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# Inference of Rock Phosphate, Phosphorus Solubilizing Bacteria and Lime on Phosphorus Content and Economic Yield of Green Gram

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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

**Aim:** To study the effect of Rock Phosphate, Phosphorus Solubilizing Bacteria, and Lime on phosphorus content in the soil, Phosphorus Uptake, and Economic yield of green gram (var. DGGS-4).
**Study Design:** This experiment was conducted through a completely randomized design with 10 treatments and 3 replications.

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**Place and Duration of Study:** The research was conducted at the Department of Soil Science and Agricultural Chemistry, Central Agricultural University, Imphal between February 2019- November 2019.

**Methodology:** Available phosphorus content in soil was estimated spectrophotometrically by Bray and Kurtz No. 1 method, the Active Phosphorus was determined by the addition of Saloid-bound phosphate, Aluminum phosphate, iron phosphate, and calcium phosphate, and total Inorganic P is the summation of all the inorganic forms of P. Total Phosphorus (Total P) in Soil was estimated by using Murphy-Riley solution and 5M NaOH and the intensity of yellow color was read at 730 nm in a spectrophotometer, organic P was calculated from the difference between total phosphorus and total mineral P, and the uptake of phosphorus was computed from the data on P concentration and dry matter yield.

**Results:** The release and fixation pattern of different forms of phosphorus, its uptake, and the economic yield of green gram were significantly affected by the application of rock phosphate singly or combination with PSB and lime. Comparing among the different treatments, significantly higher accumulation of available Phosphorus, Phosphorus uptake, and economic yield were recorded in soil treated with Rock phosphate in combination with *Phosphocare*, *Bacillus megatherium*, and lime which is at par with treatment with Rock phosphate in combination with *Bacillus megatherium* and lime.

**Conclusion:** The investigation revealed that the release and fixation pattern of different forms of phosphorus, its uptake, and the yield of green gram are significantly affected by the application of RP singly or combination with PSB and lime.

*Keywords: Phosphorus; phosphorus solubilizing bacteria; lime; rock phosphate; economic yield.*

## 1. INTRODUCTION

The word Phosphorus is taken from Greek, the word *phos* means light, and *phoros* means bearer. The word Phosphorus itself gives meaning that it glows because of its slow combustion when it comes in contact with the air. It was discovered in ancient Rome and through the ages it lost its secret, but this is a mystery. It was discovered by German alchemist Henning Brandt in 1669 [1].

Phosphorus is the second essential nutrient for plants next to nitrogen. It is absorbed by the plants in two forms $HPO_4^{2-}$ and $H_2PO_4^-$. It is also known as the key to life because the plant life cycle cannot complete due to its deficiency. It influences plant metabolic processes like signal transduction, photosynthesis, respiration, transport, and storage of energy in the form of Adenosine triphosphate (ATP) and Adenosine diphosphate (ADP) [2]. Various forms of Al, Fe, Mg, and Ca elements combine with inorganic phosphorus making it unavailable for plants and microbes. The organic Phosphorus which is in unavailable form for plants is converted to available form by soil microbes and enzymes released by the plant roots through mineralization processes. Soil P is a finite, non-renewal, and limited resource, and the reserves of P in the world are gradually being depleted [3]. Only 10-20% of the P applied with fertilizer is taken up by plants in the year of application because the majority of applied P is rapidly fixed or precipitated into poorly available forms [4]. The uptake of P from colloidal Al-P is considerably higher than colloidal Fe-P in acid soil which is due to a faster rate of crystallization of Fe-P than Al-P and a greater reduction in the surface area [5]. In India, the Iron-P and aluminum-P were higher in the recent alluvial soils than in old alluvial [6].

