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# Study of Release Pattern of Phosphorus in Soils: Incubated with Organic Acids and Different Origin of Rock Phosphate

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

In this study the hypotheses assumed was assessed through incubation study in lab. Soil samples were pre incubated at 25 °C for 1 week prior to actual incubation to stabilize the microbial activity. Moisture content of soil was adjusted to 60 % of water holding capacity by adding deionized water. There were three Rock Phosphates of origin Jhabua, Udaipur-I, Udaipur-II and eleven treatments including the control and soils were analysed at six incubation periods (15, 30, 45, 60, 90 and 120 days); with three replications to see the effectiveness of different RPs treated with organic acids and zeolite with phosphate solubilizing bacteria (PSB) viz., *Pseudomonas fluorescence.* The incubation experiment showed a positive impact of the organic acids and FYM with increasing days of incubation gaining highest peak at 60 days in their ability to release P from all the RP sources.

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Data from 45 to 60 Days After Incubation (DAI) were found most suitable for P availability point of view. URP II showed maximum available P with gluconic acid@20 mM (13.28 kg $ha^{-1}$) with an increase of 39% followed by FYM @5 tonne $ha^{-1}$ with available P (13.10 kg $ha^{-1}$) and increase of 50.97% over control. JRP responded maximum with FYM @ 5 tonne $ha^{-1}$(9.05 kg $ha^{-1}$) available P with an increase of 36.35% followed by oxalic acid. The URP I maintained highest available P with oxalic acid@0.5 M (10.59 kg $ha^{-1}$) with an increase of 44.51% followed by FYM @5 tonne $ha^{-1}$.

*Keywords: Organic acids; incubation; jhabua; Udaipur-I; Udaipur-II; rock phosphates (RPs); zeolite; gluconic acid.*

## 1. INTRODUCTION

Phosphorus (P) being one of the major essential nutrient elements and the $10^{th}$ most abundant element in the earth's crust limits the growth of more than 40% of the world's arable land [1]. Phosphorus plays an essential role in cell division and development, energy transport (ATP/ADP), signal transduction, macromolecular biosynthesis, photosynthesis and respiration. At pH 7.22 the ratio of inorganic orthophosphates $HPO_4^{-2}$ and $H_2PO_4^-$ are equal but anything below, $H_2PO_4^-$ becomes the main form of P adsorption. These inorganic forms are the primary source of P for plants and microorganisms in soil solution. The concentration of soluble P in soil (grassland and forest) ranges from 0.05 to 10 mg/L (as more than 80% of P is immobile).

Soils contain very little total P 0.02 to 0.5 % (w/w), of which only 0.1 % is available to plants [2]. A high level of nutrient deficiency is noticed due to its complex chemistry in soil. Soon after application of fertilizers in soils, P is fixated into various insoluble compounds on active surfaces of soil. Due to adsorption and precipitation reaction phosphate becomes immobile in agricultural soil based on the soil characteristics with $Ca^{+2}$ in calcareous soils., $Fe^{+3}$ and $Al^{+3}$ in acidic soils [3], or is adsorbed to the surfaces of calcium carbonate, aluminum oxide, iron oxide, and aluminum silicate. These metal ion complexes precipitate about 80 % of added P fertilizer. Hence, the recovery efficiency of P is not more than 20 % of applied P in the world soils [4]. Moreover, the primary minerals (oxyapatite, hydroxyapatite, and apatite) of P are having a poor solubility.

It is necessary to continually replenish orthophosphate removed by plants or microbes from the solid phase to sustain the plant growth or P needs to be applied to soils as soluble P fertilizers. Considering the low recovery of applied and native P and the high cost of chemical phosphatic fertilizers in addition to an increasing concern about environmental degradation, it is important to find viable solution to increase P fertilizer use efficiency [5].

