**Enzyme Activity and Nutrient Availability in Highly Calcareous Soil as Affected by Multiple Phosphorus Sources for Soybeans**

**ABSTRACT**

A field experiment was conducted to study “enzyme activity and nutrient availability in highly calcareous soil as affected by multiple phosphorus sources for soybeans” at Research Farm, College of Agriculture, Pune during kharif 2023. The object of this experiment was to study comparative efficiency of phosphorus application through phosphorus rich organic manure (PROM), single super phosphate and diammonium phosphate (DAP) for soybean on enzyme activities and nutrient availability in highly calcareous soil. This experiment consisted of two organic sources (PROM and vermicompost) and two inorganic sources of phosphorus (SSP and DAP) along with their levels @ 50, 75 and 100 % imposed for soybean in highly calcareous soil. The treatments comprised T1 - absolute control, T2 - RDF (50:75:45 kg ha-1 N: P2O5: K2O), T3 - 50% P2O5 through PROM, T4 - 75% P2O5 through PROM, T5 - 100% P2O5 through PROM, T6 - 100% P2O5 through DAP + FYM @12.5 t ha-1, T7 - 100% P2O5 through SSP + FYM @12.5 t ha-1 and T8 - 100% P2O5 through vermicompost replicated thrice in randomized block design imposed to soybean in highly calcareous soil.

Results on post harvest fertility status of highly calcareous were concluded that incorporation of PROM and vermicompost for the replacement of 100 % P2O5 found superior for build up available nitrogen, phosphorus and potassium. Further, incorporation of 100 % P2O5 through PROM reported 75.48%, 122.07 %, 169.95 % and 104.09 % increase in DTPA extractable Fe, Mn, Zn and Cu over absolute control respectively. The data on enzyme activities in highly calcareous soil revealed that incorporation of P2O5 either through organic and inorganic sources recorded higher urease (30.93 and 29.22 µg NH4+-N g-1 soil hr-1), acid (6.07 and 6.74 µg PNP g-1 soil hr-1) and alkaline phosphatase (11.34 and 10.34 µg PNP g-1 soil hr-1) and dehydrogenase (14.30 and 10.40 µg TPF g-1 soil 24 hr-1) activity at 50 % flowering of soybean than that assayed at harvest of soybean. It is inferred that incorporation of PROM or vermicompost for the substitution of either 75 or 100 % P2O5 reported significantly higher activities of enzymes in calcareous soil than that of inorganic fertilizers (SSP and DAP) or recommended dose of fertilizer or absolute control.

***Keywords:***PROM, Vermicompost, calcareous soil, urease, acid and alkaline phosphatase and dehydrogenase and soybean

1. **INTRODUCTION**

Nutrient use efficiency reduction in various soils, imbalanced fertilizer application, less nutrient response ration, multi-nutrient deficiencies, reducing organic matter content, intensive agriculture, injudicious use of irrigation water, less soil testing based fertilizer application etc are the major reasons for reducing availability and uptake of nutrients from soil. P is the major plant nutrient and considered one of the primary nutrient limiting crop yields. Therefore application of phosphatic fertilizers is essentially required to maximize crop yields. The overall P use efficiency of applied phosphatic fertilizer such as SSP, DAP, TSP etc. is lower than optimal and only 15 to 20 per cent of applied phosphorus is recovered by the first crop, because of the formation of insoluble P compounds in soil. Despite the application of phosphatic fertilizers only 15-20 % is utilized by crops in the first year with the rest becoming part of the soil's residual P pool. Further, inorganic phosphate fertilizers application not totally soluble in soil matrix due to precipitation reactions takes place with Al and Fe in acidic while Ca in alkaline calcareous soils. In highly calcareous soils phosphorus (P) availability and uptake by plants can be significantly affected by several factors, leading to various problems for crop production. Here are some of the key issues related to phosphorus in highly calcareous soils have a high calcium carbonate content which can lead to phosphorus fixation. Calcium ions (Ca2+) in the soil solution can react with phosphate ions (PO4-) to form insoluble calcium phosphate compounds, reducing the availability of phosphorus for plant uptake.

Integrated Nutrient Management (INM) consisting organic and inorganic fertilizers along with biofertilizer can enhance P use efficiency in various soils. In organic amendments like compost, farmyard manure, vermicompost, phosphorus rich organic manure and green manures which can improve soil structure, enhance microbial activity and increase the availability of phosphorus. Organic manures such as compost, farmyard manure, and poultry manure contain significant amounts of phosphorus in organic forms, which undergo gradual mineralization and release, providing a sustainable and slow-release source of phosphorus for plant uptake. Phosphorus Rich Organic Manure (PROM) offers a promising solution to address the challenges of phosphorus management in highly calcareous soils.

The use of rock phosphate as an alternative for P fertilizer is gaining attention in recent years for sustainable agriculture through microbial solubilisation particularly in acidic soil. Further, enrichment of P through rock phosphate or phosphorite in compost or FYM is gaining attention of various farmers. The incorporation of organic residues either singly or in conjunction with a cheap source of mining element as rock phosphate along with phosphate solubilizing microorganism (*Pseudomonas, Bacillus* and *Rhizobium*) helps to improve the availability of phosphorus in alkaline as well as in calcareous soil Subehia (2001).

