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# Bioactivation of Legacy Phosphorus in Calcareous Soil and its Impact on Maize Yield

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## ABSTRACT

**Background:** Over the years, the excessive P fertilizer application to crop fields resulted in the build up of insoluble phosphorus pool in soil known as legacy phosphorus. This represents a vast and largely untapped resource in soil. The current study was conducted to evaluate the potential of some P activators (Phosphorus solubilising bacteria, phytase, humic acid, farmyard manure) on increasing the plant availability of legacy P in calcareous soil.

**Methods:** A field experiment was conducted with maize hybrid COH(M) 6. The P activators were combined and applied along with the different doses of P fertilizer (100%, 75% and 50% soil test dose of single super phosphate).

**Result:** Farmyard Manure (FYM) application with Humic acid (HA) showed the increased Olsen P under 100% and 75% of P application. The phosphorus activation response of FYM+HA applied plots showed a higher value than other treatments. The maize yield and P uptake obtained with FYM and HA were statistically comparable at 100% and 75% soil test dose of P. The findings have illustrated that even the reduced amount of P fertilizer application can support the crop growth due to the release of legacy P if they are applied along with P- activators such as FYM and HA.

**Key words:** FYM, Humic acid, Legacy P, P activators, Phosphorus.

## INTRODUCTION

Phosphorus (P) is a non-substitutable nutrient for crop growth. It is an essential primary macronutrient indispensable in several physiological and biochemical processes. It is the major limiting nutrient in many agroecosystems; therefore, P deficiency is a constraint for global crop production and is estimated to impact >40% of agricultural soils (Zheng, 2010). The mobility of phosphorus (P) in the shallow subsurface is a matter of critical importance and considerable complexity. Often it is applied as chemical fertilizer to the field to improve crop production. A significant fraction of applied fertilizer P is usually fixed as an insoluble fraction due to various soil reactions.

Phosphorus accumulation is most commonly evident in arid soil conditions due to the low P use efficiency associated with the low rate of solubility and availability. This mainly includes the calcareous soils, characterized by the abundance of $Ca^{2+}$ ions, alkaline pH and presence of calcite ($CaCO_3$). All these factors promote the higher Ca- associated P precipitation, thereby reducing the P use efficiency and inducing P deficiency (Dhillon *et al*., 2017). The interaction of P with calcite surfaces results in the initial adsorption and subsequent precipitation of meta-stable dicalcium phosphate (DCP, $CaHPO_4$) and its hydrolytic conversion into octa calcium phosphate [OCP, $Ca_8(HPO_4)_2(OH)_2$] and more stable hydroxyl apatite [HAp, $Ca_{10}(PO_4)_6(OH)_2$] (Von Wandruszka, 2006). To overcome this soil P precipitation/adsorption process and to maintain the optimal P concentration in soil solution, P fertilizers are dumped in excessive amounts exceeding the crop needs. Under such long-term P fertilizer application, P exceeding the rate of crop removal accumulates in large quantities of "legacy P" in calcareous soil (Menezes-Blackburn *et al*., 2018).

So far, the soil legacy P can be considered a substantial secondary P resource, which could prolong the P crisis (Owen *et al*., 2015). It has been suggested that this accumulated P in calcareous soil would be sufficient to sustain crop yields for 100 years if it were made available (Zhu *et al*., 2018). The environmental behavior of P is also a function of its speciation, which is directly linked to P solubility, reactivity and bio-availability. For example, Fe- P is sensitive to reducing conditions, whereas Al-P and Ca-P precipitates are more likely to be sensitive to pH changes (Yan *et al*., 2017). Therefore, legacy P availability in calcareous soils is

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greatly affected by a series of pH-dependent abiotic reactions that influence the ratio of soluble-to-insoluble P pools in the soil (DeLuca *et al*., 2015).

