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Research Article

Effect of phosphorus activators on soil legacy phosphorus availability and its uptake by maize hybrid COH(M) 6 in calcareous soil

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Abstract
Continuous application of phosphatic fertilizers in association with its low recovery results in the insoluble legacy P buildup in agricultural soils. In this study, a field experiment was conducted with maize hybrid COH(M) 6 to know the effect of different P activators (Farmyard Manure, Humic acid, Phosphorus Solubilising Bacteria, and Phytase) on increasing the legacy phosphorus (P) availability. The P activators were combined and applied along with the different doses of P fertilizer (100%, 75%, and 50% soil test dose of P fertilizer). The results showed that the application of Farmyard manure (FYM) and Humic acid (HA) significantly ($p < 0.05$) increased the soil available P (18.54 kg ha^{-1}) and notably reduced the calcium P fraction (93.08 mg kg^{-1}). The application of FYM and HA with 100% soil test dose of P showed a similar grain (9.98 kg ha^{-1}) and stover P uptake (12.67 kg ha^{-1}) response as that of FYM and HA with 75% soil test dose of P. The study suggested that FYM and HA application has activated the fixed calcium phosphorus in soil and increased its availability for crop utilization. The findings have illustrated that even the reduced dose of P fertilizer application can support the nutrient uptake if they are applied along with P- activators such as FYM and HA. This could promote soil health by reducing the P overload and further P loss in soil.

Keywords: Farmyard manure, Humic acid, Legacy phosphorus, P- activators

INTRODUCTION

Phosphorus (P) is an essential nutrient for crop growth, 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. However, all those soils contain a considerable amount of total P, and the plant-available form of phosphorus (negatively charged primary and secondary orthophosphate ions) is in low concentrations (<1%) in soil solution.Even though the inorganic and organic forms of P fertilizers are applied to agricultural soils often to replenish this pool, orthophosphate ions in soil solution rapidly react with soil components and transform into other forms that are not available to plants (Alotaibi *et al*., 2021). Shortly after P fertilizer application, the added P associates with dominant ions in soils like calcium, iron, and aluminum, resulting in an insoluble P pool. Thus most of the applied P remains in the soil due to the long-term application of mineral and organic P sources. It has been suggested that accumulated P in agricultural soils can sustain crop yields worldwide for 100 years if available (Roberts & Johnston, 2015).

Phosphorus accumulation is most commonly evident in arid soil conditions due to the low P use efficiency of these soils and the low solubility and availability rate. This mostly includes the calcareous soils, characterized by the abundance of Ca^{2+} ions, alkaline pH, and calcite (CaCO3) presence. All these factors promote the higher Ca- associated P precipitation, thereby reducing the P use efficiency and inducing P deficiency (Dhillon *et al*., 2017). In order 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 amounts in agricultural soil, widely known as "legacy P" (Blackburn *et al*., 2018).

Historically, manufactured water-soluble phosphate derived from mined rock phosphate has played a significant role in replenishing the soil P pool. The recent reports claim that the source is rapidly diminishing, and this finite source will be depleted in 50 -100 years. The remaining reserves will be of lower quality and higher cost. The increasing demand for agricultural commodities increases the demand for phosphorus sources resulting in a "potential phosphate crisis" globally (Daneshgar*et al*., 2018). To overcome these conflicts, the only solution is to find ways to make use of soil legacy P as a possible P source for plants.

Unfortunately, most legacy P in soil is not readily available to plants. To increase the availability of this P to crops, the soil has to be manipulated by applying various amendments called P-activators (Zhu *et al*., 2018). The P activators are organic in nature, which include microorganisms, enzymes, organic acids, and manure. The P-activators follow several action mechanisms, including dissolution/precipitation, sorption/desorption, and mineralisation/immobilisation. On the one hand, it promotes the solubilisation of soil inorganic P by producing organic acids. On the other hand, the organic functional group of P activators occupies the P-binding sites reducing the P fixation in soil (Teng *et al*., 2020).In the present study, the performance of P-activators such as Farmyard manure (FYM), Humic acid (HA), 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 hybrid COH (M) 6.