**2. MATERIAL AND METHODS**

The experiment was conducted on highly calcareous soil (12.47% CaCO₃) with alkaline pH @ 8.18) and at Post Graduate Research Farm, College of Agriculture, Pune. The soil, classified as Inceptisol (*Vertic Haplustepts* family), was medium-deep black, 70-80 cm deep and dominated by smectite clay. Initial soil testing revealed a low organic carbon (0.62%), with very low available nitrogen (113.50 kg ha-1), low available phosphorus (10.79 kg ha-1) and very high available potassium (536 kg ha⁻¹). The DTPA-extractable micronutrients were also determined showing 1.18 mg kg⁻¹ Fe, 3.08 mg kg⁻¹ Mn, 2.28 mg kg⁻¹ Zn, and 2.24 mg kg⁻¹ Cu in highly calcareous soil.

The soybean variety KDS-726 (*Phule Sangam*) was used as the test crop for the experiment. Phosphorus Rich Organic Manure (PROM) was prepared at the Vermicompost Yard, Division of Soil Science, College of Agriculture, Pune. The recommended fertilizer dose (50:75:45 kg ha⁻¹ of N, P₂O₅, and K₂O) was applied, except in the absolute control plots. Phosphorus was provided from various organic sources *viz.,* PROM and vermicompost and inorganic sources viz., DAP and SSP. Nitrogen and potassium were supplied through urea and muriate of potash, respectively.

PROM, vermicompost and FYM were applied as basal dose before sowing and their proximate analysis was carried out before the experiment (Table 1). In order to assess the soil enzyme activities at 50% flowering of soybean, separate composite samples were collected from each plot in cloth bags and processed for analysis. Representative soil samples from each treatment were collected after harvest of soybean and processed for analysis. These collected soil samples were analysed for fertility status after harvest of soybean.

The soil KMnO4-N, Olsen’s-P and N *N* NH4OA-K was analyzed by methods given by Subbia and Asijia (1956), Watanabe and Olsen (1965) and Knudsen *et al.,* (1982) respectively. DTPA extractable micronutrients (Fe, Mn, Zn and Cu) was assessed by atomic absorption spectrophotometer method prescribed by Lindsay and Norvell (1978). Soil urease activity was assessed by following method given by Tabatabai and Bremner (1972) while acid and alkaline phosphatase activity was assessed by using method advocated by Eivazi and Tabatabai (1977). However, activity of soil dehydrogenase was assayed by using method recommended by Casida *et al.,* (1964). The data generated during the course of investigation were tabulated and statistically processed as per Panse and Sukhatme (1985).

**3. RESULTS AND DISCUSSION**

**3.1 Effect of Different Sources and Levels of Phosphorus on Nutrient Availability in Calcareous Soil (Table 2 And 3)**

**3.1.1 Major Nutrients**

Soil fertility status in terms of available nitrogen, phosphorus and potassium were significantly influenced by the application of P2O5 through PROM, vermicompost, DAP and SSP for soybean in highly calcareous soil. Data revealed that available nitrogen, phosphorus and potassium as influenced by the application of P2O5 either through PROM, vermicompost, DAP and SSP were ranged from 121.88 to 168.58 kg ha-1, 6.18 to 21.64 kg ha-1 and 507 to 874 kg ha-1 in post harvest calcareous soil, respectively. Result showed that application of 100 % P2O5 through vermicompost recorded significantly higher soil available N (168.58 kg ha-1), P (21.64 kg ha-1) and K (874 kg ha-1) which was statically at par for N (167.22 kg ha-1) and K (869 kg ha-1) availability in soil with 100 % P2O5 through PROM **(Table 2).**

In case of soil available N and K an increasing trend was reported in control (121.88 and 507 kg ha-1) than initial (113.50 and 536 kg ha-1). Application of 100 % P2O5 through vermicompost reported 48.52 % increase (from 113.50 to 168.58 kg ha-1) over initial and 38.31 % (from 121.88 to 168.58 kg ha-1) over absolute control for post harvest soil available N. Increasing trend for soil available nitrogen to the tune of 47.33 %, 32.25 % and 30.29 % over initial was observed with 100 %, 75 % and 50 % P2O5 application through PROM for soybean respectively.

Increasing magnitude for soil available phosphorus was also noted over initial in all treatments under study except absolute control (6.80 kg ha-1), 100 % P2O5 throughSSP (7.52 kg ha-1) and 50 % P2O5 through PROM (9.14 kg ha-1). Data revealed that significantly higher soil available phosphorus (21.64 kg ha-1) was observed with the application of 100 % P2O5 : vermicompost while this treatment was found statistically at par with 100 % P2O5 : PROM (18.46 kg ha-1). The intensity of increase in soil available phosphorus was reported to the tune 100.55 % (from 10.79 to 21.64 kg ha-1) over initial and 218.23 % (from 6.80 to 21.64 kg ha-1) over control with the application of 100 % P2O5 through vermicompost. Application of 100 % P2O5 through PROM noted rising trend for available phosphorus in highly calcareous soil @ 71.08 % over initial (from 10.79 to 18.46 kg ha-1) and 171.47 % (from 6.80 to 18.46 kg ha-1) over absolute control. Lower soil available phosphorus was observed in absolute control (6.80 kg ha-1) which was followed by 100 % P2O5 through SSP (7.52 kg ha-1) than rest of the treatment under study.