Unfortunately, most legacy P in the soil will not be available for plants to take up easily. Hence the soil has to be manipulated to increase the availability of this P to crops. The manipulation can be done by various P activation mechanisms, including dissolution/precipitation, sorption/desorption and mineralisation/immobilisation. The P activators (P as) can be divided into three types *viz*., 1. Bio- inoculants and Bio-Fertilizers (microorganisms and enzymes), 2. Organic matter (Organic acids, manure), 3. Zeolite and other materials (Teng *et al*., 2020). In this paper, the performance of P-activators such as farmyard manure, humic acid, phytase and phosphorus solubilising bacteria in increasing the legacy P availability in a P deficient calcareous soil was explored via a field experiment with maize.

## MATERIALS AND METHODS

### Study area and sampling

The study area is located in the black soil region of Perianaickenpalayam in the Coimbatore district (11°N and 76°E). The soil has received the long-term regular application of P fertilizers and was expected to have high levels of legacy P. The soil samples collected at a depth of 0- 20 cm were air-dried, ground and passed through a 2mm sieve before further analysis and their initial characteristics were; pH - 8.20, Organic carbon- 5.5 g $kg^{-1}$, Free $CaCO_3$ - 14%, Total P- 0.42%, Olsen P- 8.1 kg $ha^{-1}$.

### Field experiment

A field experiment was conducted with maize (*Zea mays* L.) hybrid CO(H)M 6 as a test crop in a randomised block design (RBD) with three replications. The treatment details are given in Table 1. The soil samples were collected on the critical stages of crop *viz*., Knee-high stage ($30^{th}$ day), Tasseling stage ($60^{th}$ day), Milky stage ($90^{th}$ day) and Harvest stage ($105^{th}$ stage) and subjected to laboratory analysis of Olsen P (Olsen *et al*., 1954). At the harvest stage of maize, grain and stover yield were recorded and P uptake was calculated.

In addition, to elucidate the effect of P activators on available P (AP), P activation response (PAR) (Teng *et al*., 2020) based on AP increase was calculated, where PAR is defined as:

$$PAR = \frac{AP_T - AP_0}{AP_0}$$

$AP_T$- Available P concentration in P activator treated soil.
$AP_0$- Available P concentration in control.

### Statistical analysis

Each treatment in this study was applied in a randomised design. Statistical Package of Social Sciences (SPSS) was employed for statistical analysis. The data recorded were analysed statistically by analysis of variance techniques appropriate for randomised block design (RBD) as suggested by Gomez and Gomez (1984). Means were compared and grouped by the least significant difference test ($p<0.05$).

## RESULTS AND DISCUSSION

### Effect of P-activators on Olsen P

Soil phosphorus at different critical stages of the crop showed a significant difference among the treatments, as shown in Fig 1. The available phosphorus status of soil showed a decreasing trend along the crop growth stages due to the uptake of P by the crop. Among the different P-activator combinations, the farmyard manure and humic acid application showed the higher available phosphorus in all the stages. The treatment $T_3$ (FYM+HA with 100% soil test dose of P) showed higher mean soil available P (18.54 kg $ha^{-1}$), but it was statistically comparable with $T_6$ (FYM+HA with 75% soil test dose of P). The treatment $T_3$ and $T_6$ recorded a 50.48% and 49.58% increase in soil available P over the control. Kaleeswari *et al*. (2007) reported that the decomposition of organic manure releases organic acid, which decreases adsorption and fixation. The organic acid is also involved in the complexing of Calcium, resulting in increased P release from the calcium phosphate fraction. The increased solubilisation of organic acids is due to the drop in soil pH, soil cations chelating and competition with phosphate for adsorption sites in the soil solution (Singh *et al*., 2022). The addition of humic acid to alkaline soil significantly increased the water-soluble phosphate and strongly retarded the formation of occluded phosphate by creating the competition between the humic acid and phosphorus for soil sorption sites (Yang *et al*., 2019).

