__Original Research Article__

Inference of Rock Phosphate, Phosphorus Solubilizing Bacteria and Lime on phosphorus content and Economic yield of green gram

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.

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, Aluminium phosphate, iron phosphate, and calcium phosphate, 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, 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 either singly or in combination with Phosphorus Solubilizing Bacteria and lime. Comparing among the different treatments, significantly higher accumulation of Available Phosphorus and 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 yield of green gram are significantly affected by the application of RP either singly or in combination with PSB and lime.

Keywords: Phosphorus, Phosphorus Solubilizing Bacteria, lime, and Rock phosphate and 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 a meaning that it glows because of its slow combustion when it comes in contact with the air. It was discovered in ancient Rome than through 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 the plant metabolic processes like signal transduction, photosynthesis, respiration, transport, and storage of energy in the form of ATP and ADP [2]. Various forms of Al, Fe, Mg, Ca elements combines with inorganic phosphorus and make 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 greater reduction in surface area [5]. In Indian, the iron-P and aluminum-P were higher in the recent alluvial soils than in olds alluvial [6].

In the present agricultural scenario, the high cost of conventional water-soluble phosphatic fertilizers like SSP and DAP restricts their use in developing countries like India. Thus, phosphatic fertilizers can be substituted by rock phosphates. Crop response to phosphate rock application is strongly dependent upon the rate of dissolution of rock [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 are called plant growth-promoting rhizobacteria [10, 11, 12]. 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] amelioration of soil acidity through liming [19]. Liming improves base saturation of the soil, increases the soil pH to near neutrality, inactivates Al, Fe, and Mn. reduce P fixation [20], stimulates microbial activity leading to mineralization of organic nitrogen and fixation 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 sub-family 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, peduncle 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.

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 *PSB - Phosphorus Solubilizing Bacteria

*RP - Rock Phosphate *RD- Recommended Dose

* PSB_1 – *Phosphocare* * PSB_2 – *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 muriate of 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 treatment 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 dry. 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 [23].

Available phosphorus content in soil was estimated spectrophotometrically by Bray and Kurtz No. 1 method as described by [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, Aluminium phosphate, iron phosphate, and calcium phosphate [25].

2.2 Total Inorganic P

Inorganic P is the summation of all the inorganic forms of P.

2.3 Total Phosphorus (Total P) in Soil

Two grams of 0.5 mm sieved soil was 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 [26].

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 analysis of variance technique for comparing the effects of the treatments. The significance of various effects was tested at a 5% level of probability [27].

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 (figure1). Results signified that an increasing trend of Active - P up to 30th day followed by a decline till harvest was observed in all the treatments except in T_4 where it increases at 15th 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 [28, 29, 30]. However, the decrease in the concentration might be due to crop utilization [31, 32, 33, 34, 35]. 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. This is at similarity with the findings of Tiecher, Dos Santos, and Calegari [36] and Tian [37]. Among all the treatments most significant treatment is T_3 followed by T_4 and T_2 on 60th day and at harvest. Comparatively, 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 15th and 30th days after sowing respectively. The treatments which are applied with PSB and lime show significantly lesser concentration of Active-P compared with rock phosphate added soil without lime and PSB. The addition of PSB and lime reduces P fixation in soil.