Post harvest soil available potassium status also impacted significantly by the all treatments under study. Rising trend for soil available potassium was observed in all the treatment expect absolute control. Addition of 100 % P2O5 through vermicompost recorded significantly higher available K (874 kg ha-1) which was found to be statistically at par with 100 % P2O5 through PROM (869 kg ha-1) and 75 % P2O5 through PROM (821 kg ha-1).

Post harvest soil nutrient availability for nitrogen, phosphorus and potassium were found to be increased over initial due to the addition of various P-substitution treatments through PROM, vermicompost, SSP and DAP, while magnitude of increase was more pronounced with organic manure P2O5 substituted treatments. The P-substitution through PROM and vermicompost not only supplied P but also provided other nutrient in considerable amount **(Table 2 & 3)** that might be one of the reason for higher soil buildup for nitrogen, phosphorus and potassium. Further, higher nutrient status in calcareous soil after harvest of soybean might be attributed to organic matter added through manure which leads to release of organic acids during decomposition that resulted into more rate of mineralization or solubilization. Khan *et al.* (2023) also reported increasing trend for 0.5 *M* NaHCO3 (pH 8.5) extractable P with combine application of liquid acidified organic manure with SSP and DAP in the following order as DAP>RP>SSP along with PSB. Our findings were also agreeing with Billah *et al.* (2020) with rock phosphate enriched compost + *Rhizobacteria* under wheat. Application of rock phosphate enriched compost with poultry manure resulted 36 % increase in NO3-N while 15 % for neutralnormalNH4OAc extractable potassium. Potassium availability in post harvest soil was higher that might be due to direct addition of potassium into soil available pool through organic manure and further reduction in K fixation also. These results are supported by Billah *et al.* (2020).

Higher availability of P in post harvest calcareous soil was reported with the application of 100 % P2O5 through vermicompost (21.64 kg ha-1) followed by 100 % P2O5 (18.46 kg ha-1) and 75 % P2O5 (15.24 kg ha-1) through PROM than synthetic fertilizers like SSP and DAP. This could be attributed to organic acids released by compost that might have reduced P exchange sites through chelation resulted into more soluble P in rhizosphere as compare to SSP. Further microbial community in the root rhizosphere might have taken part to release Ca-fixed P through organic acid production resulted into higher availability. These results are supported by Billah *et al.* (2020). Co-application of liquid acidulated organic manure + rock phosphate along with PSB reported higher increase in soil extractable phosphorus (194.4 %) by Khan *et al.* (2023).

In calcareous soil added phosphatic fertilizer in very short span converted into unavailable or stable form and this restricts plant growth. Addition of organic manure + rock phosphate and vermicompost ensures microbial breakdown of stable or insoluble P into soluble form (Noor *et al.* 2021). They reported high P in solution with the addition P rich organic manure. Our findings are consistent with Shen *et al.* (2011). They reported root induced changes involves release as proton to acidify rhizosphere, carboxylate exudation to mobilize sparingly available P by chelation and ligand exchanges and further secretion of phosphatase or phytase to mobilize organic P (Inositol phosphate) by enzymes. The acidification of rhizosphere pH is also related to soil buffering capacity, microbial activities lead to release of fixed P in calcareous soil. Our results are also confirmed by Jamal *et al.* (2023).

Phosphorus availability is also trigged by alkaline phosphatase activity which plays dominant role for conversion of fixed soil phosphorus to plant available form. Further addition of PROM or vermicompost in this study for P-substitution might have increased carbon and nitrogen in soil leads to maximum growth of microbes that is interlinked with P mineralization.

**3.1.2 DTPA – Fe, Mn, Zn and Cu**

Post harvest DTPA-extractable micronutrient status in calcareous soil as influenced by various sources and levels of P through PROM, vermicompost, SSP and DAP were impacted significantly under soybean cultivation. **(Table 3).** DTPA-extractable Fe, Mn, Zn and Cu in post harvest soils were ranged from 1.54 to 2.71 ppm, 2.22 to 4.93 ppm, 2.33 to 5.79 ppm and 2.20 to 4.49 ppm respectively with application of various P-sources. Data revealed that application of various P treatments reported slight increasing trend in concentration of DTPA extractable Fe, Mn, Zn and Cu in post harvest soils noted over initial and absolute control. Significantly higher DTPA Fe (2.71 ppm), Mn (4.93 ppm), Zn (5.79 ppm) and Cu (4.49 ppm) in soil at harvest of soybean were reported with the application of 100 % P2O5 through PROM.

It could be observed from the data that substitution of P @ 75 % or 100 % through PROM and 100 % P2O5 *via* vermicompost recorded higher concentration of DTPA-Fe, Mn, Zn and Cu than rest of the treatment under study. Application of 100 % P2O5 *via* PROM reported 129 % increase (1.18 to 1.53 ppm) over initial and 43.33 % over absolute control in soil at harvest of soybean. Decreasing trend in DTPA Mn, Zn and Cu were reported to the tune of 27.92 %, 6.57 % and 1.81 % in absolute control over initial respectively. However, increasing trend for DTPA Fe @ 75.48 %, Mn 122.07 %, Zn 169.95 % and Cu 104.09 % in post harvest soils over absolute control were noticed with 100 % P2O5 application through PROM. Similar increasing trend for DTPA Fe, Mn, Zn and Cu were also recorded for P substitution through either SSP or DAP.