MATERIALS AND METHODS

Study area and sampling

The study area is located in the black soil region of Perianaickenpalayam in the Coimbatore district (11ºN & 76ºE). 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}, Saloid P- 18.42 mg kg^{-1}, Reductant soluble P - 9.61 mg kg^{-1}, Calcium P –91.46 mg kg^{-1} , Iron P - 25.89 mg kg^{-1} and Aluminium P - 17.22 mg kg^{-1}.

Field experiment

A field experiment was conducted with maize (*Zea mays* L.) hybrid CO(H)M 6 as a test crop. The experiment was conducted 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}

Table 1. Treatment details

T_1	Soil test based NK (Control)
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

day), Tasseling stage (60th day), Milky stage (90th day), and Harvest stage (105th stage) and subjected to laboratory analysis of Olsen P (Olsen *et al*., 1954), Soil pH (Jackson, 2005) and P fractions (Peterson and Corey, 1966; Mehta *et al*., 1954). At the harvest stage, grain and stover P uptake was also calculated.

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 (CD < 5%).

RESULTS AND DISCUSSION

Effects of P-activators on available P (Olsen P)

Soil available P at critical stages of the crop is given in Fig. 1. The application of different P activators and different doses of P fertilizers showed variation in soil available phosphorus. Higher available P was observed in treatment with the application of Humic acid and FYM with 100% P soil test dose (T_3) (18.54 kg ha^{-1}), and it was found to be on par with treatment T_6 (FYM and Humic acid with 75% P soil test dose) and T_5 (Phytase and Humic acid with 100% P soil test dose) at 5% significant level. The available phosphorus content of soil followed a gradually decreasing trend along the growth stages due to the uptake of P by crop. In the Harvest stage, the treatment T_3 and T_6 showed a 50.48%and 49.80 %increase in soil available P over the control (T_1). The increased P contents with the addition of FYM are due to mineralization and higher water content. Organic manures after decomposition releases organic acids and increases P bioavailability by the dissolution of native and fixed P (Masood *et al*., 2013). The application of P fertilizer at 75% recommended soil test dose along with P activators was statistically comparable with 100% soil test P dose. Similar to inorganic P fertilization, P-activators likeFYM and Organic acids can also increase plant-available P in soil (Marschner, 2011). This may be due to the complexing action of FYM and Humic acid due to its strong binding ability in both solid and solution phases with ions like iron, aluminium, and calcium (Otieno *et al*., 2018). Humic materials are generally not a major source of P, but they have a mobilizing effect on the subsurface adsorbed P.The humic acid application increases the amount of water-soluble phosphate and strongly retards the formation of occluded phosphate in soil (Jing *et al*., 2020).

Effect of P-activators on soil pH

The soil pH showed a decreasing trend along thecrop growth stages. Different treatments showed variation in soil pH as depicted in Fig. 2. Comparatively, the treatment T_6 (Farmyard manure and Humic acid with 75% soil test dose of P fertilizer) showed a more significant decrease in soil pH (8.06)at the harvest stage, which