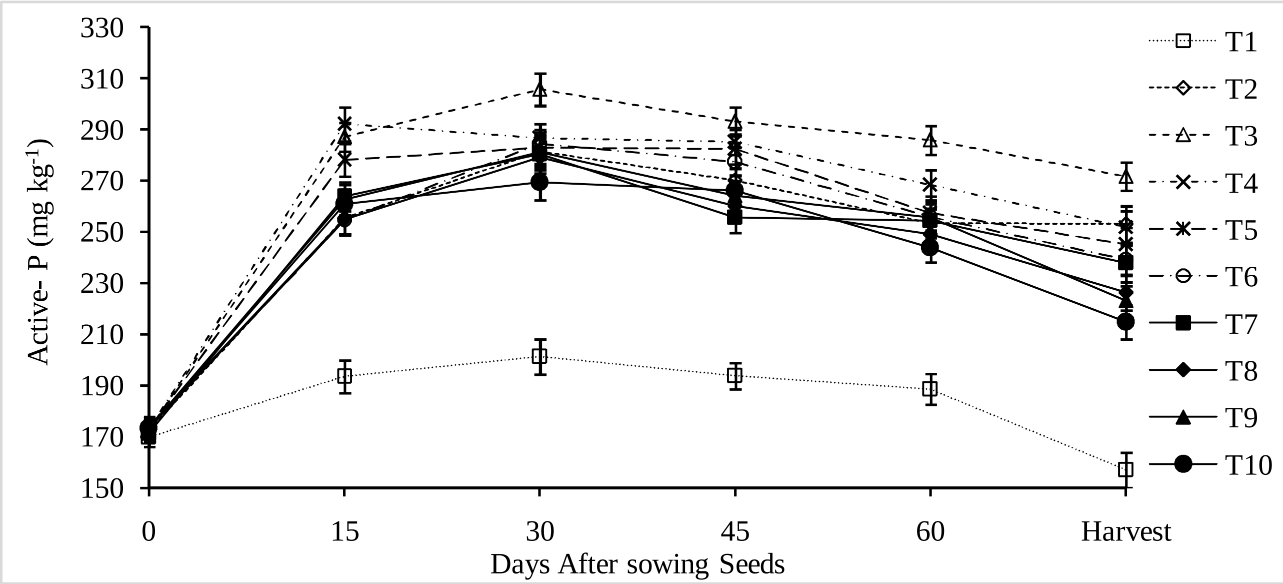


Figure 1: Changes in Active- P (mg kg^{-1}) content in green gram grown in rock phosphate fertilized soil applied with PSB and lime (error bar shows the standard error of mean)

3.2 Total Inorganic –P

Data illustrated in (figure 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 30th day and decline till harvest in all the treatments except in T_1, T_5, T_6, and T_{10} which shows an increase up to 45th day and decrease till harvest. The increasing trend might be due to the transformation of applied P or organic P to inorganic forms [28] [29] [30]. The decline might be due to the release of these forms into available P and finally uptake by green gram [33] [34]. Statistically more accumulation of total Inorganic-P was observed in all treatments with respect to control at different stages of crop growth. Similar finding on higher content of total Inorganic-P in P treated soil was also reported by Jalali and Ranjbar [38, 36, 30, 37] Among all the treatments T_4 shows the maximum amount of total inorganic P followed by T_3 and T_2 on the 30th day and at harvest, respectively. No significant difference was observed between T_4 and T_3 on the 15th, 45th, and 60th days.

