



# Nutrient Release Pattern and Soil Enzyme Activities of Calcareous Soil as Influenced by Phosphate Rich Organic Manure

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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

A study was conducted at Post Graduate Laboratory, Division of Soil Science, College of Agriculture, Pune to study the nutrient release pattern and soil enzyme activities of calcareous soil as influenced by various levels of phosphorus using organic and inorganic sources. The experiment was conducted in randomized block design with seven treatments replicated three times. The treatments comprised as T<sub>1</sub>- Absolute control, T<sub>2</sub> - RDF (50:75:45 N: P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O kg ha<sup>-1</sup>) through DAP, T<sub>3</sub> - RDF (50:75:45 N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O kg ha<sup>-1</sup>) through SSP, T<sub>4</sub>- 25 % P<sub>2</sub>O<sub>5</sub> through PROM, T<sub>5</sub>-50% P<sub>2</sub>O<sub>5</sub> through PROM, T<sub>6</sub> -75% P<sub>2</sub>O<sub>5</sub> through PROM and T<sub>7</sub> -100% P<sub>2</sub>O<sub>5</sub> through PROM and

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RDF of soybean was used for incubation. The study demonstrated that applying 100% P<sub>2</sub>O<sub>5</sub> through Phosphate Rich Organic Manure (PROM) significantly improved soil properties, by reducing soil pH and calcium carbonate, increasing organic carbon, and enhancing the availability of macronutrients (nitrogen, phosphorus, potassium) and micronutrients (iron, manganese, zinc, copper). Enzyme activities, including urease, dehydrogenase and alkaline phosphatase were improved in calcareous soils, correlating positively with nutrient availability, particularly with phosphorus and micronutrients such as iron and manganese. PROM application was more effective than chemical fertilizers in promoting nutrient mobilization and improving soil enzyme activity over a 120-day incubation period. Strong correlations were observed between enzyme activity and micronutrient availability, especially in treatments receiving 100% P<sub>2</sub>O<sub>5</sub> through PROM, indicating its potential to enhance both nutrient cycling and soil fertility.

**Keywords:** PROM; incubation; nutrient release; enzyme activities and correlation.

## 1. INTRODUCTION

Phosphorus (P) is the second most essential macronutrient for plants, after nitrogen [1] and is crucial for root development, nodulation, nitrogen fixation, and various physiological functions such as energy transfer, cell division, and photosynthesis. However, in calcareous and alkaline soils, phosphorus availability is limited due to its fixation with calcium, iron, and aluminium, leading to poor plant uptake. Lack of phosphorus in calcareous and alkaline soils is a major global problem. In calcareous/alkaline soils, phosphorus availability to plant roots is restricted by its reduced mobility in soils and higher fixation [2]. Phosphorus solubilizing microorganisms (PSB) and soil enzymes like phosphatases play a critical role in converting immobilized P into plant-available forms, especially in phosphorus-deficient soils. Non-traditional phosphorus fertilizers like Phosphate Rock (PR) and Phosphate Rich Organic Manure (PROM) offer sustainable alternatives to conventional chemical fertilizers by maintaining

phosphorus availability for longer periods and enhancing crop productivity. PROM, in particular, has been recognized as an eco-friendly solution, approved by the Government of India, to improve phosphorus availability in calcareous soils. Considering India's significant rock phosphate availability, it is necessary to optimize the utilization of PROM in calcareous soil so as to improve phosphorus use efficiency and sustainable agriculture.

## 2. MATERIALS AND METHODS

An incubation study was conducted in PG Laboratory, Division of Soil Science, College of Agriculture, Pune. The soil samples for the incubation study were obtained from the PG Instructional Farm at the College of Agriculture, Pune. The soil samples were analyzed for their chemical properties using standard analytical methods.

