



Comparative Analysis of Soil Phosphorus Determination Methods and Their Correlation with Plant Phosphorus in Standing Wheat Crops

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ABSTRACT

This study compared the accuracy of various soil phosphorus assessment methods to measure the soil's ability to supply plants with phosphorus over a brief period in the field. Twenty individual soil samples were collected from a standing wheat (*Triticum aestivum L*) crop at depths ranging from zero to twenty centimeters. An equivalent plant spike sample was also procured from the soil sampling fields. In comparison to the wet acid digestion method used to detect phosphorus in plants, several methods were utilized to assess phosphorus in the soil, including resin extractable phosphorus, AB-DTPA extractable phosphorus, NaHCO₃ extractable phosphorus, water-soluble phosphorus in suspension, and paste. The levels of variation and deficiency of phosphorus, which were found by different methods followed different patterns as shown by the fact that, AB-DTPA method finds phosphorus deficiency in 20% of samples while on the other hand, Olsen method finds phosphorus deficiency in 80% of samples. Even with such a small sampling area, none of the procedures showed a significant correlation with any other method that might account for uneven variation among the samples when determined by distinct procedures. However, corrections were observed to a certain degree between ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) extractable and resin, as well as between other procedures and the plant P scale. Both resin and ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) had a strong relationship with plant phosphorus, with the former showing a significant correlation of 0.48 and 0.21, respectively. Hence Resin and AB-DTPA methods are recommended for the determination of phosphorus under certain soil and plant conditions.

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Introduction

Wheat (*Triticum aestivum L.*) is prominent among the grains of the Poaceae family and is cultivated globally on a large scale. In Pakistan specifically, wheat is widely grown in various regions, especially in regions of Punjab. Wheat has always played a substantial role in the development of human civilization and is recognized as the most important staple grain all over the world. The grains of wheat are consumed directly or indirectly through human diets, while wheat straws serve as valuable fodder for animals. Most people over the globe consume wheat as a staple food and it provides 73% of the protein and calories they need. (Bashir et al., 2015).

Phosphorus serves an essential role as a micronutrient in sustaining life cycles. (Marschner, 2012; Ding et al., 2020; Hopkins, 2020). The availability of Phosphorus to the plant is reduced if the soil has high calcium content. A

significant portion of phosphorus fertilizer when applied to the soil tends to bind to the calcite surfaces. This makes the Phosphorus temporarily available to the plants. This can potentially lead to a decrease in crop yield (Saeed et al., 2021). To determine the availability of plant's phosphorus (P) in the soil, several soil chemical parameters such as pH and limestone concentration play a significant role (Fixen and Bruulsema, 2014; Lindsay, 2001). Calcareous soils frequently lack a sufficient supply of plant-available phosphorus (P). This occurs because of the reduced solubility, which is caused by fixation and sorption, (Lindsay, 2001; Hopkins et al., 2014; (Boukhalfa-Deraoui et al., 2015; Hopkins, 2020; Mihoub and Boukhalfa-Deraoui, 2014). Hence this low solubility of Phosphorus reduces the efficiency of P fertilizer (Jamal et al., 2018; Mihoub and Boukhalfa-Deraoui, 2014). Thus, most

calcareous soils require significant exogenous P replenishment to maintain stable yields. Soil testing serves the purpose of guiding effective and efficient soil nutrient management by utilizing the correlations between soil test results and crop responses to applied nutrients. It is essential to observe that not all available extractants have a standard calibration between the number of extractables.