Higher DTPA-Fe, Mn, Zn and Cu in post harvest calcareous soil were recorded with P-substitution either with PROM or vermicompost which might be due to higher content of this in these inputs. Further low molecular weight organic acids *viz* citric, malic, succinic, malonic and maleic acids which are released during decomposition of organic matter or microbial exudation leads to lowering the pH of soil and resulted into higher solubilization of metallic Fe, Mn, Zn and Cu in soil. These results were also supported by Adeleke *et al.* (2016). They attributed three processes of soil organic acids for solubilization of insoluble nutrients are acidification, chelation and exchange reactions. Organic acids oriented from root exudates and microbial decomposition plays vital role in reducing pH of rhizosphere soil resulted into higher metal solubility in soil solution leads to higher nutrient acquisition by plant roots. Further organic acids forms complexes with metals (Fe, Mn, Zn and Cu) that might have facilitated their solubility and availability in soil. Organic acid exuded by plant roots reduces sorption, which decreases precipitation of Fe, Mn, Zn and Cu.

Results of this experiment are consistent with Meena *et al.* (2023). They also observed higher availability of Fe, Mn, Zn and Cu with the co-application of rock phosphate + poultry manure + phosphorus solubilizing bacteria in soil. As Fe, Mn, Zn and Cu has a strong tendency to organometallic complexes as chelation leads to enhance their availability. Addition of organic manure leads to higher microbial population and their activity involved in mineralization and transformation of insoluble metal cations to soluble (plant usable) and further they also reduced fixation of metal cations in calcareous soil resulted into higher availability of DTPA Fe, Mn, Zn and Cu. In case of P-substitution through DAP and SSP reported lower availability of DTPA Fe, Mn, Zn and Cu which may be attributed to fixation in soil. These results are also in accordance with Khan *et al.* (2023).

**3.2 Effect of Different Sources and Levels of Phosphorus on Enzyme Activities in Calcareous Soil**

**3.2.1 Urease**

Data revealed that urease activity in calcareous soil was noted a reduction trend at both flowering and harvest stage of soybean over initial (33.25 µg NH4+-N g-1 soil hr-1) (Table 4).

The activity of urease at flowering were ranged from (25.93 to 30.93 µg NH4+-N g-1 soil hr-1) and at harvest from (22.30 to 29.22 µg NH4+-N g-1 soil hr-1) of soybean. However, It could be further observed from the data that activity at flowering stage of soybean was found higher than at harvest.

Application of 100 % P2O5 : PROM (30.93 µg NH4+-N g-1 soil hr-1), 75 % P2O5 : PROM (30.68 µg NH4+-N g-1 soil hr-1) and 100 % P2O5 : vermicompost (30.67 µg NH4+-N g-1 soil hr-1) reported significantly higher and statistically at par for urease activity in soil at flowering. However minimum urease activity (25.93 µg NH4+-N g-1 soil hr-1) was reported in absolute control treatment at flowering followed by RDF (27.65 µg NH4+-N g-1 soil hr-1). Application of either 50, 75 or 100 % P2O5 though PROM or 100 % P2O5 through vermicompost or DAP or SSP recorded statistically at par results for soil urease activity in highly calcareous soil at flowering except in absolute control and RDF.

Nutrient cycling in soil involves physical, chemical, biological and bio-chemical alteration and mineralization being mediated by soil flora, fauna and plant roots. Catalytic activities of soil enzymes are largely governed by organic matter contained in soil. Application of phosphorus either @ 50, 75 and 100 % through PROM for soybean in calcareous soil reported higher urease activity at 50 % flowering of soybean than at harvest. Higher urease activity in calcareous soil amended with PROM probably resulted due to the addition of organic manure which enhanced the microbial population resulted in higher secretion of urease. The reduction in urease activity at flowering and at harvest over initial which might be due to tillage practices like experimental layout, water channel and ridges and furrow for conduct of experiment causes losses of organic carbon and nitrogen too. Higher urease activity in organic manure P substituted (PROM and vermicompost) treatments resulted from an increase in soil organic matter content and microbial population resulting into secretion of urease.

Highly and significantly linear correlation recorded between urease activity and soil organic matter (r = 0.9713\*\*) by Chang *et al.* (2007). It could be observed from the data that, reduction trend for urease activity in highly calcareous soil was observed at harvest of soybean, but the magnitude of reduction was more pronounced in those treatments received only inorganic fertilizers (RDF, DAP or SSP) than organic sources of P like PROM and vermicompost. Chang *et al.* (2007) reported lower urease activity in chemical fertilizer application treatment (as similar to that of control treatment) because the presence of inorganic form of N makes the synthesis of enzyme unnecessary. Similar kind of decreasing trend in urease activity is also reported by Balakrishna *et al.* (2007) with the addition of halophytic compost, FYM and phosphobacteria. They concluded after 65 days of cultivation period urease activity reduced to the tune of 26 %. Further they also reported positive correlation among urease activity in soil, total organic matter and nitrogen content.