The PROM was prepared at Vermicompost Yard, Division of Soil Science, College of Agriculture,

**Table 1. Proximate analysis of PROM**

Sr. No	Parameters	Unit	PROM
1	pH (1:10)	-	7.18
2	EC	(dS m <sup>-1</sup> )	1.74
3	Moisture	(%)	24.02
4	Organic Carbon	(%)	21.06
5	Total N	(%)	0.78
6	Total P	(%)	14.57
7	Total K	(%)	0.37
8	Total Fe	(mg kg <sup>-1</sup> )	12.70
9	Total Mn	(mg kg <sup>-1</sup> )	0.67
10	Total Zn	(mg kg <sup>-1</sup> )	2.69
11	Total Cu	(mg kg <sup>-1</sup> )	0.46
12	C:N ratio	-	24:1
13	C:P ratio	-	1.44:1

Pune. The recommended dose of fertilizer for soybean (50:75:45 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O) was applied except to absolute control. The recommended dose of phosphorus was supplied through various sources such as PROM as an organic source and DAP and SSP as an inorganic source. The nitrogen and potassium were supplied through urea and muriate of potash, respectively. The proximate analysis of PROM was done before start of incubation.

Plastic bowls of 2 kg capacity were filled with 1kg (2 mm sieved) soil in one hundred twenty six bowls for seven treatments, three replications and six stages of sampling. The moisture was maintained at field capacity. The soil was mixed thoroughly with PROM, DAP and SSP as per treatments. The set of experiment was incubated for 0, 15, 30, 60, 90 and 120 days in three replications each. The incubation study was conducted by using factorial completely randomized design [3].

### 3. RESULTS AND DISCUSSION

#### 3.1 Chemical Properties of Soil as Influenced by Levels of PROM

Soil calcareousness influenced pH, which decreased from 8.22 at 0 days to 8.13 over 120 days. Treatments with 100% and 75% P<sub>2</sub>O<sub>5</sub> through PROM significantly lowered soil pH, with 100% P<sub>2</sub>O<sub>5</sub> showing the greatest reduction. Organic matter played an important role in reducing the pH of both soils as negative correlation between pH and organic matter is reported by Talashilkar *et al.* [4]. Soil calcareousness increased EC at 0 and 15 days (0.39 dS m<sup>-1</sup>), with no significant impact from P<sub>2</sub>O<sub>5</sub> levels initially. By 30 days, RDF through SSP and 100% P<sub>2</sub>O<sub>5</sub> recorded higher EC (0.38 dS m<sup>-1</sup>). At 60 days, RDF through DAP and SSP reported the higher EC (0.46 dS m<sup>-1</sup>), followed by 100% and 75% P<sub>2</sub>O<sub>5</sub> through PROM. At 90 and 120 days, RDF through DAP maintained the highest EC (0.49 and 0.51 dS m<sup>-1</sup>). Due to application of inorganic and organic sources across the treatments, the variation in the EC is observed over the period of 120 days of incubation.

At 0 days, P<sub>2</sub>O<sub>5</sub> levels had no effect on soil organic carbon. From 15 days onward, 100% P<sub>2</sub>O<sub>5</sub> through PROM significantly increased organic carbon, peaking at 0.75% by 120 days, comparable to 75% P<sub>2</sub>O<sub>5</sub> through PROM throughout and 50% P<sub>2</sub>O<sub>5</sub> at 15 and 90 days.

The application of rock phosphate with organic materials in gypsiferous soils increased the organic carbon and phosphorous status of soil [5]. At 0 and 15 days, calcareous soil recorded high CaCO<sub>3</sub> (12.44% and 12.25%), which was remained unaffected by PROM treatments. From 30 days onward, 100% P<sub>2</sub>O<sub>5</sub> through PROM significantly reduced CaCO<sub>3</sub> levels, reaching 11.42% at 30 days and 11.25% at 60 days, and at par with 75% and 50% P<sub>2</sub>O<sub>5</sub> through PROM treatments. During 90 and 120 days, CaCO<sub>3</sub> content was reduced, with 100% P<sub>2</sub>O<sub>5</sub> through PROM achieving the lower levels (10.58% and 9.80%), comparable to the 75% and 50% treatments. The reactivity of rock phosphate increases as carbonates are converted into mineral apatite was recorded by Qureshi *et al.* [6].

#### 3.2 Macronutrient and Micronutrient Release in Calcareous Soil as Influenced by Levels of PROM

##### 3.2.1 Macronutrients

The available nitrogen from 0 to 120 days was increased to 145.42 kg ha<sup>-1</sup> from 112.81 kg ha<sup>-1</sup>. Application of RDF through DAP consistently recorded higher nitrogen, rising from 143.76 kg ha<sup>-1</sup> at 15 days to 158.97 kg ha<sup>-1</sup> at 120 days. Pandey *et al.* [7] reported that ammonical nitrogen, the first available organic form of nitrogen for plants and microorganisms, was significantly higher over the incubation study compared to the control.