In the present agricultural scenario, the high cost of conventional water-soluble phosphatic fertilizers like Single Super Phosphate (SSP) and Di-ammonium Phosphate (DAP) restricts their use in developing countries like India. Thus, phosphatic fertilizers can be substituted by rock phosphates. Crop response to phosphate rock application strongly depends upon rock dissolution rate [7]. The phosphate rock can be recommended for direct use as it is economic, has a longer residual effect, and has potential use in plantation and long duration crops, minimizes phosphorus fixation in acid soils, has considerable liming action by reducing soil acidity, increases the availability of other essential nutrient elements including calcium to plants [8]. Phosphorus availability can be improved from rock phosphate by using microbial processes [9].

Non-symbiotic bacteria which are closely associated with plant roots improve the growth and development of plants by different mechanisms and are called plant growth-promoting rhizobacteria [10,11,12]. Phosphorus Solubilizing Bacteria (PSB) plays a sustainable and environmentally friendly role in dissolving phosphatic fertilizers and bound phosphorus thereby enhancing plant growth, phosphorus uptake, and yield [13,14,15,16]. In India 49 million ha of acid below 5.6 pH and 23 million ha between 5.6 pH and 6.5 pH [17]. To overcome the problem of low Phosphorus availability due to high phosphorus fixation, the suggested approaches are a selection of suitable crops adaptive to soil acidity [18] and amelioration of soil acidity through liming [19]. Liming improves the base saturation of the soil, increases the soil pH to near neutrality, inactivates Al, Fe, and Mn, reduces P fixation [20], and stimulates microbial activity leading to the mineralization of organic nitrogen and fixation of atmospheric nitrogen. There is a need for raising the soil pH beyond the point of neutralizing exchangeable aluminum, particularly for legumes [21].

Green gram (*Vigna radiate* L.) is popularly known as "Moong Dal" in India and is a tiny circular-shaped bean that is green in color. It is one of the main pulse crops in India. It belongs to the family Leguminosae and the subfamily Papilionaceae. It is an erect sub-erect deep-rooted, much-branched, somewhat hairy annual herb with a height ranging from 30-130 cm. Leaves are alternate, trifoliate, petiole long, stipules ovate, leaflets ovate up to 12x10 cm. Flowers are in axillary racemes, peduncles up to 13 cm in length with clusters of 10-12 flowers, corolla yellow in color sometimes curved, 5-10 cm long. The seeds contain a higher proportion of lysine than any other legume seeds. The seeds are processed and consumed as cooked whole beans or splits (dhals), sprouts, immature seeds, and flour and are used in various recipes. The objective is to study the effect of applied rock Phosphate Phosphorus Solubilizing Bacteria, and lime on phosphorus content and economic yield of green gram.

## 2. MATERIALS AND METHODS

During the *Pre-Kharif* season of 2021, the investigation was conducted in pots at the Department of Soil Science and Agricultural Chemistry, College of Agriculture, CAU, Imphal to investigate the effect of applied rock phosphate in the presence or absence of phosphorus solubilizing bacteria and lime on phosphorus content and economic yield of Green Gram (var. DGGS-4). The experiment was conducted in a completely randomized block design replicated thrice. The treatments were as follows:

| | | |
|---|---|---|
| $T_1$ | = | Control |
| $T_2$ | = | 100 % RD* of $P_2O_5$ from SSP* |
| $T_3$ | = | 100 % RD of $P_2O_5$ from RP* |
| $T_4$ | = | 100 % RD of $P_2O_5$ from RP + $PSB_1$* |
| $T_5$ | = | 100 % RD of $P_2O_5$ from RP + $PSB_2$* |
| $T_6$ | = | 100 % RD of $P_2O_5$ from RP + $PSB_1$ +$PSB_2$ |
| $T_7$ | = | 100 % RD of $P_2O_5$ from RP + Lime |
| $T_8$ | = | 100 % RD of $P_2O_5$ from RP + $PSB_1$ + Lime |
| $T_9$ | = | 100 % RD of $P_2O_5$ from RP + $PSB_2$+ Lime |
| $T_{10}$ | = | 100 % RD of $P_2O_5$ from RP + $PSB_1$ +$PSB_2$ + Lime |