### Effect of P-activators on P-activation response

P-activation response (PAR) is an important indicator of soil P availability and the transformation of P-fractions. P- Activation Response of different P activators was calculated from the available P content of treated and control soil. The mean PAR of different treatments is given in Fig 2. The application of farmyard manure and humic acid with a 100% soil test dose of P ($T_3$) showed a higher PAR (1.04), which was statistically comparable with $T_6$ ( FYM+HA with 75% soil test dose of P) (1.01).Yang *et al*. (2018) reported a higher PAR on the addition of organic acids in soil. The activating capability of $P_i$ increased by the addition of organic acids in soil due to the acidification effect of the protons on the fixed P pools. The activation response varies for each P- activator based on its activation mechanism, soil properties, type and amount used. The higher P- activation response indicates that more P is available for plant growth. PAR represents the degree of difficulty with which the transformation between the different P fractions and available P occurs (Yang *et al*., 2019). Sun *et al*. (2018) reported a higher PAR in the fertilizer applied treatments. The Olsen P and P-activation responses are positively related to the P- balance. The PAC depends

**Table 1:** Treatment details.

| $T_1$ | Soil test based NK |
|---|---|
| $T_2$ | $T_1$+SSP @ 100% recommended soil test dose |
| $T_3$ | $T_1$+$PA_1$ (FYM+HA)+SSP @ 100% recommended soil test dose |
| $T_4$ | $T_1$+$PA_2$ (PSB+Phytase)+SSP @ 100% recommended soil test dose |
| $T_5$ | $T_1$+$PA_3$( Phytase+HA)+SSP @ 100% recommended soil test dose |
| $T_6$ | $T_1$+$PA_1$ (FYM+HA)+SSP @ 75% recommended soil test dose |
| $T_7$ | $T_1$+$PA_2$ (PSB+Phytase)+SSP @ 75% recommended soil test dose |
| $T_8$ | $T_1$+$PA_3$( Phytase+HA)+SSP @ 75% recommended soil test dose |
| $T_9$ | $T_1$+ $PA_1$ (FYM+HA)+SSP @ 50% recommended soil test dose |
| $T_{10}$ | $T_1$+$PA_2$ (PSB+Phytase)+SSP @ 50% recommended soil test dose |
| $T_{11}$ | $T_1$+$PA_3$( Phytase+HA)+SSP @ 50% recommended soil test dose |

FYM- Farmyard manure @ 12.5 t $ha^{-1}$, HA- Humic acid @ 3 kg $ha^{-1}$, Phytase @ 2 kg $ha^{-1}$ and PSB- Phosphorus solubilising bacteria @ 2 kg $ha^{-1}$.

**Table 2:** Effect of P-activators on maize yield (kg $ha^{-1}$).

| Treatments | Yield (kg $ha^{-1}$) | |
|---|---|---|
| | Grain | Stover |
| $T_1$- Soil test-based NK | 2114$^{g}$ | 4864$^{g}$ |
| $T_2$- $T_1$+SSP @ 100% soil test dose | 4900$^{de}$ | 6912$^{e}$ |
| $T_3$- $T_1$+$PA_1$+SSP @ 100% soil test dose | 6613$^{a}$ | 9814$^{a}$ |
| $T_4$ - $T_1$+$PA_2$+SSP @ 100% soil test dose | 5896$^{c}$ | 8861$^{c}$ |
| $T_5$ - $T_1$+$PA_3$+SSP @ 100% soil test dose | 6205$^{b}$ | 9417$^{b}$ |
| $T_6$- $T_1$+$PA_1$+SSP @ 75% soil test dose | 6412$^{ab}$ | 9624$^{ab}$ |
| $T_7$ - $T_1$+$PA_2$+SSP @ 75% soil test dose | 5123$^{d}$ | 7960$^{d}$ |
| $T_8$ - $T_1$+$PA_3$+SSP @ 75% soil test dose | 5961$^{c}$ | 8949$^{c}$ |
| $T_9$ - $T_1$+$PA_1$+SSP @ 50% soil test dose | 4672$^{e}$ | 5879$^{f}$ |
| $T_{10}$ - $T_1$+$PA_2$+SSP @ 50% soil test dose | 3986$^{f}$ | 5112$^{g}$ |
| $T_{11}$- $T_1$+$PA_3$+SSP @ 50% soil test dose | 4110$^{f}$ | 5640$^{f}$ |
| Mean | 5090 | 7548 |
| SEd | 113.73 | 123.31 |
| CD (5%) | 237.23 | 257.22 |