At 0 days, calcareous soil recorded available phosphorus 11.89 kg ha<sup>-1</sup>, with no significant treatment effects. By 15 days, RDF through DAP showed the highest phosphorus (12.18 kg ha<sup>-1</sup>). From 30 to 120 days, 100% P<sub>2</sub>O<sub>5</sub> through PROM consistently achieved the highest phosphorus levels, peaking at 16.33 kg ha<sup>-1</sup>, similar to 75% P<sub>2</sub>O<sub>5</sub> through PROM. Kumar *et al.* [8] reported that the increased availability of phosphorus in organically amended soils results from a large reduction in phosphorus sorption.

At 0 and 15 days, calcareous soil had high potassium (483.06–483.89 kg ha<sup>-1</sup>), which was unaffected by PROM treatments. From 30 to 120 days, potassium levels increased, with RDF through DAP consistently achieving the highest values, peaking at 502.44 kg ha<sup>-1</sup> by 120 days. K content is more readily available in manured soil than in unmanured soil was also observed by Brar *et al.* [9].

**Table 2. Influence of soil calcareousness and PROM on periodical changes in pH and electrical conductivity of soil**

Levels of P <sub>2</sub> O <sub>5</sub>	Days After Incubation											
	pH						EC (dSm <sup>-1</sup> )					
	0	15	30	60	90	120	0	15	30	60	90	120
B <sub>1</sub>	8.23	8.22	8.21	8.17	8.17	8.15	0.39	0.38	0.35	0.39	0.40	0.41
B <sub>2</sub>	8.21	8.20	8.18	8.16	8.15	8.13	0.40	0.41	0.37	0.46	0.49	0.51
B <sub>3</sub>	8.22	8.21	8.19	8.17	8.17	8.14	0.39	0.41	0.38	0.46	0.48	0.49
B <sub>4</sub>	8.24	8.23	8.21	8.19	8.16	8.15	0.38	0.38	0.36	0.40	0.41	0.43
B <sub>5</sub>	8.21	8.20	8.19	8.16	8.14	8.13	0.39	0.39	0.37	0.41	0.43	0.44
B <sub>6</sub>	8.20	8.19	8.18	8.13	8.13	8.12	0.39	0.39	0.37	0.43	0.44	0.46
B <sub>7</sub>	8.20	8.19	8.17	8.12	8.12	8.10	0.38	0.40	0.38	0.44	0.46	0.48
Mean	<b>8.22</b>	<b>8.20</b>	<b>8.19</b>	<b>8.16</b>	<b>8.15</b>	<b>8.13</b>	<b>0.39</b>	<b>0.39</b>	<b>0.37</b>	<b>0.43</b>	<b>0.44</b>	<b>0.46</b>
C.D. at (5%)	NS	0.009	0.012	0.013	0.009	0.015	NS	NS	0.033	0.033	0.033	0.03
SE(m) <sub>±</sub>	0.007	0.003	0.004	0.004	0.003	0.005	0.005	0.01	0.01	0.01	0.011	0.01

**Table 3. Influence of soil calcareousness and PROM on periodical changes in organic carbon and calcium carbonate content of soil**