The solubilization of fixed P should be done properly for enhancing the fertilizer use efficiency to increase the available P in soil solution. At the same time, solubilization of fixed P requires proton ($H^+$), which can be obtained through chemical or microbial intervention. Organic acids are generally responsible for P solubilization as various P solubilizers could enhance it without harming the environment. Organic forms of P in soil have been shown in several studies to significantly contribute to plant nutrient availability. The impact of the microbes on soil organic P solubilization is unsurprising, because microbial P can constitute a large fraction of the soil organic P. [6]. The different types of organic manure increase the microbes, release acids in the root rhizosphere and help to solubilize phosphorus. PSB viz., *Pseudomonas fluorescence* mobilize insoluble inorganic phosphates from their mineral matrix to the bulk soil where they can be absorbed by plant roots. In turn, the plants supply root-borne carbon compounds, mainly sugars, which can be metabolized for bacterial growth [7].

The world's phosphorus (P) reserves are depleting at an increasing rate, and certain reports estimate, by 2050 there will be no more soil P reserve [1]. Since P fertilizers are imported from other countries and make them more expensive in India, and due to their high cost, only wealthy farmers can use them for their crops. Therefore, farmers need low-cost alternatives, or they need to use appropriate forms of fertilizers for their crops. In the Indian context, about 49.3% of districts are reported to have low available P content based on 9.6 million soil test values [1]. Thus, today's input-intensive agriculture necessitates the use of P-fertilizers to maintain soil fertility and crop productivity. As a result, one of the top research priorities is to develop alternative sources of P-fertilizers that are of the proper source and form for application in different soils.

The application of rock phosphates directly to the soils has yielded some positive results in acidic soils, but the efficacy is almost negligible in neutral and alkaline soils. However, reports of Syrian RP proved effective P fertilizer in alkaline soil of pH 7.72 and for maize grown in an acidic soil of pH 3.95. Keeping in view the considerable expense involved in importing raw material for manufacturing P fertilizers, it is imperative to find suitable ways to improve the solubility and efficiency of indigenous rock phosphates.

## 2. MATERIALS AND METHODS

Incubation study was carried out studied out in 2018-19 (Sep-Dec) at Department of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur. Soil samples were pre incubated at 25 °C for 1 week prior to actual incubation to stabilize the microbial activity. A known weight of soil (100 g oven-dry weight basis) were taken, air dried, sieved, mixed thoroughly and passed through 2-mm sieve transferred into 500 ml capacity jars. Moisture content of soil was adjusted to 60 % of water holding capacity by adding deionized water to compensate the loss due to evaporation. There were three Rock Phosphates of origin Jhabua, Udaipur-I, Udaipur-II and 11 treatments including the control and soil samples were drawn at 15 days interval (15, 30, 45, 60, 90 and 120 days), after adding organics; with three replications to see the effectiveness of different RPs treated with organic acids and zeolite with phosphate solubilizing bacteria (PSB) viz., *Pseudomonas fluorescence*.

### 2.1 Collection of Materials used for Incubation Study

Three rock phosphates (RP) were used for the present study, out of which two Udaipur grade I (URP I) with 26% Total P and Udaipur grade (URP II) with 27.48% Total P were used and one was from Jhabua Meghnagar Madhya Pradesh with 11.68% Total P. Udaipur RP (URP) were obtained from Jhamarkotra, Rajasthan State Mines and Minerals Ltd, Udaipur, Rajasthan, India. All the three RPs were used for the incubation study to see the release pattern of P from soil. Zeolite was collected from Bijapur, Bangalore, Karnataka (500 Rs $Kg^{-1}$) PSB Liquid broth of *Pseudomonas flouresence* from Microbes Research and Production Centre, JNKVV, Jabalpur and 0.28 % Total P content in Farm Yard Manure (FYM) JNKVV, Jabalpur