Testing the soil is a typical practice for determining nutrient needs and assessing current nutrient levels. This enables more precise nutrient prescription and enhanced nutrient utilization efficiency. However, most of these techniques are narrow in scope, requiring additional steps and resources to determine the concentration of a single nutrient. As a result, they are impractical for use in large-scale soil testing laboratories. Because of their capacity to extract many nutrients at once, they need the rapid assessment of many samples. Multi-nutrient extractants provide a realistic choice in the face of these challenges. (SHRIVASTAV et al., 2023). Commonly the practice of prescribing nutrients is prevalent and is employed. Mostly this practice is based on soil analysis. To achieve the intended yields, it is necessary to determine the appropriate nutrients. For this, it is necessary to assess the nutrient availability of the soil. The accuracy of any technique used to estimate nutrient availability in soils is affected by soil characteristics such as organic matter concentration, temperature, and mineral composition. Soil test values for individual nutrients and crop yield may not be reliably predicted by simple correlation coefficients alone when there are variations in both the soil and the applied nutrients under field conditions. Therefore, multiple regression analyses, considering all the essential nutrients (N, P, and K), can be used as an alternate tool for assessing the efficacy of various soil test methods simultaneously by using R² values. The efficacy of several soil testing methods in practice has been assessed. Soil characteristics such as organic matter concentration, temperature, and mineral composition affects The accuracy of any technique, which is used to estimate nutrient availability in soils. (Velayuthan et al., 1984). The screening process plays a vital role in identifying the appropriate soil testing method (Mosi and Lakshminarayanan, 1985). Water-extractable P (WEP), calcium chloride-extractable P (CCEP), and Olsen-extractable P (OEP) can be used to extract the accessible P forms in soils, whereas total P can be seen as an indicator of the soil's Preserve and the accumulation of P owing to extensive P application. WEP and CCEP are used to assess P leaching from soils, while OEP is employed as an agronomic indicator. Since standard P extraction methods are thought to depict labile P fractions more accurately, it is important to do research into the P background level using these methods. In this study, we analyzed the relationship between plant phosphorus (P) levels and the characteristics of phosphorus (P) in the soil solution. The main goals of this study were focused on the wheat crop grown at the Research Farm in the Peshawar region of the University of Agriculture. Specifically, we aimed to investigate the connection between different forms of soil extractable phosphorus (AB- DTPA extractable P, Olsen P, water-soluble P, Resin extractable P, and paste extractable P) and the concentration of phosphorus in wheat plants.

Materials and methods

Soil and Plant Sample Collection

A study was conducted to maximize the determination and absorption of phosphorous (P) by plants to improve the yield and health of wheat. The experiment involved collecting samples from the Research Farm of the Agriculture University Peshawar during the spring season. Plant samples were obtained at the flowering i.e. the anthesis stage in wheat. The samples were subsequently dried for laboratory analysis. At each sampling site, 10 to 15 leaves were collected from the plants. Soil samples were also collected from a depth of 0-20 centimeters from three to four different sites at a single experimental location. These soil samples were mixed to create a composite soil sample, which was then combined with the plant samples from the respective site. Along with this, subsamples were taken at a variety of depths and then combined using stainless steel augers at each sampling site. These soil samples were then collected, sealed in labeled plastic bags and transported to a lab for testing. (Beygi and Jalali, 2018). Beygi and Jalali, (2018) conducted a study where they reported on the sampling and analysis of prevalent soil properties. Furthermore, GPS readings were recorded at each site for accurate location information. In the later processes a comparison was made between the plant and soil samples to assess the phosphorous concentration.

This comparison sought to ascertain the concentration of Soil-Solution-P in the leaves of wheat plants. The ultimate goal was to improve understanding and use of P-determination and absorption by plants, which would result in higher agricultural output and healthier plant characteristics.