**3.2.2 Acid and Alkaline Phosphatase**

The data regarding acid and alkaline phosphatase activity in calcareous soil revealed increasing trend in all the treatments over initial (4.24 µg PNP g-1 soil hr-1) and (7.95 µg PNP g-1 soil hr-1) respectively. Further it is observed, that slight reduction trend in acid and alkaline phosphates activity with all the treatments at harvest stage than assayed at flowering stage of soybean.( Table 5).

Acid phosphatase activity in calcareous soil at flowering stage varied significantly and ranged from 4.29 to 6.38 µg PNP g-1 soil hr-1 while from 4.17 to 6.74 µg PNP g-1 soil hr-1 at harvest of soybean. Significantly higher acid phosphatase activity was reported with the application of 100 % P2O5 through vermicompost which was followed by 100 % P2O5 through PROM. However, the activity of same enzyme at harvest of soybean were higher in case of 75 and 100 % P2O5 through PROM, DAP and vermicompost.

In case of alkaline phosphatase activity in highly calcareous soil which were ranged from 8.04 to 11.34 µg PNP g-1 soil hr-1 at flowering while 4.43 to 10.34 µg PNP g-1 soil hr-1 at harvest of soybean. Significantly higher and statistically at par activity of alkaline phosphatase were reported with the application 100 % P2O5 : PROM (11.34 µg PNP g-1 soil hr-1) and (10.34 µg PNP g-1 soil hr-1) and in those plots received 100 % P2O5 through vermicompost (11.01 µg PNP g-1 soil hr-1) and (10.1 µg PNP g-1 soil hr-1) at 50 % flowering and at harvest of soybean respectively. Further it could be observed from the data that the activity of alkaline phosphatase was slightly higher at flowering than assayed at harvest of soybean. But the intensity of reduction was more pronounced in the plots received either RDF, SSP or DAP or even no fertilizer treatment (absolute control).

Organic matter decomposition, root exudates and microflora origin organic acids released in alkaline calcareous soil might have played vital role for enhancing the activity of acid and alkaline phosphatase in the treatment received P-substitution through PROM and vermicompost. These results are also supported by Gorde *et al.* (2022). Further both acid and alkaline phosphates activities in calcareous soils were significantly higher in manure P added treatments (PROM and vermicompost) than those received inorganic P through SSP and DAP. These results are in accordance with Chang *et al.* (2007). They noted positive linear correlation for acid phosphatase activity with the application of compost (r = 0.9995\*\*). Similar results were also reported by Rocabruna *et al.* (2024) and Meena and Biswas (2015). In this study higher alkaline phosphatase activity in alkaline calcareous soil was observed than acid phosphatase which probably due to sensitivity of phosphatase for soil pH. Similar results for higher acid phosphatase activity than alkaline phosphatase in acid soil was noted by Kalembasa and Symanowics (2012). Manure and biochar application significantly increased enzyme activities in soil than that of inorganic fertilizer application. Higher degree of variation in acid phosphatase activity was observed with organic manure addition than inorganic fertilizer application (Amadou *et al.,* 2020).

**3.2.3 Dehydrogenase**

At flowering, dehydrogenase activity was ranged from 11.57 to 14.26 µg TPF g-1 soil 24 hr-1 while 8.06 to 10.65 µg TPF g-1 soil 24 hr-1 at harvest stage of soybean in highly calcareous soil (Table 6). Application of 100 % P2O5 : PROM (14.30 µg TPF g-1 soil 24 hr-1) or vermicompost (14.26 µg TPF g-1 soil 24 hr-1) were reported significantly higher dehydrogenase activity and they are statistically at par with each other. Similar trend for dehydrogenase activity with the same treatment was reported at harvest of soybean also. Lower dehydrogenase activity was noticed at harvest stage than that of 50 % flowering stage of soybean. Diminishing trend in the dehydrogenase activity was more dominant in amendment of P through either RDF, DAP or SSP than PROM and vermicompost. No fertilizer application treatments (absolute control) reported minimum dehydrogenase activity at both the stages.

Oxidation of organic matter in terms of dehydrogenase activity was measured at flowering and at harvest of soybean grown in highly alkaline calcareous soil with the application of organic and inorganic sources of P. Data presented in Table 6 indicated slight reduction trend in soil dehydrogenase activity over initial at flowering as well as at harvest stage of soybean. However, the magnitude of reduction was more pronounced in the treatment received phosphorus through inorganic fertilizers like SSP and DAP than PROM and vermicompost. Cultivation practices, tillage operations, weeding and more opening of soil etc. may have lost organic carbon from soil there by reduction in total carbon that might be the cause for reduction of dehydrogenase activity at both growth stages than initial. Similar results are also quoted for reduction in the dehydrogenase activity due to loss of organic carbon by Prasanna *et al.* (2012). They observed lower dehydrogenase activity in 5 % organic manure treated soil than that of 10 %. The magnitude of increase in dehydrogenase activity was more pronounced in organic manure (PROM and vermicompost) P-substituted treatment than those inorganic P through SSP and DAP.

Higher dehydrogenase activity in calcareous soil was reported with the application of P-rich organic manure which might be due to higher soil microbial respiration along with quantity and quality of manure used in this experiment. It could be ascribed from this experiment that rate of mineralization is not only depends on quantity of organic manure but also on quality. In this case amendment of P-rich organic manure with lower C:N ratio (24:1) (table 1) might have enhanced microbial population its activity and metabolic rate leads higher dehydrogenase activity in soil. Further soil microbial respiration is strongly linked to dehydrogenase activity, indicating high metabolic processes and nutrient cycling in the soil. Addition of PROM as P sources leads to higher microflora with their activities resulted higher synthesis of dehydrogenase.