**Table 1. Initial soil analysis**

| Soils | S |
|---|---|
| Latitude | N-23$^0$12'17.6" |
| Longitude | E-79$^0$57'17.6" |
| pH | 6.16 |
| EC($dSm^{-1}$) | 0.63 |
| OC($gkg^{-1}$) | 9.60 |
| $CaCO_3$($gkg^{-1}$) | 65.00 |
| CEC[cmol(p+) $kg^{-1}$] | 46.58 |
| Clay (%) | 22.50 |
| N (Kg $ha^{-1}$) | 232.52 |
| K(Kg $ha^{-1}$) | 287.52 |
| Zn($mgkg^{-1}$) | 0.70 |
| Olsen-P(Kg $ha^{-1}$) | 5.04 |

### 2.2 Chemical Analysis of Rock Phosphate

#### 2.2.1 Total phosphorus

For determination of total P (TP), 1.0 g of the RP sample was taken in 250 ml conical flask and to this 25 ml of concentrated nitric acid ($HNO_3$) and 20 ml of $HClO_4$ (70%) was added and kept on a hot plate for 30 min, to oxidize any organic material related to RP. Once cooling the contents to temperature let the contents become clear with dense white fumes. Once complete digestion of the RP samples, add 50 ml of distilled water and filter through Whatmann No. 1 filter paper. Final volume of the sample was created up to 100 ml and the P content in the acid digest was measured at 440 nm using spectrophotometer following the quality procedure of vanadomolybdo phosphoric acid yellow color technique (Jackson, 1973).

#### 2.2.2 Analysis of Phosphorus in soil after incubation

Soil tests for available P were analyzed on 15 days interval at 15 ,30 ,45 ,60 , 90 and 120 days during incubation. All soil samples were sieved (2mm) after air-drying (23 °C) to constant moisture content in the laboratory. The available P in soil was extracted using the Olsen's reagent (0.5 M $NaHCO_3$) [8] for the neutral soil. The P content in the extracts was determined using the ascorbic acid blue colour method [9]. The intensity of the blue color developed was determined by a spectrophotometer at 660 nm wavelength.

**Table 2. Treatment details**

| $S_1$-$R_1$ | $S_2$-$R_1$ | $S_3$-$R_1$ |
|---|---|---|
| JRP@80 kg/ha P | URP I@80 kg/ha P | URP II@80 kg/ha P |
| $T_1$: Control (RDF) | $T_1$: Control (RDF) | $T_1$: Control (RDF) |
| $T_2$: PSB @1 ml/100 g soil | $T_2$: PSB @1 ml/100 g soil | $T_2$: PSB @1 ml/100 g soil |
| $T_3$: Cow urine@ 1ml | $T_3$: Cow urine@ 1ml | $T_3$: Cow urine@ 1ml |
| $T_4$: FYM@ 5 tonne/ha | $T_4$: FYM@ 5 tonne/ha | $T_4$: FYM@ 5 tonne/ha |
| $T_5$: Zeolite @1g/kg soil | $T_5$: Zeolite @1g/kg soil | $T_5$: Zeolite @1g/kg soil |
| $T_6$: Gluconic acid @20 mM | $T_6$: Gluconic acid @20 mM | $T_6$: Gluconic acid @20 mM |
| $T_7$: Oxalic acid @ 0.5 M | $T_7$: Oxalic acid @ 0.5 M | $T_7$: Oxalic acid @ 0.5 M |
| $T_8$: Citric acid @2 mg/L | $T_8$: Citric acid @2 mg/L | $T_8$: Citric acid @2 mg/L |
| $T_9$: Ammonium sulphate + zeolite | $T_9$: Ammonium sulphate + zeolite | $T_9$: Ammonium sulphate + zeolite |
| $T_{10}$: Zeolite + gluconic acid | $T_{10}$: Zeolite + gluconic acid | $T_{10}$: Zeolite + gluconic acid |
| $T_{11}$: Ammonium sulphate @20 kg/ha | $T_{11}$: Ammonium sulphate @20 kg/ha | $T_{11}$: Ammonium sulphate @20 kg/ha |

### 2.3 Treatments

Incubation experiment was laid with soil superimposed with 3 different grade RPs alongwith all 11 treatments in a Completely Randomized Design (CRD) layout in 3 replications.