Data Analysis

Several characteristics, including soil phosphorus (P) levels, were evaluated through a thorough examination of soil and plant samples in this study. Several methodologies were employed to ascertain the P levels, encompassing the AB-DTPA extractable P method proposed by (Havlin and Soltanpour, 1981), the Olsen P method as per Olsen (1954), water-soluble P determined using the paste extract technique outlined by Gardner, (1986), and resin-P based on Murphy and Riley's approach (1962). Additionally, the concentration of phosphorus in plant leaves was measured to complement the soil analysis. Water-soluble phosphorus content was measured by taking 1 gram of soil and vigorously mixing it with 5 millilitres of purified water for a duration of 30 minutes (Jalali et al., 2023). To calculate the Olsen Extractable Phosphorus (OEP), a soil sample weighing 1 gram was vigorously mixed with 20 milliliters of Olsen solution (0.5 M NaHCO₃) for a duration of 30 minutes (Olsen et al., 1982). The samples acquired using the aforementioned techniques underwent centrifugation and filtration. To analyse the total phosphorus content, 0.2 g of soil was subjected to furnace treatment at 550 °C for 1 hour. Following this, 25 ml of 1 N HCl was introduced, and the mixture was heated for 15 minutes (Andersen, 1976). Using a UV-visible spectrophotometer and the colorimetric method developed by (Murphy and Riley, 1962), the P concentration in each of the aforementioned extracts was determined.

Results

AB-DTPA extractable phosphorus

The soil's AB-DTPA extractable phosphorus (P) content ranges from 2.1 to 5.7 mg kg⁻¹, with an average value of 3.9 mg kg⁻¹. (Table.1) The standard deviation of the P content is low at 1.1, indicating a small variation among the samples. Among the samples analysed, 20% of the soils are deficient in P, while the remaining 80% have P levels in the low to medium range. When compared to the standard values in (Table. 2), there are insufficient P levels in any of the samples. The formation of complexes is responsible for P deficiency in calcareous soils.

$$\text{AB - DTPA P (mg kg}^{-1}\text{)} = \frac{\text{Readings} \times \text{voulme made}}{\text{Weight of soil}}$$

Olsen Extractable Phosphorus

Soil Olsen P levels varied from 4.2 to 11.2 mg kg⁻¹ (mean: 7.7 mg kg⁻¹) with a small range and standard deviation of 1.8. (Table. 1) Out of the 20 samples, 80% were deficient in P, while the remaining 20% had medium levels. No soil samples indicated toxicity or adequate P levels.

Water Soluble Phosphorus in Paste and Suspension

The soil's water-soluble phosphorus (P) content ranged from 0.7 to 1.3 mg kg⁻¹, with an average value of 1.1 mg kg⁻¹. (Table. 1) The variation in water-soluble P values was relatively small, with a standard deviation of 1.1. Additionally, the paste P content in the soil ranged from 0.1 to 0.3 mg L⁻¹, with a mean value of 0.2 mg L⁻¹. The paste P values also showed a narrow range of variation, with a standard deviation of 0.04

Resin Extractable Phosphorus (Bioavailable-Phosphorus)

The soil's Resin P levels varied from 3.9 to 9.0 mg L⁻¹ mg L⁻¹, averaging 5.0 mg L⁻¹. The range of Resin P values was relatively small, with a standard deviation of 1.5 (Table. 1).

Plant extractable Phosphorus

Plant P varied from 0.1% to 0.3%, averaging at 0.2% with a narrow range and a standard deviation of 0.05 (Table. 1).

Discussions

Extraction of P through AB-DTPA

The AB-DTPA extractable phosphorus (P) analysis of the soil shows a relatively narrow P content range, averaging 3.9 mg kg⁻¹ with a low standard deviation of 1.1. This suggests the consistent availability of P. However, 20% of the soils are deficient in P, which negatively impacts plant growth and soil fertility. None of the samples meet the sufficient P levels indicated in Table 2, highlighting the need to address this deficiency for sustainable agriculture and higher crop yield. The deficiency in calcareous soils is caused by complex formation due to high pH levels, resulting in P immobilization. Implementing soil management strategies such as adjusting pH, adding organic matter, and using P fertilizers can improve P availability and nutrient uptake. Similarly, increasing the levels of applied P enhances the water-soluble AB-DTPA extractable P. AB-DTPA extractable phosphorus (P) concentration levels rise during initial growth stages, but in subsequent periods, P values can increase due to fixation and other complexation processes occurring gradually (Griffith, 1983).