**SSP - Single super phosphate*
**RP - Rock phosphate*
** $PSB_1$ – Phosphocare*
**PSB - Phosphorus solubilizing bacteria*
**RD- Recommended dose*
** PSB2 – Bacillus megatherium*

Each of the pots was filled with 5 Kg of air-dried soil. In each experimental pot, a recommended dose of 20 kilograms of N $ha^{-1}$ in the form of urea and 20 kg $K_2O$ $ha^{-1}$ in the form of muriatic potash was applied and thoroughly mixed with the soil. Rock phosphate and SSP were administered to the pots as phosphorus sources according to different sets of treatments based on the prescribed amount (40 kg $P_2O_5$ $ha^{-1}$) for the test crop green gram (variety DGGS-4). Two PSBs were used to treat green gram seeds. $PSB_1$ (Commercial) strain from the market and $PSB_2$ strain from the lab (*Bacillus megatherium*). The inoculated seeds were dried in the shade and sowed as soon as they were dried for 12 hours. In each pot, five green gram seeds were sowed. Following germination, a single seedling was retained throughout the experiment. The soils of each treatment were humidified at 60% of the water-holding capacity during the entire experiment.

The soil samples were collected on the $0^{th}$, $15^{th}$, $30^{th}$, $45^{th}$, and $60^{th}$ days after sowing seeds and at harvest from the rhizosphere region of a green gram to estimate the phosphorus content. The lime requirement is calculated by SMP (Shoemaker Mclean Pratt) buffer method [22], two weeks before liming is done to the soil so that it reacts with soil mass according to different sets of treatments. The pH of the soil was estimated by using a glass electrode Systronic pH meter with a water suspension ratio of 1:2.5 as described by Jackson [23].

Available phosphorus content in soil was estimated spectrophotometrically by Bray and Kurtz No. 1 method as described by Bray and Kurtz [24]. The whole seeds were used to record the economic yield after drying at 65 to 70°C to constant dry weight. The economic yield was recorded and expressed in grams per plant.

### 2.1 Active P

The Active Phosphorus was determined by the addition of Saloid-bound phosphate, Aluminum phosphate, iron phosphate, and calcium phosphate [25].

### 2.2 Total Inorganic P

Inorganic P is the summation of all the inorganic forms of P and the inorganic P fractions were determined by ascorbic acid method [26].

### 2.3 Total Phosphorus (Total P) in Soil

Two grams of 0.5 mm sieved soil were weighed and transferred to a 300 ml platinum crucible and 30 ml of 60 percent $HClO_4$ was added and digestion was carried out on a sand bath at 150°C till the dense fumes of $HClO_4$ evolved. When digestion was completed, the flask was removed and cooled. 50 ml of distilled water was added to the flask and the solution was filtered into a 250 ml volumetric flask and volume was made with distilled water. An aliquot from this was used for estimation of total P by using Murphy-Riley solution and 5M NaOH and the intensity of yellow color was read at 730 nm in a spectrophotometer [23].

### 2.4 Organic Phosphorus

The organic P was calculated from the difference between total phosphorus and total mineral P as suggested by Mehta et al. [27].

### 2.5 Phosphorus Uptake

The uptake of phosphorus was computed from the data on P concentration and dry matter yield using the formula

P uptake in plant (mg plant$^{-1}$) = P conc.in plant (mg kg$^{-1}$) x dry matter yield (g plant$^{-1}$) x (1/1000)

Data obtained from the experiment were statistically analyzed through the analysis of variance technique for comparing the effects of the treatments. The significance of various effects was tested at a 5% level of probability [28].