## 3. RESULTS AND DISCUSSIONS

### 3.1 Effect of Organics and Incubation Days on the P Released from Soils when Applied with JRP as P Source

The trend of results show clear cut that organics enhanced the P availability significantly at different days of incubation. Data in Table 3, at different days of incubation show that, available P status has been influenced significantly by treatments and days of incubation but not by their interaction. Among the treatments, the highest available-P was recorded in the treatment T4-FYM @ 5 tonnes $ha^{-1}$ (9.05 kg $ha^{-1}$) which was closely equal to the treatment T7: Oxalic acid @ 0.5 M (8.95 kg $ha^{-1}$) and T9-Ammonium Sulphate + zeolite (8.91 kg $ha^{-1}$). The highest available P (8.83 kg $ha^{-1}$) was recorded at 60 Days After Incubation (DAI) which showed superiority over 15, 30, 90 and 120 DAI. However, treatment 45 and 60 DAI were statistically at par with each other. Thus it is obvious from the data that 45 to 60 DAI is the most suitable one for P availability point of view. Sharp peak (31.14%) was recorded over the period of incubation at 60 days. The per cent increase was observed maximum in treatment T4: FYM@5tonne $ha^{-1}$ (36.35%) and followed by T7: Oxalic acid@0.5 M (34.88%) over recommended dose of fertilizer (RDF). Similar results were reported by Sharma et al. [10], Jamal et al. [11], Wang et al. [12] and Sarangi et al. [13].

### 3.2 Effect of Organics and Incubation Time on the P Released from Soils when Udaipur Rock Phosphate (URP-I) Applied as P Source

Data in Table 4, at different days of incubation show that, available P status has been influenced significantly by treatments and days of incubation but not by their interaction. The highest available-P was recorded in the treatment T7: Oxalic acid @ 0.5 M (10.89 kg $ha^{-1}$) which was found statistically at par with T4: FYM @ 5 tonnes $ha^{-1}$ (10.59 kg $ha^{-1}$). The highest available P (10.10 kg $ha^{-1}$) was recorded at 60 DAI which was found statistically superior to 15, 30, 90 and 120 DAI, however equal to 45 DAI.

The release of P showed maximum during 45 to 60 days after incubation. Sharp peak (32.34%) was recorded over the period of incubation at 60 days. The increase was observed maximum in treatment T7: Oxalic acid@0.5 M (48.57%) followed by T4: FYM@ 5 tonne/ha (44.51%) over RDF.

### 3.3 Effect of Organics and Incubation Time on the P Released from Soil when Applied Udaipur Rock Phosphate II (URP-II) as P Source

Data in Table 5, at different days of incubation show that, available P status has been influenced significantly by treatments and days of incubation but not by their interaction. Among the treatments, the highest available-P was recorded in the treatment T6: Gluconic acid @ 20mM (13.28 kg ha$^{-1}$) which was closely equal to T4-FYM @ 5 tonnes ha$^{-1}$ (13.10 kg ha$^{-1}$) and T7-Oxalic acid@ 0.5 M (12.70 kg ha$^{-1}$).The highest available P (11.70 kg ha$^{-1}$) was recorded at 60 DAI which was found statistically superior to 15 and 120 DAI. Treatments at 30, 45 and 90 DAI were statistically at par with each other.

The release of P showed maximum during 45 to 60 days after incubation. Sharp peak (32.34%) was recorded over the period of incubation at 60 days. The increase was observed maximum in treatment T6: Gluconic acid @ 20mM (39%) and followed by T7: Oxalic acid@0.5 M (50.18**%)** over RDF. In P solubilization, organic acids are the main contributors [14,15]. Secretion, quantitatively and qualitatively, of organic acids by beneficial microorganisms is mainly gene-dependent but could also be influenced by the ecosystem environmental properties [16]. Organic acids can convert insoluble forms of P into bioavailable forms by creating acidic microsites that lower the pH thus releasing P from calcium ions, chelating metal ions that typically immobilize P, and occupying exchange sites on soil and mineral ions. Gupta [17] in a study found that applied soluble-P was fixed in 24 hr but gradually increased upto 30-45 days depending upon the type of soil and thereafter it remain constant. The P adsorption was increased substantially up to 30 days [18].