Higher activities of urease, acid and alkaline phosphatase and dehydrogenase were noted in PROM or vermicompost based P substitution which might be due to organic matter addition to the soil which act as an energy source for microbes leads to higher population and activity. Similar results were also reported by Jadhav *et al.* (2024). They concluded that application of 7.5 t ha-1 FYM along with phytase enzyme application @ 3600 IU were found superior for higher acid and alkaline phosphatase and dehydrogenase at 50 % flowering and harvest of soybean. Gorde *et al.* (2022) also recorded higher microbial population and soil enzyme activity with the application of 100 % RDN through 33 % from each FYM, vermicompost and neem cake. Chang *et al.* (2007) also concluded that soil microbial population, its biomass and enzyme activities were significantly increased with the application of compost treated soil than only chemical fertilizer added. Higher soil enzyme activities were reported in plots received PROM and vermicompost which may have released organic acids during decomposition of organic matter like maleic, fumaric etc. that lowered the pH of calcareous soil to the neutral range. Higher P availability through P-rich organic manure added to soybean might have enhanced and proliferated root development and biomass leads to stimulation of enzymes resulted by higher microbial activities and production of root exudates that acted on a substrate for enzyme.

**4. CONCLUSION**

The present study revealed that the application 100% P2O5 through either PROM or vermicompost were found beneficial for soil buildup not only for N, P and K but also found superior for DTPA-Fe, Mn, Zn and Cu. Further activities of soil enzymes like urease, acid and alkaline phosphatase along with dehydrogenase were also found positively with application of 100% P2O5 through PROM or vermicompost.

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**References**

1. Adeleke, R., Nwangburuka, C. and Oboirien, B. (2016) Origins, roles and fate of organic acids in soil: A review. *South African Journal of Botany,* **108**, 393-406.
2. Amadou, A., Song, A., Tang, Z., Li, Y., Wang, E., Lu, Y., Liu, X., Yi, K., Zhang, B. and Fan, F. (2020) The effect of organic and mineral fertilization on soil enzyme activities and bacterial community in the below and above ground parts of wheat. *Agronomy,* **10,** 1452.
3. Balakrishna, V., Venkatesan, K. and Ravindran, K. C. (2007) The influence of halophytic compost, farm yard manure and phosphobacteria on soil microflora and enzyme activities. *Plant Soil Environment,* **53**(4), 186-192.
4. Billah, M., Khan, M., Bano, A., Nisa, S., Hussain, A., Dawar, K. M., Munir, A. and Khan, N. (2020) Rock phosphate enriched compost in combination with rhizobacteria; a cost-effective source for better soil health and wheat productivity. *Agronomy,***10**, 1390.
5. Casida, L. E. Hr., Klein, D. A. and Santoro, T. (1964) Soil dehydrogenase activity. *Soil Science,* **98**, 371-376.
6. Chang, E., Chung, R. and Tsai, Y. (2007) Effect of different application rates of organic fertilizer on soil enzyme activity and microbial population. *Soil Science and Plant Nutrition,* **53**, 132-140.
7. Eivazi, F. and Tabatabai, M. A. (1977) Phosphatase in soils. *Soil Biology and Biochemistry*, **9**, 167-172.
8. Gorde, N. B., Zade, S. P., Bodke, V. S. and Nakhate, P. S. (2022) Effect of organic farming on enzymatic activity and microbial population of soybean. *The Pharma Innovation Journal,* **11**(12), 2182-2186.
9. Jadhav, A. B., Taggu, B., Suradhkar, R., Patil, A. V. and Khire, J. M. (2024) Study on enzyme activities and nutrient availability in calcareous soil as influence by phytase and fym levels under soybean cultivation. *International Journal Current Microbiological Applied Science,* **13**(01), 42-52.
10. Jamal, A., Saeed, M. F., Mihoub, A., Hopkins, B. G, Ahmad, I. and Naeem, A. (2023) Integrated use of phosphorus fertilizer and farm yard manure improves wheat productivity by improving soil quality and P availability in calcareous soil under subhumid conditions. *Frontiers Plant Science,* **14**, 1034421.
11. Kalembasa, S. and Symanowics, B. (2012) Enzymatic activity of soil after applying various waste organic materials, ash and mineral fertilizers. *Polish Journal Environmental Studies,* **21**(6), 1635-1641.
12. Khan, K. S., Naveed, M., Qadir, M. F., Yaseen, M. and Siddiqui, M. H. (2023) Bio-organically acidified product- mediated improvements in phosphorus fertilizer utilization, uptake and yielding of *zea mays* in calcareous soil. *Plants,* **12**, 3072.
13. Knudsen, D., Peterson, G.A. and Pratt, P.F. (1982) Lithium, sodium potassium. *In*: Methods of soil analysis, part-2. Page, A.L. (Ed.), Madison, Wisconsin, USA, 225-245.
14. Lindsay, W. L. and Norwell, W.A. (1978) Development of DTPA soil test for zinc, iron, manganese and copper, *Soil Science Society of America Journal*, **42**, 421-428.
15. Meena, D., Meena, H. R., Jain, D. Kumar, T., Meena, A. J., Khardia, N. and Chundawat, D. S. (2023) Effect of different phosphorus sources on soil physio-chemical property dynamics under chickpea crop. *International Journal Plant Soil Science,* **35**(19), 240-249.
16. Meena, M. D., and Biswas, D. R. (2015) Effect of rock phosphate enriched compost and chemical fertilizers on microbial biomass phosphorus and phosphorus fractions. *African Journal of Microbiology Research,* **9**(23), 1519-1526.
17. Noor, K., Sarwar, G., Shah, S. H., Muhammad, S., Zafar, A., Manzoor, M. Z. and Murtaza, G. (2021) Formulation of phosphorus rich organic manure from rock phosphate and its dose optimization for the improvement of maize (*Zea mays L.*). *Journal of Plant Nutrition*, **44**(1), 96-119.
18. Panse V.A. and Sukhatme P.V. (1985) Statistical methods for Agricultural workers, Revised Edn. ICAER, New Delhi.
19. Prasanna, N. R., Sumathi, T., Sai Gopal, D. V. R. and Narasimha G. (2012) Influence of organic manure on soil microbial and enzyme activities. *ISSN : 0974 – 7435,* **6,** 8-9.
20. Rocabruna, P. C., Domene, X., Matteazzi, A., Figl, U., Fundneider, A., Martinez, M. F., Venir, E., Robatscher, P., Preece, C., Penuelas, J. and Peratoner, G. (2024) Effect of organic fertilisation on soil phosphatase activity, phosphorus availability and forage yield in mountain permanent meadows. *Agriculture Ecosystem and Environment,* **368**, 109006.
21. Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W. and Zhang, F. (2011) Phosphorus dynamics: from soil to plant. *Plant Physiology,* **156**, 997-1005.
22. Subbiah, B.V. and Asijia, G. L. (1956) A rapid procedure for estimation of available nitrogen in soil. *Current Science*, **25**, 259-260.
23. Subehia, S. (2001) Direct and residual effect of Udaipur rock phosphate as a source of P to wheat soybean cropping system in western Himalayan soil, *Research on Crop,* **2**, 297-300.
24. Tabatabai, M. A. and Bremner, J. M. (1972) Assay of urease activity in soils. *Soil Biology and Biochemistry*, **4**, 479-487.
25. Watanabe, F.S. and Olsen, S.R. (1965) Test of ascorbic acid method for determination of phosphorus in waters and NaHCO3, extracts from soil. *Soil Science Society of America* Proceedings, **29**, 677-678.

