P in an organic production system was also investigated using principal component analysis (PCA), a multivariate statistical approach (Mukherjee et al. 2021).

A PCA using a biplot model (Fig. 4) revealed that the P retrieved by the various methods differed significantly. Results showed a more significant association of CA-extracted P with the plant (P %, P uptake, pod yield, and DMY) and soil attributes (OC and pH) as compared to the Olsen method (Fig. 4). P derived by other methods did not show any relation with plant parameters.



**Fig. 3** Soil parameters (OC and pH) and their association with **a** BEDTA P, **b**  $K_2CO_3$  P, **c** CA P, **d** 2-KGA P, and **e** Olsen P respectively. P for phosphorus, BEDTA P = basic EDTA (0.5 M NaOH + 0.5 M EDTA) estimated P,  $K_2CO_3$  P = 1% potassium carbonate estimated P, CA P = 1% citric acid estimated P, 2-KGA P = 0.5 M

2-ketogluconic acid estimated P, Olsen P = 0.5 M  $NaHCO_3$  estimated P, and OC = organic carbon.  $R^2$  indicated the coefficient of determination. \* and \*\* indicated significant association at 0.05 and 0.01 levels of confidence respectively

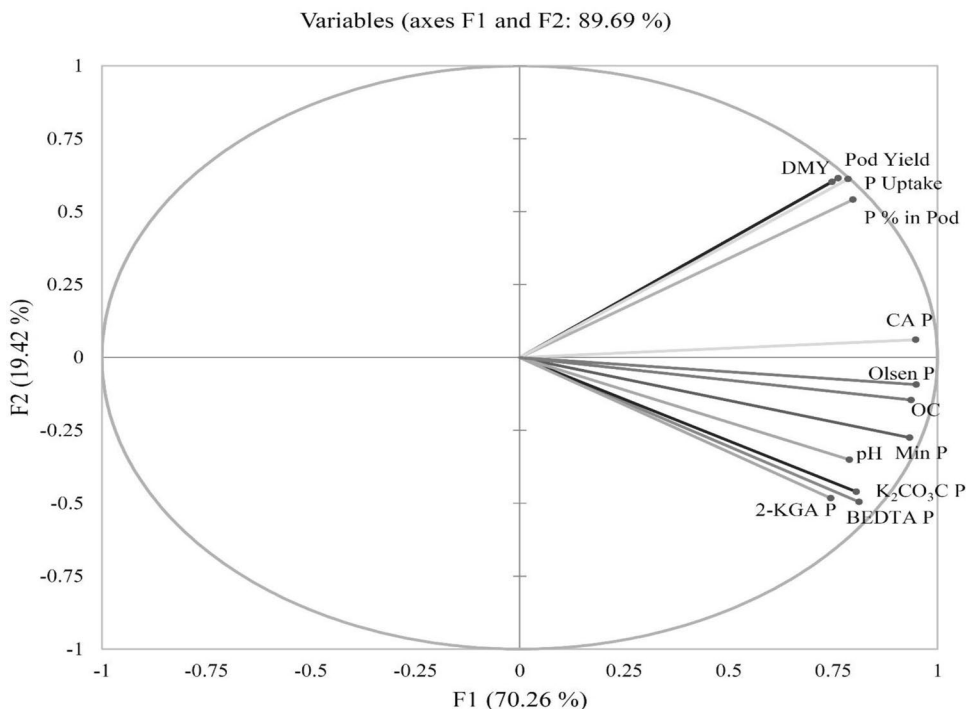
PCA result exhibited that the first two components within the variables explained 90% (Fig. 4) of the overall variation in P extracted by various methods and their relation with plant and soil parameters, with principal component 1 (PC1) accounting for 70% of the total variance and showed a significant loading on each variable. Furthermore, the principal component eigenvectors scoring (Table 6) revealed that the P estimated by CA came in first (lowest rank-sum score: 4), followed by the Olsen technique.

### 3.6 Estimation of Sensitivity of an Extraction Procedure Toward Different Treatments (Fertilizers Input)

A reliable extraction technique should detect little input variations by extracting varying amounts of P for a diverse range of treatments. For this, we calculated a ratio between the highest and lowest extracted P values by different methods for the treatments (excluding CK) to understand the sensitivity of any procedure toward the changes in inputs.