Levels of P <sub>2</sub> O <sub>5</sub>	Days After Incubation											
	Organic carbon (%)						Calcium carbonate (%)					
	0	15	30	60	90	120	0	15	30	60	90	120
B <sub>1</sub>	0.55	0.55	0.55	0.58	0.63	0.64	12.42	12.42	12.45	12.37	12.30	12.24
B <sub>2</sub>	0.55	0.56	0.67	0.59	0.65	0.66	12.46	12.42	12.33	12.23	11.81	10.75
B <sub>3</sub>	0.57	0.57	0.65	0.62	0.66	0.68	12.44	12.33	12.36	12.08	11.75	10.58
B <sub>4</sub>	0.57	0.57	0.56	0.64	0.68	0.69	12.44	12.30	12.28	11.82	11.33	10.50
B <sub>5</sub>	0.56	0.58	0.59	0.66	0.70	0.71	12.42	12.17	12.15	11.33	11.00	10.08
B <sub>6</sub>	0.56	0.59	0.61	0.69	0.71	0.73	12.46	12.08	11.91	11.31	10.75	10.07
B <sub>7</sub>	0.57	0.60	0.63	0.70	0.73	0.75	12.42	12.00	11.42	11.25	10.58	9.80
Mean	0.56	0.58	0.61	0.64	0.68	0.70	12.44	12.25	12.13	11.77	11.36	10.58
C.D. at (5%)	NS	0.03	0.032	0.023	0.021	0.027	NS	NS	0.472	0.48	0.455	0.615
SE(m) <sub>±</sub>	0.008	0.01	0.01	0.007	0.007	0.009	0.149	0.176	0.151	0.154	0.146	0.197

**Table 4. Influence of soil calcareousness and PROM on release of nitrogen and phosphorus in soil**

Levels of P <sub>2</sub> O <sub>5</sub>	Days After Incubation											
	Nitrogen (kg ha <sup>-1</sup> )						Phosphorus (kg ha <sup>-1</sup> )					
	0	15	30	60	90	120	0	15	30	60	90	120
B <sub>1</sub>	112.89	122.36	116.63	118.99	120.38	123.61	11.92	11.71	11.95	12.17	12.43	12.68
B <sub>2</sub>	113.71	143.76	145.53	146.28	152.23	158.97	11.90	12.18	12.67	13.02	15.71	16.11
B <sub>3</sub>	112.59	136.31	142.57	143.29	147.77	152.55	11.91	12.14	12.37	12.48	14.97	15.28
B <sub>4</sub>	112.89	123.52	126.29	137.81	139.35	140.38	11.91	11.74	12.22	12.25	12.97	14.03
B <sub>5</sub>	112.49	125.73	137.81	139.35	140.38	142.80	11.92	11.85	12.27	12.37	13.34	14.78
B <sub>6</sub>	112.94	128.23	139.35	140.38	142.80	143.29	11.91	11.95	12.99	14.28	15.76	16.31
B <sub>7</sub>	112.13	129.93	140.29	142.80	150.22	156.34	11.76	12.12	13.00	14.30	15.84	16.33
Mean	112.81	129.98	135.50	138.42	141.87	145.42	11.89	11.95	12.50	12.98	14.43	15.07
C.D. at (5%)	NS	3.952	5.203	0.532	1.049	0.503	NS	0.029	0.02	0.038	0.193	0.052
SE(m) <sub>±</sub>	3.831	1.268	1.67	0.171	0.337	0.162	0.148	0.009	0.006	0.012	0.062	0.017

**Table 5. Influence of soil calcareousness and PROM on potassium and iron in soil**

Levels of P <sub>2</sub> O <sub>5</sub>	Days After Incubation											
	Potassium (kg ha <sup>-1</sup> )						Iron (mg kg <sup>-1</sup> )					
	0	15	30	60	90	120	0	15	30	60	90	120
B <sub>1</sub>	482.05	480.60	482.21	483.30	486.34	486.23	2.90	2.89	2.89	2.89	2.89	2.92
B <sub>2</sub>	484.65	488.83	490.36	493.89	499.02	502.44	2.92	2.90	2.91	2.92	2.93	2.94
B <sub>3</sub>	484.17	486.34	489.76	493.76	496.13	500.09	2.91	2.90	2.90	2.90	2.91	2.92
B <sub>4</sub>	481.73	481.73	484.41	486.34	487.43	488.83	2.91	2.90	2.95	3.01	3.11	3.47
B <sub>5</sub>	482.31	482.21	486.34	487.43	488.83	490.12	2.91	2.91	2.99	3.05	3.18	3.68
B <sub>6</sub>	482.31	483.24	487.43	488.83	491.03	491.26	2.90	2.93	3.04	3.15	3.35	4.01
B <sub>7</sub>	484.17	484.29	488.83	491.26	495.74	498.44	2.90	2.94	3.05	3.16	3.36	4.02
Mean	483.06	483.89	487.05	489.26	492.08	493.92	2.91	2.91	2.96	3.01	3.11	3.42
C.D. at (5%)	NS	NS	0.896	0.89	1.022	0.871	NS	0.029	0.034	0.016	0.011	0.009
SE(m) <sub>±</sub>	4.803	3.423	0.287	0.286	0.328	0.28	0.023	0.009	0.011	0.005	0.003	0.003