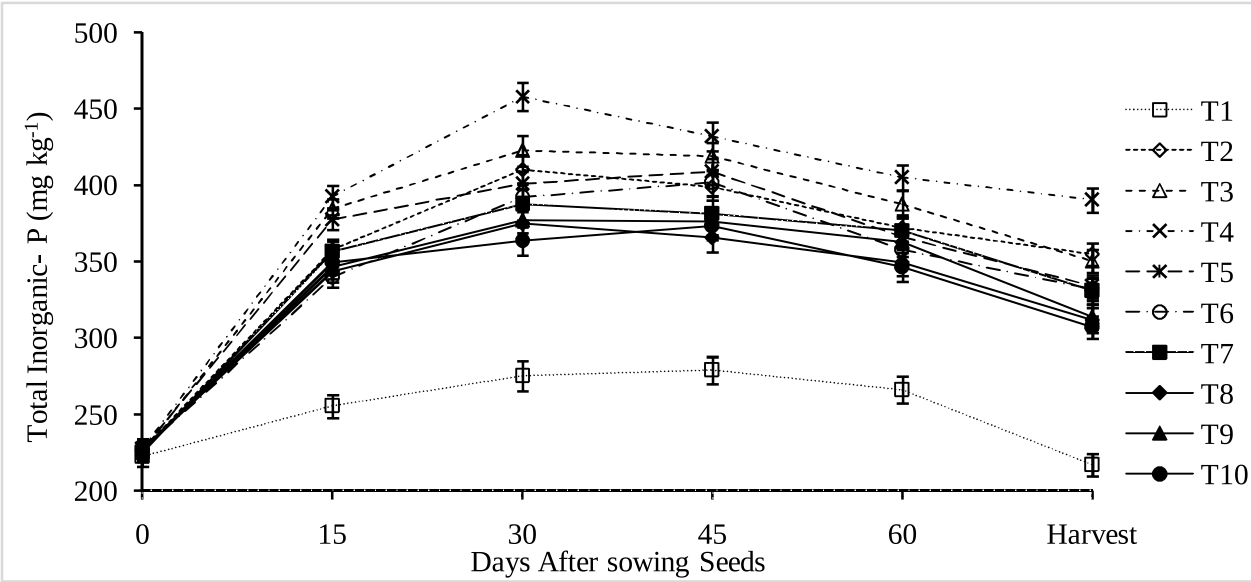
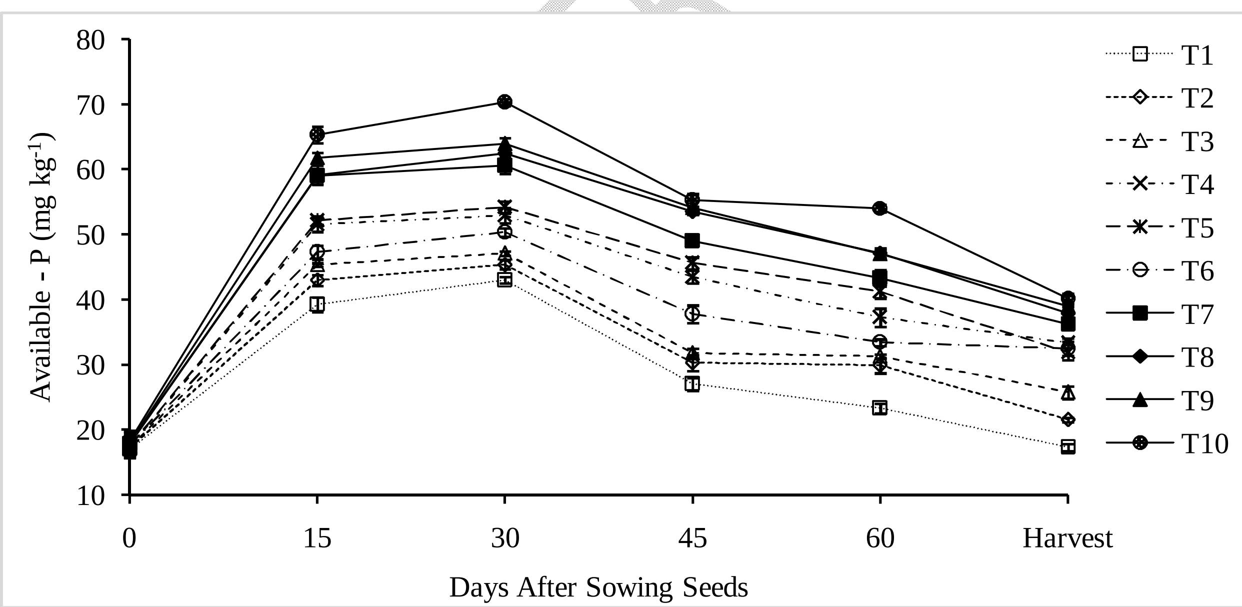


Figure 2: Changes in Total Inorganic- P (mg kg^{-1}) content in green gram grown in rock phosphate fertilized soil applied with PSB and lime (error bar shows the standard error of mean)