**Fig. 4** Evaluation of different method-derived P for explaining crop performance and soil parameter changes due to intervention imposed by principal component analysis (PCA)-biplot. P for phosphors, BEDTA P = basic EDTA (0.5 M NaOH + 0.5 M EDTA) estimated P, K<sub>2</sub>CO<sub>3</sub> P = 1% potassium carbonate estimated P, CA P = 1% citric acid estimated P, 2-KGA P = 0.5 M 2-ketogluconic acid estimated P, Olsen P = 0.5 M NaHCO<sub>3</sub> estimated P, OC = organic carbon, Min P = mineralized P, DMY = dry matter yield, P uptake = P uptake by plant, P % in pod = percentage of P present in pod. F1 (70.26%) and F2 (19.42%) represent cumulative variability percentage at F1 and F2 factor loadings



**Table 6** Evaluation and ranking of different phosphorous estimation methods by principal component analysis (PCA)

| Different methods derived P      | Eigenvectors |         |         | Regression factor Scoring |    |    | Rank-Sum | Rank |
|----------------------------------|--------------|---------|---------|---------------------------|----|----|----------|------|
|                                  | PC1          | PC2     | PC3     | F1                        | F2 | F3 |          |      |
| BEDTA P                          | 0.2798       | -0.3244 | -0.1505 | 3                         | 5  | 3  | 11       | 3    |
| K <sub>2</sub> CO <sub>3</sub> P | 0.2776       | -0.3016 | -0.2356 | 4                         | 3  | 4  | 11       | 3    |
| CA P                             | 0.3265       | 0.0398  | 0.3498  | 2                         | 1  | 1  | 4        | 1    |
| 2-KGA P                          | 0.2565       | -0.3160 | -0.4955 | 5                         | 4  | 5  | 14       | 4    |
| Olsen P                          | 0.3269       | -0.0608 | 0.0182  | 1                         | 2  | 2  | 5        | 2    |

P for phosphors, BEDTA P = basic EDTA (0.5 M NaOH + 0.5 M EDTA) estimated P, K<sub>2</sub>CO<sub>3</sub> P = 1% potassium carbonate estimated P, CA P = 1% citric acid estimated P, 2-KGA P = 0.5 M 2-ketogluconic acid estimated P, Olsen P = 0.5 M NaHCO<sub>3</sub> estimated P

The methods were ordered based on the values of each Eigenvector scored by the regression component. The first place went to the component with the highest value (factor scoring: 1), and the last place went to the component with the lowest value (factor scoring: 5). The rank-sum for each examined method was created by adding the factor scores together. The most effective strategy was determined to have the lowest rank-sum score (rank: 1)

Table 7 depicts that, in the case of CA, the ratio value was 1.50, followed by 1.49 for 2-KGA > 1.47 for Olsen method > 1.39 for K<sub>2</sub>CO<sub>3</sub> > 1.33 for BEDTA.

### 4 Discussion

Methods having different chemical mechanisms to extract soil P under an organic system were employed in the present experiment to investigate the suitability to relate with plant response and predict the changes of soil attributes. We have chosen some indices in the material and method section to

find out the most reliable method in our experiment. Each index has been discussed in this section.

Mineralization of organic P is a prime factor for P availability in the plants (Achat et al. 2010; Arenberg and Arai 2018; Bünemann et al. 2007; Li et al. 2021), and remarkably it is more applicable to the organic production system. The present investigation revealed that Min P could notably predict different plant parameters, supported by the study of Adeptu and Corey (1976). Eid 1954 also suggested that Min P plays a significant role in P availability for the plant. Furthermore, both Olsen and CA showed a significant regression relation with the Min P, which can strongly claim their

**Table 7** Estimation of sensitivity of an extraction procedure toward different treatments

| Methods                        | The highest amount of P estimation |                              | The Lowest amount of P estimation |                              | Ratio (highest/lowest) |
|--------------------------------|------------------------------------|------------------------------|-----------------------------------|------------------------------|------------------------|
|                                | Treatments                         | Value (kg ha <sup>-1</sup> ) | Treatments                        | Value (kg ha <sup>-1</sup> ) |                        |
| BEDTA                          | VC+PM                              | 335.04                       | IF                                | 252.50                       | 1.33                   |
| K <sub>2</sub> CO <sub>3</sub> | PM                                 | 262.92                       | IF                                | 188.98                       | 1.39                   |
| CA                             | FYM+VC                             | 87.59                        | IF                                | 58.30                        | 1.50                   |
| 2-KGA                          | VC+PM                              | 74.07                        | MOC+PM                            | 49.66                        | 1.49                   |
| Olsen                          | FYM+VC                             | 79.92                        | FYM                               | 54.25                        | 1.47                   |