Table 1. Concentrations of soil and plant phosphorus as determined by different protocols

S. No.	AB-DTPA P	Olsen P	Paste P	WS P	Resin P	Plant P
	mg kg-1	mg kg-1	mg L-1	mg kg-1	mg kg-1	%
1	4.76	9.54	0.32	1.20	7.89	0.27
2	3.96	7.8	0.21	1.02	6.46	0.21
3	3.84	5.14	0.18	1.11	4.45	0.16
4	4.05	6.78	0.13	1.20	3.97	0.18
5	4.88	7.57	0.23	1.27	4.16	0.24
6	5.42	8.34	0.19	1.10	5.87	0.29
7	3.17	6.73	0.24	0.93	4.95	0.19
8	5.13	9.34	0.14	1.35	5.88	0.31
9	4.67	8.76	0.23	1.12	8.66	0.35
10	2.14	5.63	0.19	0.72	4.69	0.26
11	3.84	8.54	0.24	1.21	5.88	0.25
12	3.46	8.26	0.15	1.23	5.88	0.28
13	3.66	4.21	0.26	1.03	5.48	0.32
14	2.48	5.76	0.15	0.99	5.76	0.25
15	2.45	5.13	0.21	1.32	4.95	0.26
16	2.37	9.91	0.22	0.92	4.11	0.21
17	5.58	11.23	0.21	1.26	8.40	0.32
18	3.83	8.63	0.18	1.13	6.64	0.22
19	5.68	8.9	0.22	1.23	9.02	0.34
20	3	8.34	0.19	1.12	5.400	0.26
Min	2.1	4.2	0.1	0.7	3.9	0.1
Max	5.7	11.2	0.3	1.3	9.0	0.3
Mean	3.9	7.7	0.2	1.1	5.9	0.2
St. Deviation	1.1	1.8	0.04	1.1	1.5	0.05

Table 2. Criteria for P status (mg kg⁻¹) determined by different procedures

Methods	Low	Medium	High/Adequate
AB-DTPA ext. P	< 3.0	4-7	> 7
Olsen P	<10	10-15	>15

Source: Rashid et al. 1996.

Table 3. Value of R showing the correlation of P extraction procedures with each other and plant phosphorus

	WS	Paste P	Olsen P	Resin P	Plant P
AB-DTPA P	0.59	0.15	0.54	0.60	0.47
WS P	1.00	-0.07	0.34	0.29	0.29
Paste P	-	1.00	0.12	0.12	0.21
Olsen P	-	-	1.00	0.54	0.31
Resin P	-	-	-	-	0.70

Crop growth stages and increased nutrient uptake can deplete the soil's nutrient content. Phosphorus (P) applications of 90, 135, and 180 kg ha⁻¹ resulted in considerably greater extractable P concentrations than the control and 45 kg P₂O₅ ha⁻¹ at all phases of crop development. However, there were no significant differences between the three higher P levels. This lack of significant variation at higher P levels may be regardless of the crop's growth stage or phosphorus (P) levels, the AB-DTPA extractable P deficiency persisted, resulting in values below 3.0 mg kg⁻¹ when 180 kg P₂O₅ ha⁻¹ was administered (Rashid et al., 1988; Sharif, 1985). When phosphorus is added to highly calcareous soil, it transforms into phases that cannot be extracted (Khattak, 1996; Lindsay, 1979).