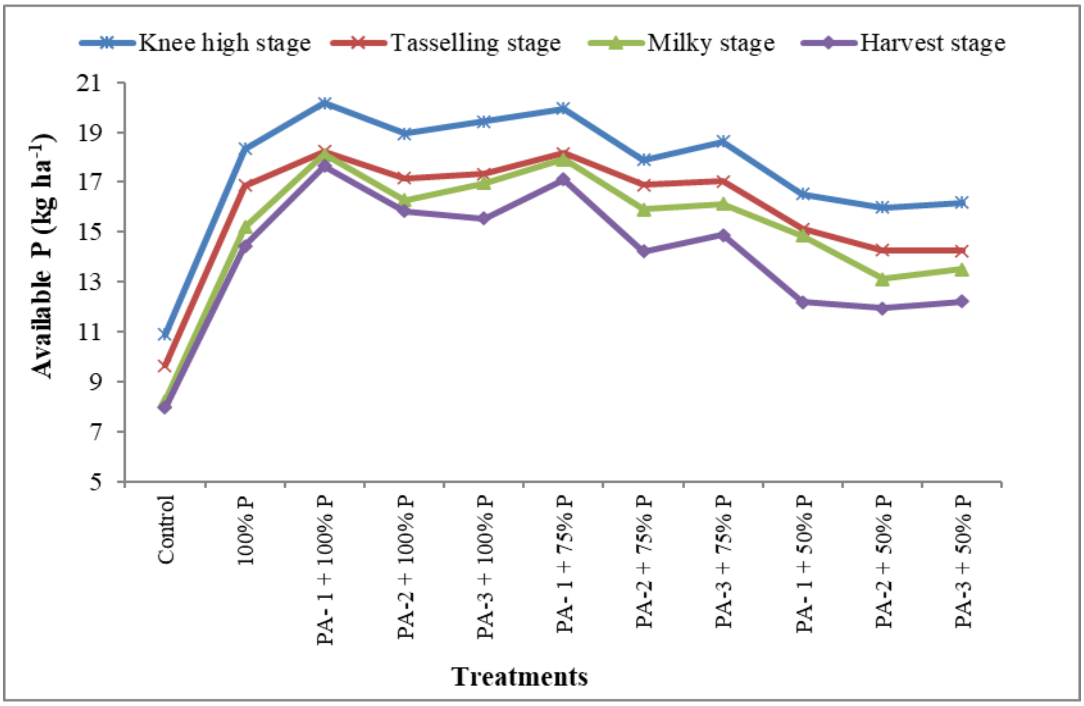

Fig. 2. *Effect of P-activators on soil pH (PA 1 – Farmyard manure (FYM) @ 12.5 t ha^{-1} + Humic acid (HA) @ 3 kg ha^{-1}, PA 2– Phosphorus Solubilising Bacteria (PSB) @ 2 kg ha^{-1} + Phytase (Phy) @ 2 kg ha^{-1}, PA 3 – Phytase (Phy) @ 2 kg ha^{-1} + Humic acid (HA) @ 3 kg ha^{-1})*

was on par with T_3 and T_6 (8.08). The treatment differences were non significant ($p<0.05$).The application of FYM and Humic acid showed a 0.5 unit decrease in pH from its initial value. Similar results were obtained by Barka *et al.* (2018). They reported a 0.5 unit reduction in soil pH after four months of applying organic manure. The decrease in soil pH is attributed to the neutralization of hydrogen ions produced by organic acids with hydroxyl groups present in soil solution resulting in the formation of water and other complex compounds (Andersen *et al*., 2013). The reduction in soil pH under the application of organic amendments like FYM, Humic acid, PSB, and Phytase may be attributed to the progress of decomposition and oxidation of organic compounds in soil. Sometimes it may also be due to the activity of soil microorganisms producing CO_2 and root exudates during the decomposition of organic matter (Orman and Kaplan, 2011).

Effect of P-activators on soil P fractions

The sequential soil P fractionation done at the harvest stage showed significant variation among different treatments. All inorganic and organic P fractions showed a decrease in P-activators applied treatments compared to their initial value, as shown in Fig. 3. Saloid P, reductant soluble P, Iron P, and Aluminium P have not shown significant differences ($p<0.05$) among the treatments. Among all the inorganic fractions, calcium phosphate showed a more substantial reduction with applying P activators. Comparatively, the effect of farmyard manure and humic acid was more pronounced, with significant variation in calcium phosphate fraction, indicating its contribution to increasing the legacy P availability. The treatment T_6 (FYM + HA + 75% soil test dose of P) showed a greater reduction in soil calcium P fraction (93.08 mg kg^{-1}). Farmyard manure and humic acid increase the availability of P in the soil by producing CO_2 and organic and inorganic acids. These products help in the conversion of insoluble P to soluble ones as they act as a carbon source, lower the soil pH, and dissolve the calcium phosphate in calcareous soils (Song *et al*., 2017). The reduction of calcium phosphate fraction in soil over time is due to the reduction of soil affinity constants and sorption capacities by adding FYM/ Humates. This is attributed to the complexing of exchangeable calcium by components of manure or the competition of P fixation sites by organic acids. The release of organic anions enhances the release of sparingly soluble P, not only from the acid-soluble pool but also from more stable residual P fractions (Wandruszka, 2006)