**Table 6. Influence of soil calcareousness and PROM on manganese and zinc in soil**

Levels of P <sub>2</sub> O <sub>5</sub>	Days After Incubation											
	Manganese (mg kg <sup>-1</sup> )						Zinc (mg kg <sup>-1</sup> )					
	0	15	30	60	90	120	0	15	30	60	90	120
B <sub>1</sub>	1.99	1.98	1.99	1.99	2.00	2.03	0.41	0.41	0.40	0.41	0.42	0.41
B <sub>2</sub>	2.01	2.00	2.00	2.00	2.01	2.01	0.42	0.43	0.42	0.43	0.43	0.43
B <sub>3</sub>	2.01	1.98	2.01	2.01	1.99	2.01	0.41	0.42	0.41	0.42	0.42	0.42
B <sub>4</sub>	1.97	1.98	2.02	2.03	2.06	2.24	0.41	0.46	0.47	0.52	0.62	0.75
B <sub>5</sub>	1.98	1.99	2.03	2.06	2.25	2.50	0.41	0.49	0.48	0.57	0.78	0.90
B <sub>6</sub>	1.99	2.02	2.04	2.07	2.34	2.53	0.41	0.51	0.55	0.68	0.79	0.91
B <sub>7</sub>	2.00	2.03	2.05	2.08	2.35	2.54	0.40	0.52	0.56	0.69	0.80	0.93
Mean	1.99	2.00	2.02	2.04	2.20	2.27	0.41	0.46	0.47	0.53	0.61	0.68
C.D. at (5%)	NS	0.007	0.008	0.007	0.011	0.01	NS	0.026	0.019	0.063	0.061	0.035
SE(m) <sub>+</sub>	0.008	0.002	0.002	0.002	0.004	0.003	0.005	0.008	0.006	0.02	0.02	0.011

### 3.2.2 Micronutrients

The iron at 0 days of incubation was 2.90 mg kg<sup>-1</sup> and from 15 days onward, application of 100% P<sub>2</sub>O<sub>5</sub> through PROM improved it to 4.02 mg kg<sup>-1</sup> by 120 days, which was at par with 75% P<sub>2</sub>O<sub>5</sub> through PROM. Safarzadeh *et al.* [10] reported that the release of iron from soil samples following the addition of organic compounds increased over time. The manganese in calcareous soil increased from 1.99 mg kg<sup>-1</sup> at 0 days to 2.54 mg kg<sup>-1</sup> at 120 days with 100% P<sub>2</sub>O<sub>5</sub> through PROM, and at par with 75% P<sub>2</sub>O<sub>5</sub> through PROM at all stages. The increased in available manganese after first week of incubation period might be due to reduction of Mn<sup>3+</sup> to Mn<sup>2+</sup> ion because decrease in organic and oxide surfaces during this period with advancement of incubation period [11]. Zinc content in calcareous soil increased from 0.41 mg kg<sup>-1</sup> at 0 days to 0.93 mg kg<sup>-1</sup> at 120 days with 100% P<sub>2</sub>O<sub>5</sub> through PROM, consistently higher than other treatments. The increased in available zinc supplying capacity was

significantly higher in organic manure treated soil compared to untreated soil was also reported by Motaghian and Hosseinpur [12].

Copper content in calcareous soil increased from 1.99 mg kg<sup>-1</sup> at 0 days to 3.02 mg kg<sup>-1</sup> at 120 days with 100% P<sub>2</sub>O<sub>5</sub> through PROM, showing higher levels than other treatments. Copper release through the mineralization of organic carbon from organic waste was responsible for the increase in copper availability in soil was reported by Tella *et al.* [13].