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 (figure 3). Available-P concentration reached maximum on the 30th day followed by a decline up to harvest in all the treatments. The increase indicated the release of phosphorus into available form [39, 40, 41]. The decrease might be due to fixation/adsorption of phosphorus onto Fe and Al oxides in acid soils and by the formation of Fe and Al phosphate complexes [42] or phosphorus uptake by crop [33, 34]. 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 [43]; Jalali and Ranjbar [38] and Wang [44]. An increase in phosphorus availability due to rock phosphate application was also reported earlier by Laskar [39]. 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. Comparatively higher concentration of Available-P was found in T_{10} sowing parity with T_9 and T_8 on 45^th^ day and at harvest. Irrespective of different sampling stages treatments applied along with lime show significantly higher concentration of Available-P over unlime treatments. This shows that liming can increase phosphorus availability by stimulating mineralization of soil organic phosphorus [45, 46]. It was also observed that irrespective of liming, the addition of PSB comparatively enhanced available P content over sole rock phosphate treatment from 30^th^ day onwards till harvest, and similar results were obtained by Sundra, Natarajan, and Hari [47]. PSB plays an important role in dissolving both fertilizer phosphorus and bound P in the 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

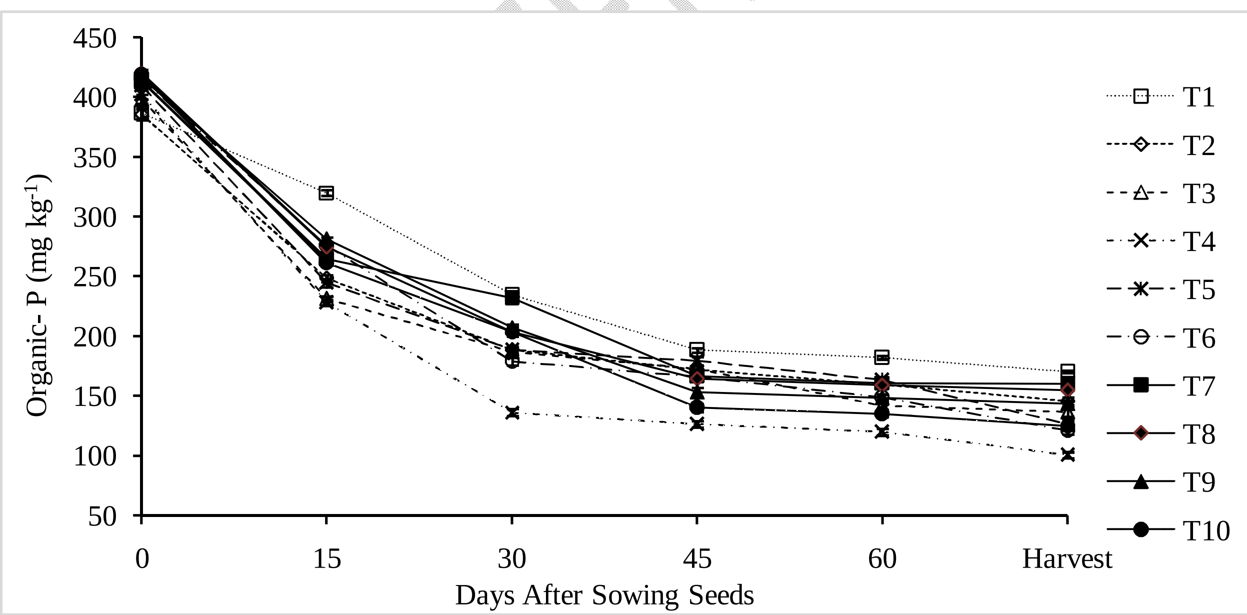


organic chelates to free the bound phosphate ion [13].

Figure 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 mean)

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 (figure 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 [48]. 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 higher rate of P-mineralization in soil treated with RP as compared to untreated soil [48]. In general, treatments that are applied along without lime shown significantly lesser Organic-P over the treatments which are applied with lime on the 30th day. Irrespective liming, rock phosphate applied with gave significantly lesser concentration of Organic-P over the treatments without PSB at harvest. Comparing among 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 [49, 50].