PA 1- Farmyard manure (FYM) @ 12.5 t $ha^{-1}$+Humic acid (HA) @ 3 kg $ha^{-1}$, PA 2- Phosphorus Solubilising Bacteria (PSB) @ 2 kg $ha^{-1}$+Phytase (Phy) @ 2 kg $ha^{-1}$, PA 3-Phytase (Phy) @ 2 kg $ha^{-1}$+Humic acid (HA) @ 3 kg $ha^{-1}$.Values with different letters indicate the significant difference using LSD at $p \leq 0.05$.

on the P geochemical fraction and the PAC was correlated with most $P_i$ fractions. Among the different inorganic pools, the labile pool can easily be activated and transformed into a biologically available P pool (Wu *et al*., 2017).

**Effect of P-activators on maize yield and P uptake**

The grain and stover yield of maize were recorded at the harvest stage and are given in Table 2. Their P uptake was calculated and depicted in Fig 3. The application of farmyard manure and humic acid with a 100% soil test dose of P ($T_3$) showed a higher grain yield (6613 kg $ha^{-1}$) with P uptake of 9.98 kg $ha^{-1}$ and stover yield (9814 kg $ha^{-1}$) with P uptake of 14.67 kg $ha^{-1}$, which was statistically comparable with $T_6$ (FYM+HA with 75% soil test dose of P). Similar results were observed by Sikka *et al*. (2018). The FYM application with a full and reduced dose of P fertilizer showed a similar yield, biomass production and P uptake. Under the conditions of reduced soil fertility, the combined application of P fertilizer and FYM are most effective because the supply of nutrients from both sources is additive. Shafi *et al*. (2020) reported a higher grain, straw yield of wheat and maximum harvest index of 43% in FYM, humic acid and fertilizer amended plots. The results revealed that the application of P with HA decreases the P-fixation and increases the water-soluble P availability to plants by making its soluble complexes and its uptake improves the storage of photosynthates in the plants. The addition of humic acid and FYM have direct as well as indirect effects on crop growth and yield, as they play an essential role in supplying and cycling nutrients in the soil and releasing the insoluble pool by its solubilising effect under the P-deficit conditions (Hussain *et al*., 2021; Kumawat *et al*., 2021). The FYM and humic acid, besides releasing their own nutrient, increases the nutrient use

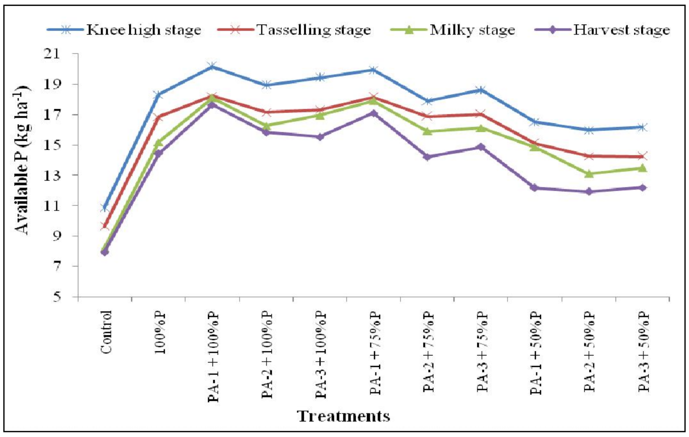


**Fig 1:** Effect of P-activators on available phosphorus (kg ha$^{-1}$).

PA 1- Farmyard manure (FYM) @ 12.5 t ha$^{-1}$+Humic acid (HA) @ 3 kg ha$^{-1}$, PA 2-Phosphorus Solubilising Bacteria (PSB) @ 2 kg ha$^{-1}$+Phytase (Phy) @ 2 kg ha$^{-1}$, PA 3- Phytase (Phy) @ 2 kg ha$^{-1}$+Humic acid (HA)@ 3 kg ha$^{-1}$.