## 3. RESULTS AND DISCUSSION

### 3.1 Active-P

Data on the amount of Active-P in green gram grown in soil fertilized with rock phosphate in the presence or absence of PSB and lime are illustrated in (Fig. 1). Results signified that an increasing trend of Active - P up to the 30$^{th}$ day followed by a decline till harvest was observed in all the treatments except in $T_4$ where it increases at the 15$^{th}$ day and reduces till harvest. This increase might be due to the transformation of applied P into less soluble forms showing that the P fixing capacity of the soil increases [29,30,31]. However, the decrease in the concentration might be due to crop utilization [32,33,34,35,36]. A critical study of the data showed that irrespective of different treatments and crop growth stages significantly higher Active-P was accumulated more in rock phosphate fertilized soil in the presence or absence of PSB and lime over untreated control. Similar findings were recorded by Tiecher, Dos Santos, and Calegari [37] and Tian [38]. Among all the treatments most significant treatment is $T_3$ followed by $T_4$ and $T_2$ on the 60$^{th}$ day and at harvest. Comparatively, a higher concentration of Active-P was observed in soil treated with $T_4$ which is at par with $T_3$ and $T_6$ on the 15$^{th}$ and 30$^{th}$ days after sowing, respectively. The treatments which are applied with PSB and lime show significantly lesser concentrations of Active-P compared with rock phosphate-added soil without lime and PSB. The addition of PSB and lime reduces P fixation in soil.

### 3.2 Total Inorganic –P

Data illustrated in (Fig. 2) show changes in the amount of total Inorganic-P in green gram grown in soil added with rock phosphate, PSB, and lime. Results revealed that Inorganic-P content increased up to the 30$^{th}$ day and decline till harvest in all the treatments except in $T_1$, $T_5$, $T_6$, and $T_{10}$ which show an increase up to the 45$^{th}$ day and decrease till harvest. The increasing trend might be due to the transformation of applied P or organic P to inorganic forms [29] [30] [31]. The decline might be due to the release of these forms into available P and finally, uptake by green gram [34] [35]. Statistically more accumulation of total Inorganic-P was observed in all treatments with respect to control at different stages of crop growth. A similar finding on higher content of total Inorganic-P in P-treated soil was also reported by Jalali and Ranjbar and others [39,37,31,36]. Among all the treatments, $T_4$ shows the maximum amount of total inorganic P followed by $T_3$ and $T_2$ on the 30$^{th}$ day and at harvest, respectively. No significant difference was observed between $T_4$ and $T_3$ on the 15$^{th}$, 45$^{th}$, and 60$^{th}$ days.

### 3.3 Available-P

Data on changes in the amount of Available-P in green gram grown in soil added with rock phosphate, PSB, and lime were shown in (Fig. 3). Available-P concentration reached a maximum on the 30$^{th}$ day followed by a decline up to harvest in all the treatments. The increase indicated the release of phosphorus into the available form [40,41,42]. The decrease might be due to the fixation/adsorption of phosphorus onto Fe and Al oxides in acid soils and by the formation of Fe and Al phosphate complexes [43] or phosphorus uptake by crops [34,35]. Further, the results revealed that irrespective of different treatments and sampling stages, there was a significant increase in available P in rock phosphate fertilized soil in the presence or absence of PSB and lime over control. This is at par with the findings of Singh [44], Jalali and Ranjbar [39], and Wang [45]. An increase in phosphorus availability due to rock phosphate application was also reported earlier by Laskar [40]. The detailed study revealed that the maximum amount of available P was recorded in $T_{10}$ followed by $T_9$ on the 15 and 30$^{th}$ day. A comparatively higher concentration of Available-P was found in $T_{10}$ sowing parity with $T_9$ and $T_8$ on the 45$^{th}$ day and at harvest. Irrespective of different sampling stages treatments applied along with lime show a significantly higher concentration of Available-P over unlime treatments. This shows that liming can increase phosphorus availability by stimulating the mineralization of soil organic phosphorus [46,47]. It was also observed that irrespective of liming, the addition of PSB comparatively enhanced available P content over sole rock phosphate treatment from the 30$^{th}$ day onwards till harvest, and similar results were obtained by Sundra, Natarajan, and Hari [48]. PSB plays an important role in dissolving both fertilizer phosphorus and bound P in soil that is environmentally friendly and sustainable. P solubilization is mainly due to the reaction between organic acids executed with phosphate binders such as Al, Fe, and Ca, or Mg to form stable organic chelates to free the bound phosphate ion [13].