### 3.3 Soil Enzyme Activities as Influenced by Levels of PROM in Highly Calcareous and Low Calcareous Soils

#### 3.3.1 Urease activity

Urease activity at 120 days was observed higher with application of 100% P<sub>2</sub>O<sub>5</sub> through PROM (47.28 µg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> hr<sup>-1</sup>), and at par with

**Table 7. Influence of soil calcareousness and PROM on copper in soil**

Levels of P <sub>2</sub> O <sub>5</sub>	Days After Incubation					
	Copper (mg kg <sup>-1</sup> )					
	0	15	30	60	90	120
B <sub>1</sub>	2.10	2.09	2.10	2.11	2.10	2.10
B <sub>2</sub>	2.10	2.15	2.12	2.10	2.13	2.11
B <sub>3</sub>	2.10	2.12	2.11	2.10	2.12	2.10
B <sub>4</sub>	2.09	2.08	2.14	2.22	2.28	2.41
B <sub>5</sub>	2.10	2.09	2.14	2.28	2.94	2.86
B <sub>6</sub>	2.09	2.13	2.20	2.74	2.96	2.99
B <sub>7</sub>	2.10	2.14	2.22	2.75	2.97	3.02
Mean	1.99	2.11	2.15	2.33	2.50	2.51
C.D. at (5%)	NS	0.021	0.06	0.105	0.075	0.111
SE(m)±	0.019	0.007	0.019	0.034	0.024	0.036

**Table 8. Influence of soil calcareousness and PROM on urease, dehydrogenase and alkaline phosphatase activity of soil**

Levels of P <sub>2</sub> O <sub>5</sub>	Days After Incubation					
	urease		Dehydrogenase		Alkaline phosphatase	
	0	120	0	120	0	120
B <sub>1</sub>	33.06	33.01	14.34	14.52	9.63	12.11
B <sub>2</sub>	33.07	35.21	14.36	16.06	9.69	14.24
B <sub>3</sub>	33.09	36.16	14.46	15.99	9.69	14.66
B <sub>4</sub>	33.08	39.77	14.34	15.84	9.63	15.35
B <sub>5</sub>	33.06	46.97	14.31	15.95	9.67	17.03
B <sub>6</sub>	33.05	47.05	14.24	16.19	9.61	17.70
B <sub>7</sub>	33.09	47.28	14.35	16.26	9.67	17.72
Mean	33.07	40.78	14.34	15.83	9.66	15.55
C.D. at (5%)	NS	1.439	NS	0.209	NS	0.406
SE(m)±	0.067	0.462	0.114	0.067	0.09	0.13

application of 75% and 50% P<sub>2</sub>O<sub>5</sub> through PROM treatments. Srinivasan *et al.* [14-16]. reported that the addition of organic material is good source of energy and carbon to heterotrophs causing an increase in enzymatic activity and a rise in population.

### 3.3.2 Dehydrogenase activity

At 120 days, 100% P<sub>2</sub>O<sub>5</sub> through PROM showed the higher dehydrogenase activity (16.26 µg TPF g<sup>-1</sup> 24 hr<sup>-1</sup>), which was at par with 75% P<sub>2</sub>O<sub>5</sub> through PROM and RDF through DAP. These results were in close confirmation with Rai and Yadav [17-19].

### 3.3.3 Alkaline Phosphatase Activity

Alkaline phosphatase activity at 120 days was highest with 100% P<sub>2</sub>O<sub>5</sub> through PROM (17.72 µg PNP g<sup>-1</sup> hr<sup>-1</sup>), comparable to 75% P<sub>2</sub>O<sub>5</sub> through PROM. Waldrip *et al.* [20,21] reported that organic manure may enhance phosphatase activity by providing soil microbes with carbon, nitrogen, and phosphorus.

## 4. CONCLUSION

Application of Phosphate Rich Organic Manure (PROM) significantly increased availability of all three major macronutrients (nitrogen, phosphorus, and potassium) and micronutrients (iron, manganese, zinc, and copper) in soil as compared to application of recommended dose of fertilizers. Reduction in the soil calcium carbonate is also recorded due to application of 100 % P<sub>2</sub>O<sub>5</sub> using PROM in calcareous soil. Moreover, the application of P<sub>2</sub>O<sub>5</sub> through PROM positively influenced microbial activities, including urease, dehydrogenase, and alkaline phosphatase, suggesting improved soil nutrient cycling and microbial health.

### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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