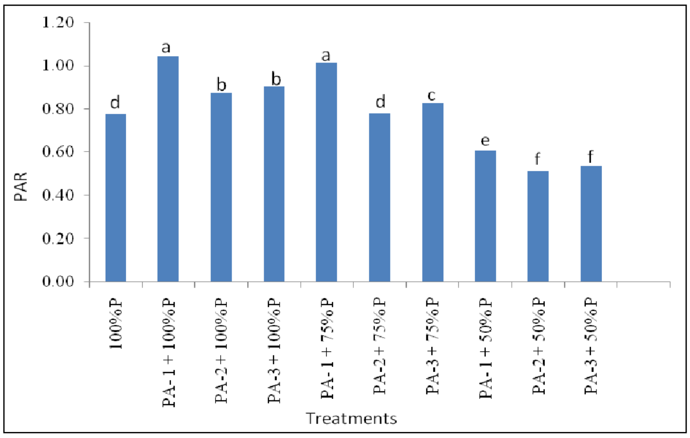


**Fig 2:** Effect of P-activators on phosphorus activation response.

PA 1- Farmyard manure (FYM) @ 12.5 t ha$^{-1}$+Humic acid (HA) @ 3 kg ha$^{-1}$, PA 2-Phosphorus Solubilising Bacteria (PSB) @ 2 kg ha$^{-1}$+Phytase (Phy) @ 2 kg ha$^{-1}$, PA 3-Phytase (Phy) @ 2 kg ha$^{-1}$+ Humic acid (HA)@ 3 kg ha$^{-1}$. Bars with different letters indicate the significant difference using LSD at p≤0.05.

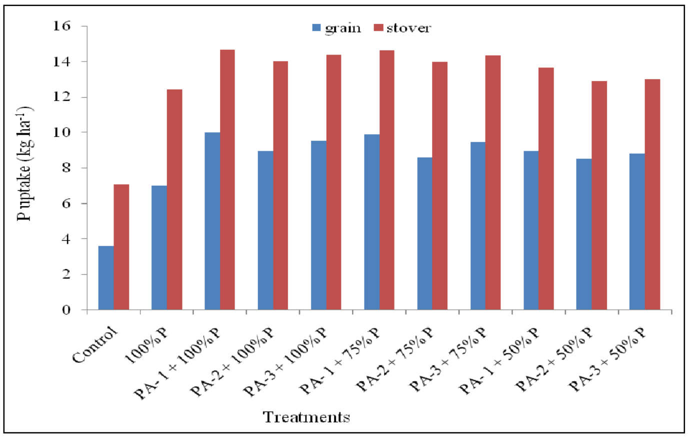


**Fig 3:** Effect of P-activators on P uptake.

PA 1- Farmyard manure (FYM) @ 12.5 t ha$^{-1}$+Humic acid (HA) @ 3 kg ha$^{-1}$, PA 2- Phosphorus Solubilising Bacteria (PSB) @ 2 kg ha$^{-1}$+Phytase (Phy) @ 2 kg ha$^{-1}$, PA 3- Phytase (Phy) @ 2 kg ha$^{-1}$+ Humic acid (HA)@ 3 kg ha$^{-1}$.

efficiency of applied inorganic fertilizers as well as solubilises the fixed pool of nutrients in the soil, thereby promoting the crop growth and yield (Venketesh *et al*., 2019).

## CONCLUSION

The study was undertaken to know the potential of different P- activators (FYM, HA, Phytase and PSB) on solubilising legacy P. The field experiment conducted with maize showed that the FYM and HA were the best P-activator combination for calcareous soil. The results of soil samples analysed for Olsen P suggested that the FYM and HA could perform better at 100% and 75% soil test dose of P fertilizer in all critical stages of the crop. The yield and uptake of maize crop at the harvest stage were also statistically comparable among $T_3$ (FYM and HA with 100% soil test dose of P) and $T_6$ (FYM and HA with 75% soil test dose of P). This shows that the P-activators have solubilised a portion of legacy P and increased its availability in soil for crop growth. The study concludes that FYM and humic acid with 75% soil test dose of P can be the efficient P management strategy for calcareous soils to reduce the P overload in soil and to promote the good soil health for sustainable agriculture.

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**Conflict of interest**

The authors declare that there is no competing interests.

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