### 3.4 Organic-P

Data on changes in the amount of Organic-P in green gram grown in soil applied with rock phosphate, PSB, and lime was illustrated in (Fig. 4). Results revealed that irrespective of different treatments Organic-P concentration declined at harvest as compared with the initial value. This shows the mineralization of Organic-P [49]. Higher amount of Organic-P was accumulated in untreated soil when compared to phosphorus-treated soil except at zero days of sowing. This might be due to a higher rate of P-mineralization in soil treated with RP as compared to untreated soil [49]. In general, treatments that are applied without lime show significantly lesser Organic-P over the treatments which are applied with lime on the 30$^{th}$ day. Irrespective of liming, rock phosphate applied gave a significantly lesser concentration of Organic-P over the treatments without PSB at harvest. Comparing the lime-treated system at harvest combined application of $PSB_1$ and $PSB_2$ recorded lower organic P content. Reports are also given that mixed inoculations of phosphate-solubilizing microorganisms enhanced the mineralization of organic phosphate [50,51].

### 3.5 Total Phosphorus (P) in Soil

Data on changes in Total-P concentration in green gram grown in soil added with rock phosphate, PSB, and lime were illustrated in (Fig. 5). The data revealed that irrespective of different treatments and sampling stages Total-P declined gradually till harvest. Total-P was recorded more in P treatments when compared to control at different stages of crop growth. Similar reports were also presented earlier [45,52,37]. Irrespective of lime and PSB addition no significant difference in Total-P value was recorded in RP-treated soils on the 15$^{th}$, 30$^{th}$, 45$^{th}$, and 60$^{th}$ DAS. However, further study of the data revealed that a statistically higher content of Total-P was found in $T_7$ followed by $T_8$ at harvest. The treatments applied along with lime showed significantly lesser accumulation of Total-P compared to the corresponding similar treatment without lime at harvest. It was also observed that comparing the RP-treated soils with or without lime separately, soil applied with the two PSBs in the combination showed significantly fewer concentrations of total P over non-PSB and single PSB treatments at harvest.

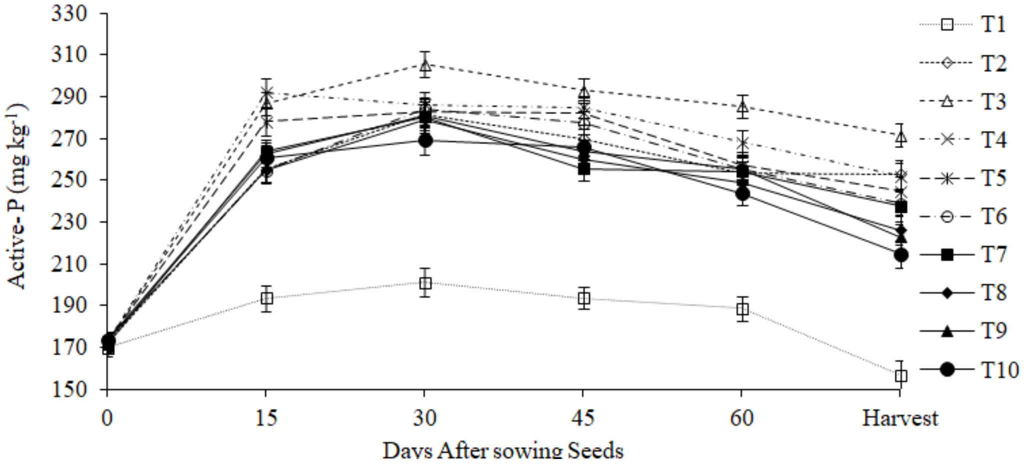


**Fig. 1. Changes in Active- P (mg kg$^{-1}$) the content in green gram grown in rock phosphate fertilized soil applied with PSB and lime (error bar shows the standard error of the mean)**