Effect of P- activators on plant P concentration and P uptake

The stover and grain P concentrations and their P uptake calculated using the dry matter production is depicted in Table 2. The results indicate that treatment T_3 with the application of FYM and Humic acid along with a 100% soil test dose of P showed the higher stover (0.197 %) and grain (0.272 %) P content as well as P uptake (22.65 kg ha^{-1}). But it was found to be statistically comparable with treatment T_6 (FYM + HA + 75% soil test dose of P) at 5% significant level. This shows that the FYM and HA have solubilised and mobilised a part

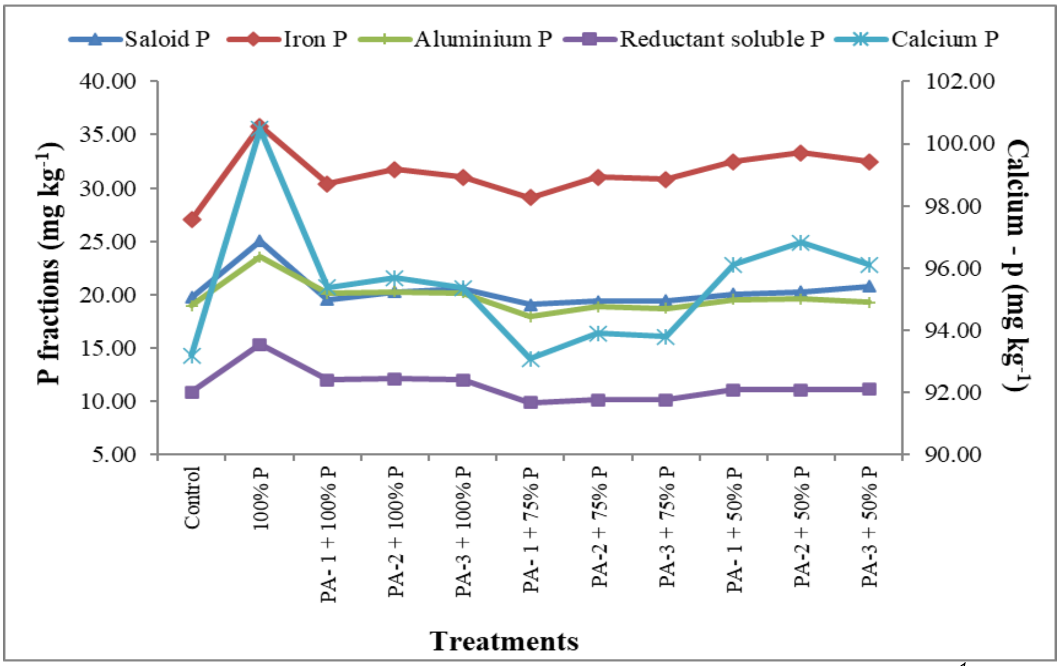

Fig. 3. *Effect of P-activators on inorganic P fractions (PA 1 – Farmyard manure (FYM) @ 12.5 t ha^{-1} + Humic acid (HA) @ 3 kg ha^{-1}, PA 2– Phosphorus Solubilising Bacteria (PSB) @ 2 kg ha^{-1} + Phytase (Phy) @ 2 kg ha^{-1}, PA 3 – Phytase (Phy) @ 2 kg ha^{-1} + Humic acid (HA) @ 3 kg ha^{-1})*

Table 2. Effect of P-activators on plant P concentration and P uptake at harvest stage