Extraction of P through Olsen

The soil samples analyzed revealed a range of Olsen P levels from 4.2 to 11.2 mg kg⁻¹, averaging 7.7 mg kg⁻¹. The narrow variation in Olsen P values (standard deviation of 1.8) indicated consistent phosphorus deficiency across the majority of the 20 samples. 80% of the samples were deficient in P, while the remaining 20% showed medium P levels. No soil samples exhibited P toxicity or adequate P levels. This P deficiency suggests a potential limitation on plant growth and development. To optimize agricultural productivity and nutrient uptake, addressing this issue through appropriate fertilization strategies is essential. Notably, excessive phosphorus application was not observed in any soil sample. However, closely monitoring P levels and adjusting fertilization practices is crucial to avoid potential environmental issues related to phosphorus runoff. In Mediterranean environments, (Matar et al., 1992) observed that Olsen P threshold values increased with greater aridity. This phenomenon was explained by the fact that P primarily moves to the roots through diffusion in the soil solution, which is hindered when the soil is dry. Additionally, reduced soil water content leads to higher ionic strength in the soil solution. This promotes P adsorption in soils when the pH exceeds a certain threshold, affecting the equilibrium between solid and water phases and decreasing P release from sorbent surfaces. Therefore, maintaining consistent soil moisture improves P utilization by crops, resulting in lower threshold values for fertilizer response. It is crucial to account for climatic conditions in each growing season.

Water-Soluble P in Paste and Suspension Extract

The water-soluble phosphorus content in the soil ranged from 0.7 to 1.3 mg kg⁻¹ (mean: 1.1 mg kg⁻¹) with a small variation. The paste phosphorus content ranged from 0.1 to 0.3 mg L⁻¹ (mean: 0.2 mg L⁻¹), also showing a narrow range of variation. Overall, both the water-soluble and paste phosphorus contents demonstrate consistent concentrations, indicating a stable and homogeneous distribution of phosphorus in the soil sample. Lata Verma and Marschner, (2013) stated that the decrease in water-soluble P in the soil at a later stage of incubation may be a result of the assimilation of readily available P (WSP) by microbes. Similar outcomes were observed in soils incubated with rock phosphate and organic matter for 75 days, where water-soluble P levels decreased significantly. Overall, the level of accessible phosphorus (P) initially decreased over the course of 28 days and then gradually rose during a later phase of the incubation period. This suggests that compost with RP charge releases P at a slow rate but over an extended duration. The decline in available P could be attributed to its temporary conversion into an unavailable form. Results like those reported by Bangar et al. (1985); Biswas and Narayanasamy, (2006) were seen after applying phosphorus-rich compost to soil.

Resin P

Resin P levels varied greatly amongst the sites tested, with concentrations ranging from 3.9 to 9.0 mg L⁻¹ (on average, 5.0 mg L⁻¹) of phosphorus. The variance in Resin P values was rather small, but it was still there, and it could have an impact on plant growth and nutrient availability. Resin P concentrations show a substantial amount of fluctuation, as indicated by a standard deviation value of 1.5. Soil type, land use practices, and environmental factors all have a role in the observed variation. Knowing this variance is critical for efficient soil management and nutrient planning since it paves the way for individualized fertilization techniques and precise interventions. A high association (R² = 0.86) was found between NaHCO₃-P and phosphorus uptake in ryegrass grown in pots (Sibbesen, 1978). For algae, it was an accurate indicator of phosphorus availability (Zhou et al., 2001). Ellis and Stanford (1988) discovered a strong (p 0.05) correlation between NaOH-P and NTA-P and the phosphorus availability in selenastrum. Fe oxide paper-P showed a strong association with the growth of Anabaena, Euglena,

Selena strum, and Ankistrodesmus ($p < 0.001$) (Sharpley, 1993). Anion resin-P also exhibited a high correlation ($R^2 = 0.90$) with P uptake in pot-grown ryegrass (Sibbesen, 1978), simulating the decrease of P at the surface of freshwater (Uusitalo et al., 2000).

P Concentration in plants Plant P concentrations in the dataset have an average value of 0.2%, indicating a relatively low concentration overall. The range of concentrations spans from 0.1% to 0.3%, suggesting variability but within a narrow range. A low standard deviation of 0.05 confirms the narrow range and indicates consistency and stability in the dataset. To understand the observed variation better, additional analysis and experiments could explore factors such as environmental conditions, nutrient availability, genetic variations, and other variables impacting P uptake and utilization by plants.