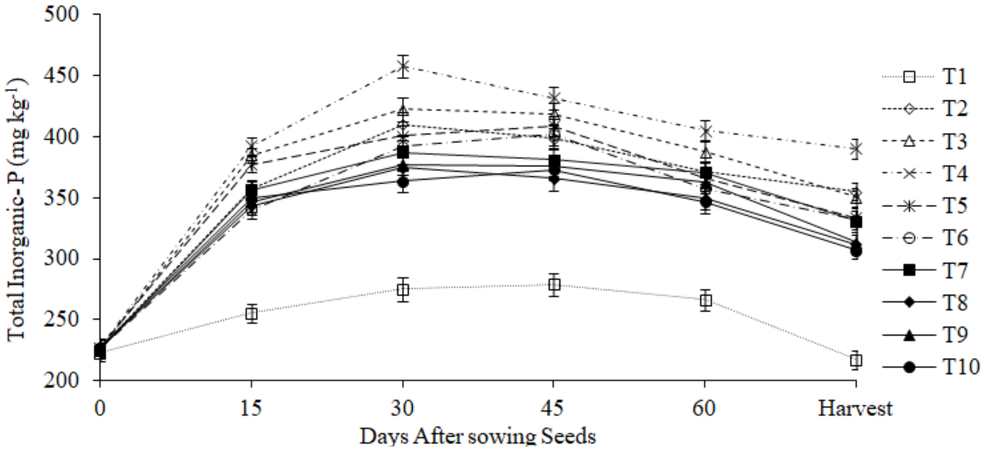


**Fig. 2. Changes in Total Inorganic- P (mg kg$^{-1}$) the content in green gram grown in rock phosphate fertilized soil applied with PSB and lime (error bar shows the standard error of the mean)**

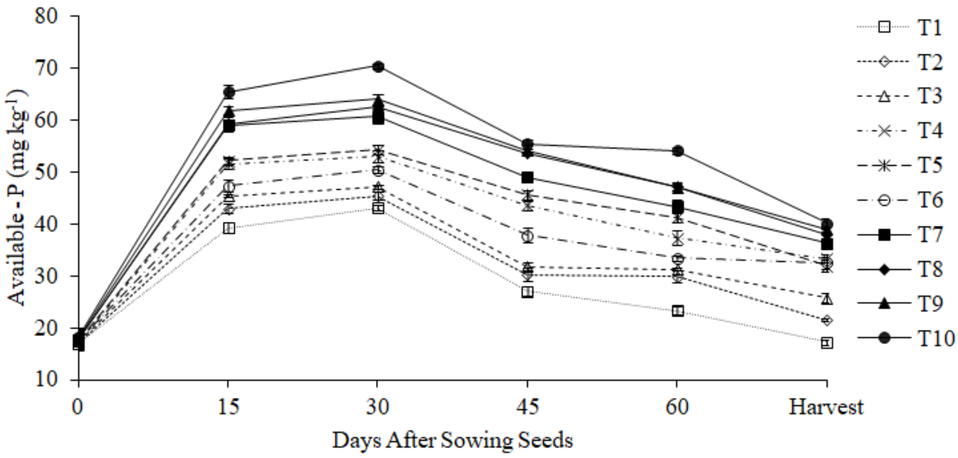


**Fig. 3. Changes in Available P (mg kg$^{-1}$) content in green gram grown in rock phosphate fertilized soil applied with PSB and lime (error bar shows the standard error of the mean)**

### 3.6 P-Uptake

Data on P-Uptake by green gram grown in soil fertilized with rock phosphate in the presence or absence of PSB and lime are illustrated in (Fig. 6). The data revealed that irrespective of different treatments there was an increasing trend of P-uptake by green gram up to harvest. This increase in uptake with crop age was examined earlier by Ikerra, Mnkeni, and Singh [53] and Setia and Sharma [54]. Statistically higher amount of P-uptake was observed in all P treatments when compared to control at different crop growth stages. This is at par with reported findings [32,34]. Further investigation signified that significantly higher P uptake by green gram was found in $T_{10}$ which is followed by $T_9$ on the 30$^{th}$ and 60$^{th}$ day and at harvest. On the 15$^{th}$ and 45$^{th}$ DAS, comparatively higher P uptake was found in soil applied with $T_{10}$ which is statistically at par with $T_9$. Comparing RP-added limed and unlimed treated systems, it was found that liming significantly increased P-uptake over related similar treatments without lime.