The low P in soil was observed in control. This may be due to the alkaline pH of the soil and it is known fact that P from RPs is not solubilized in alkaline environment/conditions and the RP stays as inert material. This inert behavior of RP in alkaline/calcareous soil has also been reported. This amount of P solubilized by PSB is quite unsatisfactory [19]. This unsatisfactory performance of PSB in solubilization of P from low grade RP may be attributed to the low organic matter status of the soil used for the study.

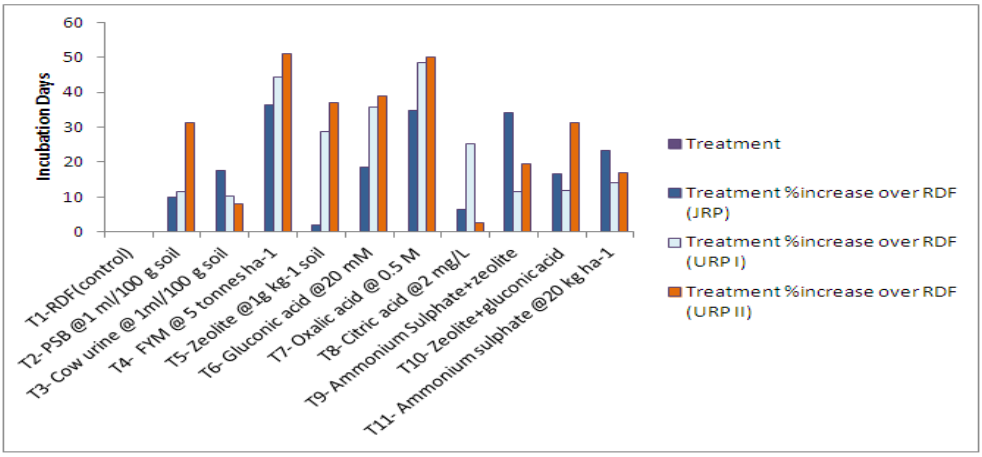


**Fig. 1. P release pattern at different Incubation days with treatments**

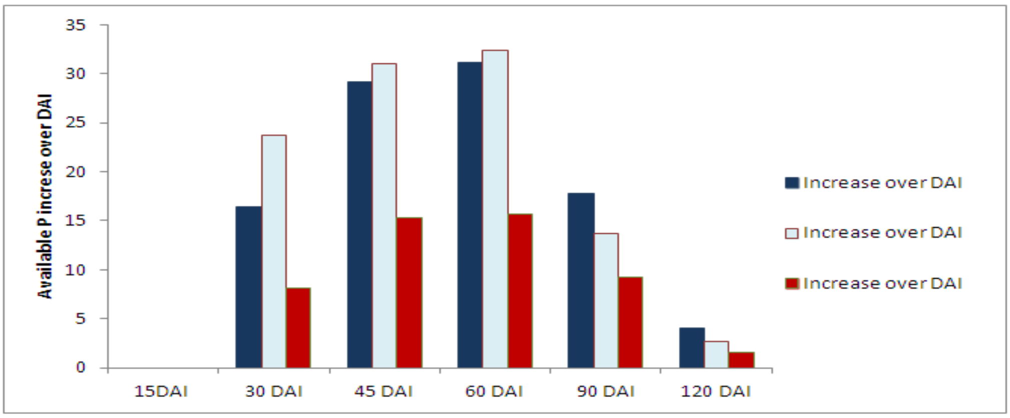


**Fig. 2. Available Olsen P increase over different DAI**

**Table 3. Phosphorus release (kg ha-1) from soils when applied from JRP as a P source along with organics**

| Treatment | Incubation days | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | **15DAI** | **30 DAI** | **45 DAI** | **60 DAI** | **90 DAI** | **120 DAI** | |
| T1-RDF(control) | 5.13 | 7.13 | 7.63 | 7.22 | 6.89 | 5.81 | 6.64 |
| T2- PSB @1 ml/100 g soil | 5.35 | 6.95 | 7.99 | 8.19 | 8.29 | 7.03 | 7.30 |
| T3- Cow urine @ 1ml/100 g soil | 5.78 | 7.38 | 8.39 | 9.87 | 8.59 | 6.81 | 7.80 |
| T4- FYM @ 5 tonnes $ha^{-1}$ | 8.40 | 8.92 | 9.40 | 9.78 | 9.03 | 8.76 | 9.05 |
| T5- Zeolite @1g $kg^{-1}$ soil | 6.79 | 6.86 | 7.52 | 6.79 | 6.54 | 6.14 | 6.77 |
| T6- Gluconic acid @20 mM | 6.26 | 7.18 | 9.80 | 9.33 | 7.83 | 6.87 | 7.88 |
| T7- Oxalic acid @ 0.5 M | 7.53 | 9.81 | 9.03 | 9.14 | 9.14 | 9.05 | 8.95 |
| T8- Citric acid @2 mg/L | 6.39 | 6.91 | 7.55 | 8.06 | 6.69 | 6.77 | 7.06 |
| T9- Ammonium Sulphate+zeolite | 8.36 | 9.91 | 9.03 | 9.75 | 8.74 | 7.68 | 8.91 |
| T10- Zeolite+gluconic acid | 6.34 | 7.59 | 9.69 | 9.07 | 7.90 | 5.86 | 7.74 |