The Concentration of Plant P with Soil P Extracted By Different Methods

The correlations between different procedures for soil P extraction were examined. AB-DTPA showed significant correlations with Water-soluble P ($R = 0.59$), Olsen P ($R = 0.54$), and Resin P ($R = 0.60$), but had weak correlations with P determined in paste extract. However, the positive correlations observed in AB-DTPA were consistent with other procedures except for paste P. Paste P had low levels that couldn't be accurately determined and varied greatly among soil samples, unlike other procedures. water-soluble P did not show significant correlations with any other extraction method. Paste P also didn't show significant correlations with any method. Olsen P only

showed significance with Resin P. These findings indicate that the correlations between methods depend on soil types, fertilizer application history, total phosphorus content in the soil, and moisture content during sampling. (Table .3) Resin P demonstrated the strongest correlation with plant P ($r^2 = 0.484$), followed by AB-DTPA extractable P ($r^2 = 0.217$). These correlations are considered significant as the R-value exceeds 0.5. Other soil P extraction methods, such as water-soluble P ($r^2 = 0.083$), Olsen P ($r^2 = 0.098$), and Paste P ($r^2 = 0.043$), exhibited weaker and less significant correlations with plant P.(Figure 1 -5) showed the graphical presentation of different values of correlation of plant P with water-soluble P, AB-DTPA phosphorus, Olsen P, Resin P and soil paste P. The resin P and AB-DTPA methods were found to be the most suitable for determining phosphorus (P) levels in the tested soil and plant conditions. Different soil characteristics can influence the variability of these results, as observed by other researchers. For example, the correlation between total plant-available P and Olsen P was not significant, indicating that Olsen P is not a reliable indicator for managing fertilizer in the tested soils. In non-calcareous soils, various methods used to estimate available P showed significant correlations with plant uptake, particularly the Morgan and water-soluble estimates. In calcareous soils, the recommended method is AB-DTPA extraction. AB-DTPA extractable P levels showed a highly significant correlation with plant uptake of P. Compared to other methods like Colwell or resin, DGT (diffusive gradients in thin films) showed a better assessment of plant-available P concentrations in soil based on previous studies.