Treatments	Total P (%)		P uptake (kg ha^{-1})		
	Grain	Stover	Grain	Stover	Total
T_1 - Soil test-based NK (Control)	0.171^{f}	0.104^{d}	3.62^{f}	5.06^{f}	8.68^{h}
T_2- T_1 + SSP @ 100% soil test dose	0.204^{d}	0.180^{b}	7.00^{e}	10.44^{e}	17.44^{g}
T_3- T_1 + PA_1+ SSP @ 100% soil test dose	0.272^{a}	0.197^{a}	9.98^{a}	12.67^{a}	22.65^{a}
T_4 - T_1 + PA_2+ SSP @ 100% soil test dose	0.254^{bc}	0.192^{a}	8.97^{c}	12.01^{bc}	20.98^{cd}
T_5 - T_1 + PA_3+ SSP @ 100% soil test dose	0.266^{ab}	0.195^{a}	9.50^{b}	12.36^{ab}	21.86^{ab}
T_6 - T_1 + PA_1+ SSP @ 75% soil test dose	0.270^{a}	0.196^{a}	9.91^{a}	12.62^{a}	22.53^{a}
T_7 - T_1 + PA_2+ SSP @ 75% soil test dose	0.246^{c}	0.188^{ab}	8.60^{d}	11.96^{bc}	20.56^{de}
T_8 - T_1 + PA_3+ SSP @ 75% soil test dose	0.264^{ab}	0.194^{a}	9.47^{b}	12.29^{ab}	21.76^{bc}
T_9 - T_1 + PA_1+ SSP @ 50% soil test dose	0.192^{de}	0.164^{c}	8.97^{c}	11.64^{c}	20.61^{de}
T_{10} - T_1 + PA_2+ SSP @ 50% soil test dose	0.189^{e}	0.156^{c}	8.53^{d}	10.88^{de}	19.41^{f}
T_{11} - T_1 + PA_3+ SSP @ 50% soil test dose	0.190^{e}	0.159^{c}	8.81^{cd}	10.97^{d}	19.78^{ef}
Mean	0.229	0.175	8.48	11.17	19.66
Sed	0.005	0.005	0.16	0.22	0.40
CD (5%)	0.012	0.012	0.33	0.47	0.84

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.

of fixed P into an available form, so their effects were comparable on 100% and 75% SSP applications. Compared to the control(T_1), the treatment T_3 and T_6 showed a 61.6 % and 61.4 % increase in P uptake, respectively. The results also reported that P-activators could work well in the presence of inorganic fertilizers.The Interaction of FYM and HA with mineral P application was significant. Apparently, an additive effect was noted between the FYM, HA, and mineral fertilizers on P uptake. Similarly, Rakotoson and Tsujimoto (2020) reported that the application of FYM with organic acid increased the biomass and P uptake of irrigated rice equivalent to that of mineral P application. The additive effects of organic and inorganic amendments are responsible for higher nutrient uptake and better plant growth. The positive impact of FYM application on P uptake could be attributed to the temporary pH reduction due to the FYM decomposition, which reduces the P sorption capacity and increases the soluble P contents in soil (Kumar *et al.*, 2013).

Conclusion

The present study concluded that FYM, Humic acid, Phytase, and PSB could act as good solubiliser of legacy P. The combined application of FYM with humic acid performed better both at 100% and 75% soil test dose of P supplied through Single Super Phosphate (SSP) fertilizer. Some studies have stated that soil P deficits trigger legacy P availability. Here, it was evidenced that the 25% deficit in P application promoted the availability of legacy P. This is because the P-activators (FYM + HA) solubilised the fixed native P into the labile pool, resulting in the hike of soil available phosphorus, plant P concentration, and P uptake. The combination of FYM and humic acid showed a synergistic effect on solubilising the calcium phosphate fraction, with the decline in pH due to the release of carboxylates, promoting the ligand-driven mineral solubilisation. These results can be a theoretical base for realizing the potential of soil legacy P, at least in P enriched black calcareous soils, to improve agricultural sustainability.

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Conflict of interest

The authors declare that they have no conflict of interest.

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