P for phosphorus, BEDTA = basic EDTA (0.5 M NaOH + 0.5 M EDTA), K<sub>2</sub>CO<sub>3</sub> = 1% potassium carbonate, CA = 1% citric acid, 2-KGA = 0.5 M 2-ketogluconic acid, Olsen method = 0.5 M NaHCO<sub>3</sub>

FYM for farmyard manure, VC for vermicompost, MOC for mustard oil cake, and PM for poultry manure, whereas IF for inorganic fertilizers where urea, di-ammonium phosphate (DAP), and muriate of potash (MOP) were applied for nitrogen (N), phosphorus (P), and potassium (K) respectively

applicability as a simplified protocol for determining P under the organic system. However, the organic nutrients used in this experiment had different Min P, possibly due to their varied chemical nature (Eghball et al. 2002) and C:N ratio (Mukherjee et al. 2021).

Our experiment investigated various P extraction methods to find the most promising one for an organic production system. BEDTA and K<sub>2</sub>CO<sub>3</sub> extractants were used to determine the organic P content in soil and their reliability in explaining the plant and soil parameters. The result revealed that irrespective of treatments, BEDTA extractant was far more capable of extracting organic P than K<sub>2</sub>CO<sub>3</sub>, as BEDTA-P was ~23% higher than K<sub>2</sub>CO<sub>3</sub>-P. The robustness of BEDTA to extract organic P can be explained by two mechanisms: first, NaOH produces electrostatic repulsion by raising the negative charge of organic components and substituting sodium for polyvalent bridging cations (Turner et al. 2005), and second, forming a metal-EDTA chelate with solubilization of complex polymerized humic compound (Cade-Menun et al. 2000; Chakraborty and Saha 2017; Mukherjee et al. 2021). This result was consistent with the outcome of Harrap (1963), where sodium EDTA extracted a higher organic P than other extractants. Furthermore, Cade-Menun and Preston (1996), Soinnie et al. (2011), and Staal and Petersen (2019) have also suggested NaOH+EDTA as an excellent extractant for estimating organic P. On the other hand, Nakamura et al. (2019) have reported the efficiency of K<sub>2</sub>CO<sub>3</sub> in P availability. But, unlike BEDTA, K<sub>2</sub>CO<sub>3</sub> mainly extracts easily decomposable chemical molecules such as Mg-phytate (Eid 1954; Jackman 1949), which might be the main reason for lower organic P extraction.

However, both BEDTA and K<sub>2</sub>CO<sub>3</sub> techniques might not take into account all the variables affecting the crop's availability of P as these inorganic extractants did not correlate with plant parameters. This statement is supported by the study of Fixen and Grove (1990). In addition, the soil and plant data originate from a field trial, which is essentially a heterogeneous system that may

impact the correlation study. Finally, unlike organic N, dissolved organic P cannot be directly accommodated by organisms other than marine bacteria (Dyhrman et al. 2006). According to Mukherjee et al. (2021), the chemical characteristics of extracted organic pools are likely to be the determining factor in plant utilizable nutrients rather than the amount of organic pool itself. The limitation is whether organic P recovered using these procedures can be utilized to assess the relevance in an organic production system.

The exudation of organic acids from plants, microbes, and decomposed organic matter can enhance P availability (Adeleke et al. 2017; Gypser and Freese 2020; Hou et al. 2018; Jones 1998). Additionally, organic acids can dissolve P either through ligand exchange or ligand-promoted dissolution (Andrino et al. 2021; Oburger et al. 2011). This experiment used two organic acids, i.e., CA and 2-KGA. CA is a primary organic acid synthesized during the decomposition of organic matter (Hayes et al. 2000). There are many studies in which CA appears to be an appropriate extractant for simulating P mobilization (Clarholm et al. 2015; Fäth et al. 2019; Manghabati et al. 2018; Vaughan et al. 1993; Wei et al. 2010). On the other hand, a high concentration of 2-KGA (20% of the rhizospheric product) is found in the rhizosphere zone, produced through the microbial action of glucose. Also, ~38% of the rhizosphere product is glucose, so the presence of 2-KGA in the organic system might play a significant role in P availability (Moghimi 1977). Around 22% higher P was extracted using CA than 2-KGA, possibly because of the carboxylic acid group present in CA has a higher chelation potential (Ivanova et al. 2006) than keto groups in 2-KGA. The chelation mechanism of CA, where it forms complexes, particularly with the metal ions like Fe<sup>3+</sup>, Al<sup>3+</sup>, or Ca<sup>2+</sup>, preventing the soil particles from absorbing P and subsequently releasing P, might be responsible for an excellent P extraction (Barrow et al. 2018; Menezes-Blackburn et al. 2021; Wang and Lambers 2020). Furthermore, CA may increase phosphatase enzyme activity and microbial