**Table 1. Proximate analysis of PROM, FYM and vermicompost**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sr. No.** | **Parameter** | **Unit** | **PROM** | **FYM** | **Vermicompost** |
| 1. | pH (1: 10) | - | 7.18 | 7.49 | 6.91 |
| 2. | EC | (dSm-1) | 1.74 | 1.66 | 2.12 |
| 3. | Moisture | (%) | 24.02 | 23.5 | 25.45 |
| 4. | Organic Carbon | (%) | 21.06 | 22.26 | 30.79 |
| 5. | Total N | (%) | 0.78 | 0.69 | 1.48 |
| 6. | Total P | (%) | 14.57 | 0.39 | 0.79 |
| 7. | Total K | (%) | 0.37 | 0.38 | 0.81 |
| 8. | Total Fe | (ppm) | 10550 | 186 | 388 |
| 9. | Total Mn | (ppm) | 600 | 35.7 | 65.7 |
| 10. | Total Zn | (ppm) | 125 | 14.8 | 18.3 |
| 11. | Total Cu | (ppm) | 30 | 4.39 | 15.2 |
| 12. | Total Ca | % | 13.63 | - | - |
| 13. | Total Mg | % | 0.52 | - | - |
| 14. | Total B | (ppm) | 30 | - | - |
| 15. | C:N ratio | - | 24:1 | 39:1 | 20:1 |
| 16. | C:P ratio | - | 1.44:1 | 57.07:1 | 38.97:1 |

**Table 2. Effect of PROM and phosphatic fertilizers on major nutrient status of soil after harvest of soybean in highly calcareous soil**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr. No.** | **Treatment** | **N**  **(kg ha-1)** | **P**  **(kg ha-1)** | **K**  **(kg ha-1)** |
| 1 | Absolute Control | 121.88 | 6.80 | 507 |
| 2 | RDF (NPK) | 142.22 | 14.02 | 718 |
| 3 | 50% P2O5 : PROM | 147.88 | 9.14 | 770 |
| 4 | 75% P2O5 : PROM | 150.11 | 15.24 | 821 |
| 5 | 100% P2O5 : PROM | 167.22 | 18.46 | 869 |
| 6 | 100% P2O5 : DAP | 150.52 | 13.18 | 726 |
| 7 | 100% P2O5 : SSP | 154.55 | 7.52 | 697 |
| 8 | 100% P2O5 : Vermicompost | 168.58 | 21.64 | 874 |
|  | SE+ | 3.48 | 1.79 | 21.28 |
|  | CD at 5% | 10.38 | 5.43 | 72.15 |
|  | **Initial** | **113.50** | **10.79** | **536** |