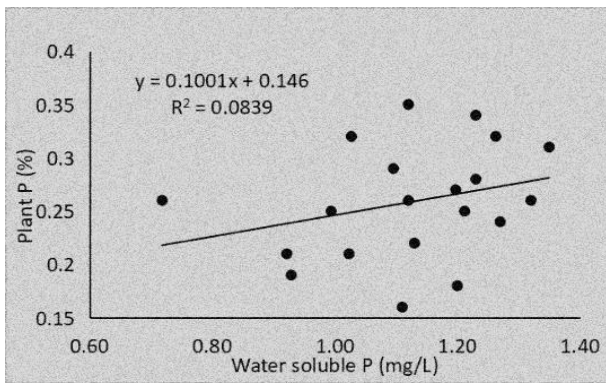


Figure 1 Correlation of Water-soluble P with plant P

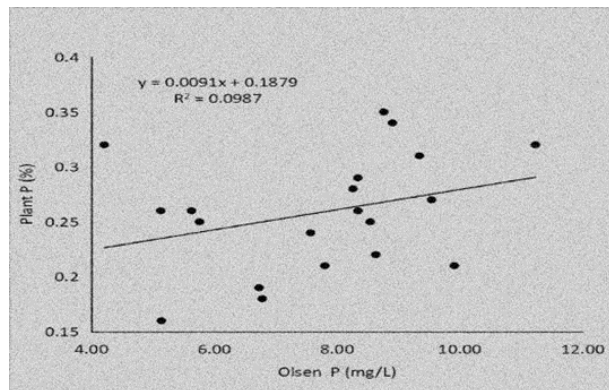


Figure 2 Correlation of AB-DTPA ext. P and plant P

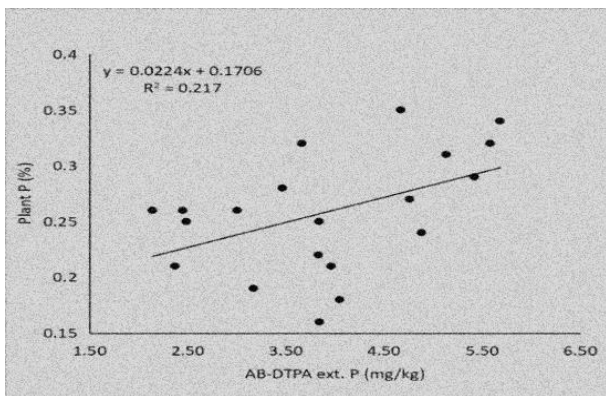


Figure 3 Correlation of Olsen P with plant P

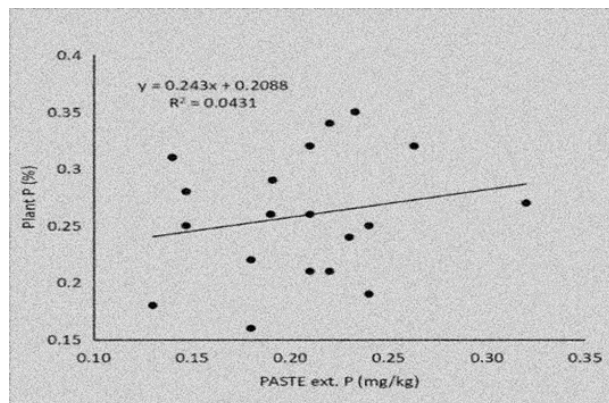


Figure 4 Correlation of Paste ext. P with and plant

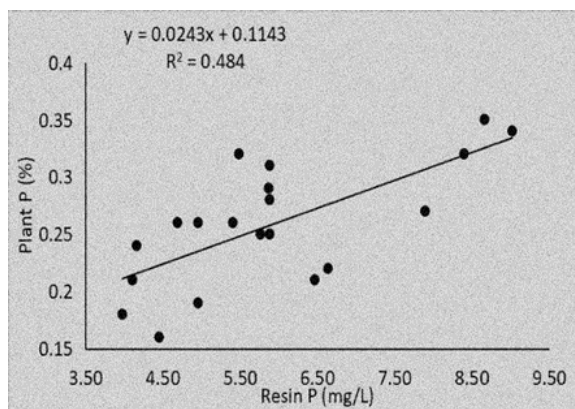


Figure 5 Correlation of Resin P with Plant P

Conclusions and Recommendations

The results of soil phosphorus extraction methods varied among the samples. AB-DTPA extractable phosphorus was deficient in 20% of the samples, while the Olsen method indicated low soil phosphorus levels in 80% of the samples. AB-DTPA phosphorus correlated strongly with water-soluble phosphorus, Olsen phosphorus, and Resin phosphorus but weakly with paste extract phosphorus. Water-soluble and paste extract phosphorus did not show significant correlations with any method, while Olsen phosphorus correlated significantly with Resin phosphorus. Resin phosphorus had the highest correlation with plant phosphorus, followed by AB-DTPA extractable phosphorus, indicating that these two methods are most suitable for phosphorus determination in the given soil and plant conditions. The recommended methods for determining and recommending fertilizer for phosphorus (P) in the soil and climate conditions are AB-DTPA ext. P and Resin P, as they show significant correlations with plant P, indicating its availability to plants. Further research should be conducted in larger areas and various agroecological zones to optimize fertilizer recommendations for different crops and soils.

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Conflict of interest

We declare no conflict of interest.

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