activity, subsequently increasing the rate at which organic P dissolves in soil (Wang and Lambers 2020; Wei et al. 2010).

On the other hand, the effectiveness of the Olsen method in determining the readily available P is well recognized (Hartmann et al. 2019), where  $\text{NaHCO}_3$  decreases the activity of  $\text{Ca}^{++}$  in solution through  $\text{CaCO}_3$  precipitation and  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$  by the formation of Al and Fe oxyhydroxides which promotes P availability (Schoenau and O'Halloran 2008; Sims 2000). However, the Olsen method determined a slightly lower amount of P, which might be due to poor extraction of some mineral compound-bound P by  $\text{NaHCO}_3$  compared to CA (Gerke 1992; Maguire et al. 2005). Generally, Olsen method focuses on inorganic P but neglects the organic P (Recena et al. 2015), contributing a considerable part to P availability that CA can measure (Wei et al. 2010).

Pearson's correlation study reflects that CA-extracted P had the strongest correlation with all the parameters, followed by  $\text{NaHCO}_3$ -extracted P. However, no other methods showed any correlation with plant parameters. A similar trend was noticed for soil parameters. In this context, CA is qualified for the most suitable method for P estimation under an organic production system in a specified soil type. Furthermore, the principal component analysis and sensitivity study also established the suitability of CA method.

## 5 Conclusion

Based on our analysis, citric acid estimated phosphorus excelled over other methods concerning crop response (P % in pod, pod yield, dry matter yield, and P uptake) and predicting changes in soil parameters such as pH and organic carbon. So, the results of this experiment substantially support our hypothesis that conventional methods of phosphorus determination are not equally applicable to an organic system. Therefore, using citric acid as a new approach to detect plant phosphorus availability can assist organic certification agencies and soil testing laboratories in their evaluation strategy and policy-making for phosphorus management under the organic farming system, particularly in the new alluvial soil. Furthermore, the approach utilized in our study offers organic farmers a practice-oriented method that can reduce phosphorus risk from the organic field and better resource management to flourish organic cultivation in the coming future to make organic farming a profitable venture. This method will be applicable to countries having vast alluvial soil, such as India. However, it should be validated under different agroecological conditions with diverse cropping systems and seasons to test the method's robustness.

**Acknowledgements** The authors acknowledge that the STCRC (Soil Test Crop Response Correlation) under AICRP (All India Coordinated Research Project) has provided the necessary funds and facilities during the research program.

**Author Contributions** Buddhadev Sarkar did the research, wrote the manuscript, and provided all the data. Niharendu Saha guided me throughout the research program. Siddhartha Mukherjee helped to design the paper, Jit Sankar Basak helped to conduct the statistical analysis, and Suchandra Dutta assisted in writing the article in proper English. Dipankar Dey and Pradip Dey helped to formulate the research methodology. All authors confirm that the author list is accurate in both content and order and that no modification to the author list can be made without the formal approval of the Editor-in-Chief. In addition, all authors will accept the Editor-in-Chief's decisions over accepting or rejecting the manuscript for publishing in the Journal of Soil Science and Plant Nutrition. This research has not been submitted for publication, nor has it been published in whole or in part elsewhere. However, we attest that all authors listed on the title page have contributed significantly, have read the manuscript, confirmed the validity and legitimacy of the data and its interpretation, and agree to its submission to the esteemed Journal of Soil Science and Plant Nutrition.

**Funding** Funding was received from the All India Coordinated Research Project (AICRP) on Soil Test Crop Response (STCR).

## Declarations

**Conflict of Interest** The authors declare no competing interests.

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