**Table 3. Effect of PROM and phosphatic fertilizers on micronutrient status of soil after harvest of soybean in highly calcareous soil**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sr. No.** | **Treatment** | **Fe**  **(ppm)** | **Mn**  **(ppm)** | **Zn**  **(ppm)** | **Cu**  **(ppm)** |
| 1 | Absolute Control | 1.54 | 2.22 | 2.13 | 2.20 |
| 2 | RDF (NPK) | 1.21 | 3.10 | 2.50 | 4.40 |
| 3 | 50% P2O5 : PROM | 1.81 | 4.12 | 4.87 | 4.24 |
| 4 | 75% P2O5 : PROM | 2.58 | 4.27 | 5.10 | 4.35 |
| 5 | 100% P2O5 : PROM | 2.71 | 4.93 | 5.79 | 4.49 |
| 6 | 100% P2O5 : DAP | 1.64 | 3.68 | 4.08 | 4.02 |
| 7 | 100% P2O5 : SSP | 1.60 | 3.27 | 3.88 | 4.14 |
| 8 | 100% P2O5 : Vermicompost | 1.81 | 4.93 | 4.88 | 4.48 |
|  | SE+ | 0.15 | 0.29 | 0.26 | 0.24 |
|  | CD at 5% | 0.46 | 0.90 | 0.87 | 0.73 |
|  | **Initial** | **1.18** | **3.08** | **2.28** | **2.24** |

**Table 4. Effect of PROM and phosphatic fertilizers on urease activity in highly calcareous soil**

|  |  |  |  |
| --- | --- | --- | --- |
| **Treat**  **No.** | **Details** | **Urease activity**  **(µg NH4+-N g-1 soil hr-1)** | |
| **Flowering** | **Harvest** |
| T1 | Absolute Control | 25.93 | 22.30 |
| T2 | RDF (NPK) | 27.65 | 23.22 |
| T3 | 50% P2O5 : PROM | 29.60 | 28.08 |
| T4 | 75% P2O5 : PROM | 30.68 | 28.28 |
| T5 | 100% P2O5 : PROM | 30.93 | 29.22 |
| T6 | 100% P2O5 : DAP | 28.30 | 26.28 |
| T7 | 100% P2O5 : SSP | 28.13 | 26.12 |
| T8 | 100% P2O5 :Vermicompost | 30.67 | 28.08 |
|  | SE± | 1.26 | 1.40 |
|  | CD at 5% | 3.81 | 4.23 |
|  | **Initial** | **33.25** | |

**Figure 1 Graph of the effect of PROM and phosphatic fertilizers on urease activity in highly calcareous**

**soil.**

**Table 5. Effect of PROM and phosphatic fertilizers on acid and alkaline phosphatase activity in calcareous soil**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treat No.** | **Details** | **Phosphatase activity (µg PNP g-1 soil hr-1)** | | | |
| **Acid** | | **Alkaline** | |
| **Flowering** | **Harvest** | **Flowering** | **Harvest** |
| T1 | Absolute Control | 4.29 | 4.17 | 8.04 | 4.43 |
| T2 | RDF (NPK) | 5.75 | 6.44 | 8.81 | 6.03 |
| T3 | 50% P2O5 : PROM | 5.39 | 5.15 | 8.88 | 8.02 |
| T4 | 75% P2O5 : PROM | 5.62 | 5.79 | 9.91 | 8.91 |
| T5 | 100% P2O5 : PROM | 6.07 | 6.74 | 11.34 | 10.34 |
| T6 | 100% P2O5 : DAP | 5.70 | 6.24 | 8.86 | 7.15 |
| T7 | 100% P2O5 : SSP | 5.33 | 5.99 | 8.35 | 7.92 |
| T8 | 100% P2O5 : Vermicompost | 6.38 | 6.33 | 11.01 | 10.1 |
|  | SE± | 0.14 | 0.39 | 0.15 | 0.13 |
|  | CD at 5% | 0.42 | 1.18 | 0.47 | 0.41 |
|  | **Initial** | **4.24** | | **7.95** | |

**Figure: 2 Graph of the Effect of PROM and phosphatic fertilizers on (a) acid and (b) alkaline phosphatase activity in highly calcareous soil.**

**Table 6. Effect of PROM and phosphatic fertilizers on dehydrogenase activity in calcareous soil**

|  |  |  |  |
| --- | --- | --- | --- |
| **Treat No.** | **Details** | **Dehydrogenase activity**  **(µg TPF g-1 soil 24 hr-1)** | |
| **Flowering** | **Harvest** |
| T1 | Absolute Control | 11.57 | 8.06 |
| T2 | RDF (NPK) | 12.82 | 8.89 |
| T3 | 50% P2O5 : PROM | 12.34 | 9.26 |
| T4 | 75% P2O5 : PROM | 13.42 | 9.62 |
| T5 | 100% P2O5 : PROM | 14.30 | 10.40 |
| T6 | 100% P2O5 : DAP | 12.48 | 9.60 |
| T7 | 100% P2O5 : SSP | 12.18 | 9.97 |
| T8 | 100% P2O5 : Vermicompost | 14.26 | 10.65 |
|  | SE± | 0.42 | 0.49 |
|  | CD at 5% | 1.28 | 1.50 |
|  | **Initial** | **15.63** | |

**Figure 3 Graph of the Effect of PROM and phosphatic fertilizers on dehydrogenase activity in highly**

**calcareous soil.**