### 3.7 Economic Yield

Data pertaining to the Economic Yield of green gram grown in soil fertilized with rock phosphate in the presence or absence of PSB and lime were illustrated in (Fig. 7). The result revealed that a statistically higher yield was recorded in green gram grown in P treated systems as compared to the control. This is at par with the several records [55,34,56,15,36]. Statistically, the maximum economic yield was found in soil treated with $T_{10}$ showing parity with $T_9$ and $T_8$. RP-treated soil applied with lime gave statistically more yield when compared to the remaining unlimed treatments. This shows that RP applied with PSB and lime gave a higher economic yield than the unlimed soils.

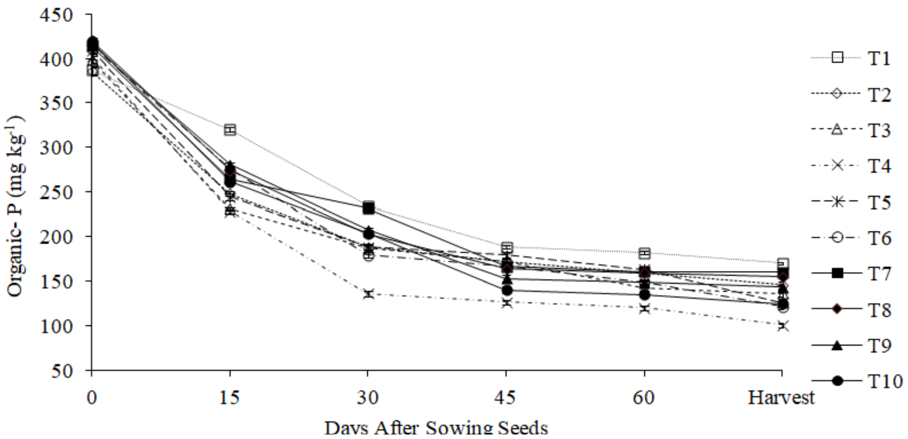


**Fig. 4. Changes in Organic P (mg kg$^{-1}$) content in green gram grown in rock phosphate fertilized soil applied with PSB and lime (error bar shows the standard error of the mean)**

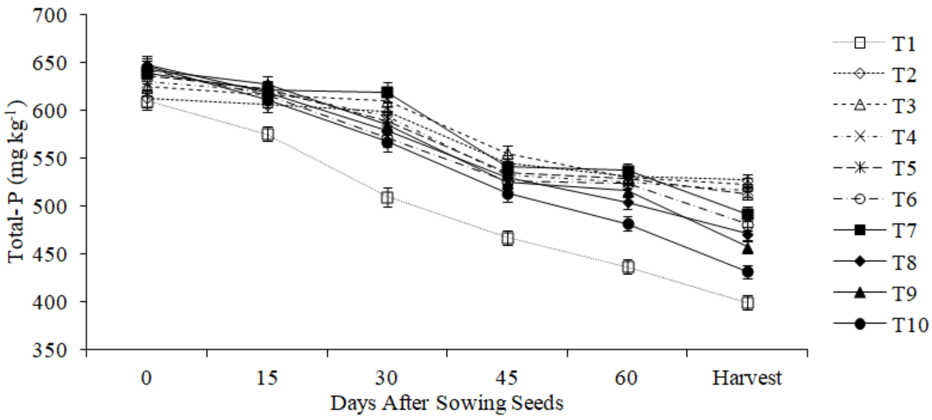


**Fig. 5. Changes in Total P (mg kg$^{-1}$) content in green gram grown in rock phosphate fertilized soil applied with PSB and lime (error bar shows the standard error of the mean)**