| T11- Ammonium sulphate @20 kg $ha^{-1}$ | 7.77 | 7.59 | 9.68 | 9.98 | 7.69 | 6.37 | 8.18 |
| Mean | 6.74 | 7.84 | 8.70 | 8.83 | 7.94 | 7.01 | 7.84 |
| | | | | | **SEm±** | **CD at 5%** | |
| Treatment(T) | | | | | 0.31 | 0.87 | |
| Incubation Day(D) | | | | | 0.23 | 0.64 | |
| Interaction (TxD) | | | | | 0.76 | NS | |

**Table 4. P release from soils when applied from Udaipur Rock Phosphate (URP-I) as a P source along with organics**

| Treatment | Incubation days | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | **15DAI** | **30 DAI** | **45 DAI** | **60 DAI** | **90 DAI** | **120 DAI** | |
| T1-RDF(control) | 6.97 | 8.25 | 7.45 | 8.00 | 7.03 | 6.28 | 7.33 |
| T2- PSB @1 ml/100 g soil | 5.93 | 9.48 | 10.61 | 9.43 | 6.96 | 6.63 | 8.18 |
| T3- Cow urine @ 1ml/100 g soil | 5.92 | 8.73 | 9.33 | 9.98 | 7.65 | 6.93 | 8.09 |
| T4- FYM @ 5 tonnes $ha^{-1}$ | 8.63 | 11.10 | 11.60 | 11.11 | 10.73 | 10.38 | 10.59 |
| T5- Zeolite @1g $kg^{-1}$ soil | 7.78 | 10.54 | 11.04 | 10.53 | 8.80 | 7.88 | 9.43 |
| T6- Gluconic acid @20 mM | 8.27 | 9.95 | 10.28 | 11.44 | 10.64 | 9.18 | 9.96 |
| T7- Oxalic acid @ 0.5 M | 10.24 | 10.94 | 12.14 | 12.10 | 9.99 | 9.93 | 10.89 |
| T8- Citric acid @2 mg/L | 8.19 | 9.42 | 10.50 | 10.84 | 8.85 | 7.32 | 9.19 |
| T9- Ammonium Sulphate+zeolite | 7.05 | 8.56 | 7.71 | 9.34 | 9.20 | 7.27 | 8.19 |
| T10- Zeolite+gluconic acid | 7.05 | 8.45 | 10.32 | 9.12 | 7.44 | 6.79 | 8.19 |
| T11- Ammonium sulphate @20 kg $ha^{-1}$ | 7.89 | 8.45 | 8.97 | 9.17 | 8.13 | 7.56 | 8.36 |
| Mean | 7.63 | 9.44 | 9.99 | 10.10 | 8.67 | 7.83 | 8.95 |
| | | | | | **SEm±** | **CD at 5%** | |
| Treatment(T) | | | | | 0.28 | 0.78 | |
| Incubation Day(D) | | | | | 0.21 | 0.58 | |
| Interaction (TxD) | | | | | 0.68 | NS | |

**Table 5. P release from soils when applied from Udaipur Rock Phosphate (URP-II) as a P source along with organics**

| **Treatment** | **Incubation days** | | | | | | **Mean** |
|---|---|---|---|---|---|---|---|
| | **15DAI** | **30 DAI** | **45 DAI** | **60 DAI** | **90 DAI** | **120 DAI** | |
| T1-RDF(control) | 8.26 | 8.35 | 9.21 | 9.07 | 8.69 | 8.48 | **8.68** |
| T2- PSB @1 ml/100 g soil | 11.29 | 11.36 | 12.10 | 11.73 | 11.77 | 10.10 | **11.39** |
| T3- Cow urine @ 1ml/100 g soil | 8.38 | 9.81 | 10.62 | 9.93 | 9.04 | 8.46 | **9.37** |
| T4- FYM @ 5 tonnes $ha^{-1}$ | 11.59 | 12.16 | 13.49 | 13.48 | 13.78 | 14.10 | **13.10** |
| T5- Zeolite @1g $kg^{-1}$ soil | 10.97 | 11.88 | 15.71 | 11.91 | 10.98 | 9.89 | **11.39** |
| T6- Gluconic acid @20 mM | 11.85 | 11.91 | 12.30 | 12.60 | 12.86 | 10.84 | **13.28** |
| T7- Oxalic acid @ 0.5 M | 11.18 | 13.28 | 13.81 | 13.32 | 13.52 | 13.07 | **12.70** |
| T8- Citric acid @2 mg/L | 8.76 | 9.81 | 9.40 | 8.96 | 8.39 | 8.08 | **8.90** |
| T9- Ammonium Sulphate+zeolite | 9.57 | 10.88 | 11.11 | 11.18 | 10.26 | 9.28 | **10.38** |
| T10- Zeolite+gluconic acid | 9.42 | 10.69 | 12.75 | 12.82 | 12.37 | 10.31 | **11.39** |
| T11- Ammonium sulphate @20 kg $ha^{-1}$ | 9.57 | 9.68 | 10.76 | 11.35 | 9.47 | 10.03 | **10.14** |
| Mean | 10.11 | 11.01 | 11.66 | 11.70 | 11.07 | 10.24 | 10.30 |
| | | | | | **SEm±** | **CD at 5%** | |
| Treatment(T) | | | | | 0.348 | 0.974 | |
| Incubation Day(D) | | | | | 0.257 | 0.719 | |
| Interaction (TxD) | | | | | 0.853 | NS | |

## 4. CONCLUSION

The results of our incubation experiment opined that among the RPs used in the study, URP-II, released the highest P at the starting of the experiment, but this P was significantly decreased with subsequent incubation periods. Udaipur II rock phosphate incubated with gluconic acid, FYM and oxalic acid recorded significantly higher amounts of available P. Thus it is obvious from the data that 45 to 60 DAI is the most suitable one for P availability point of view. If the DAI is either below or above the P availability decreases and the magnitude of decrease is more at 15 DAI than lower one to that of 120 DAI. Among the treatments, (F4) FYM @5 tonnes $ha^{-1}$ with RPs recorded significantly higher Ca-P, Fe-P, Al-P content in soil. However, in case of RP sources application of URP (II) increased phosphorus pools significantly over JRP and URP I sources.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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