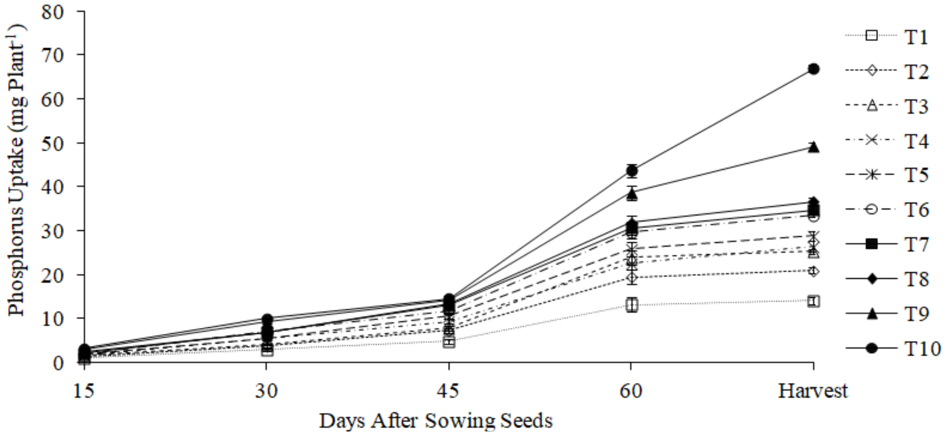


**Fig. 6. Phosphorus Uptake (mg Plant$^{-1}$) by green gram grown in rock phosphate fertilized soil applied with phosphorus solubilizing bacteria and lime (error bar shows the standard error of the mean)**

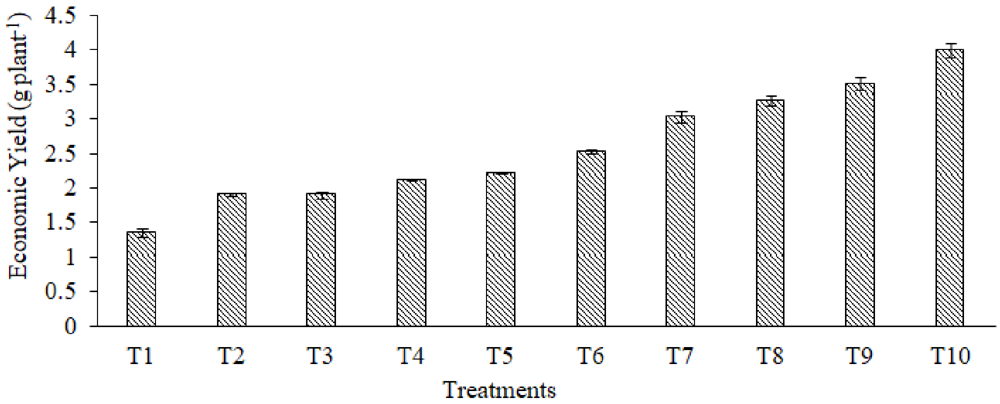


**Fig. 7. Economic Yield (g Plant$^{-1}$) of green gram grown in rock phosphate fertilized soil applied with phosphorus solubilizing bacteria and lime (error bar shows the standard error of the mean)**

## 4. CONCLUSION

Irrespective of the different treatments and sampling stages, all the P treatments showed higher accumulation of active P, total inorganic P, available P, P uptake, and economic yield when compared to the control whereas total P and Organic P reduces. The treatment which includes the combination 'RP+PSB+lime' showed statistically more accumulation of available P, P uptake, and economic yield of green gram. The application of phosphorus sources results in accelerating different forms of P content in the soil. Among different treatments increased P-uptake and economic yield was seen more in Rock phosphate applied along with PSB and lime. The investigation revealed that the release and fixation pattern of different forms of phosphorus, its uptake, and the yield of a green gram are significantly affected by the application of RP singly or combination with PSB and lime.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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