Figure 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 mean)

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 (figure 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 by [44, 51, 36]. Irrespective of lime and PSB addition no significant difference in Total-P value was recorded in RP treated soils on 15th, 30th, 45th, and 60th DAS. However, further study of the data revealed that statistically higher content of Total-P was found in T_7 followed by T_8 at harvest. The treatments applied along with lime shown significantly lesser accumulation of Total-P compared to the corresponding similar treatment without lime at harvest. It was also observed that comparing between the RP treated soils with or without lime separately, soil applied with the two PSBs in combination shown significantly fewer concentration of total P over non PSB and single PSB treatments at harvest.

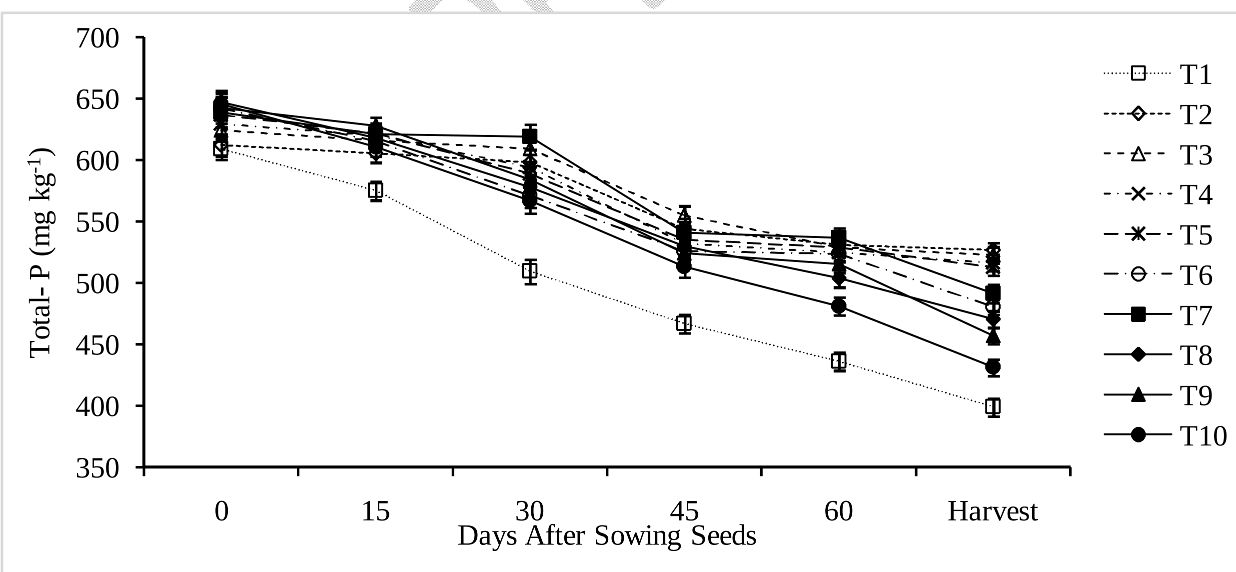


Figure 5: Changes in Total P (mg kg^{-1}) content in green gram grown in rock phosphate fertilized soil applied with PSB and lime (error bar shows the standard error of mean)

3.6 P-Uptake

Data on P-Uptake by green gram grown in soil fertilized with rock phosphate in presence or absence of PSB and lime are illustrated in (figure 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 [52] and Setia and Sharma [53]. 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 the findings of [31, 33]. Further investigation signified that significantly higher P uptake by green gram was found in T_{10} which is followed by T_9 on 30th and 60th day and at harvest. On 15th and 45th DAS, comparatively higher P uptake was found in soil applied with T_{10} which is statistically at par withT_9. Comparing between RP added limed and unlimed treated system, it was found that liming significantly increased P-uptake over related similar treatment without lime.

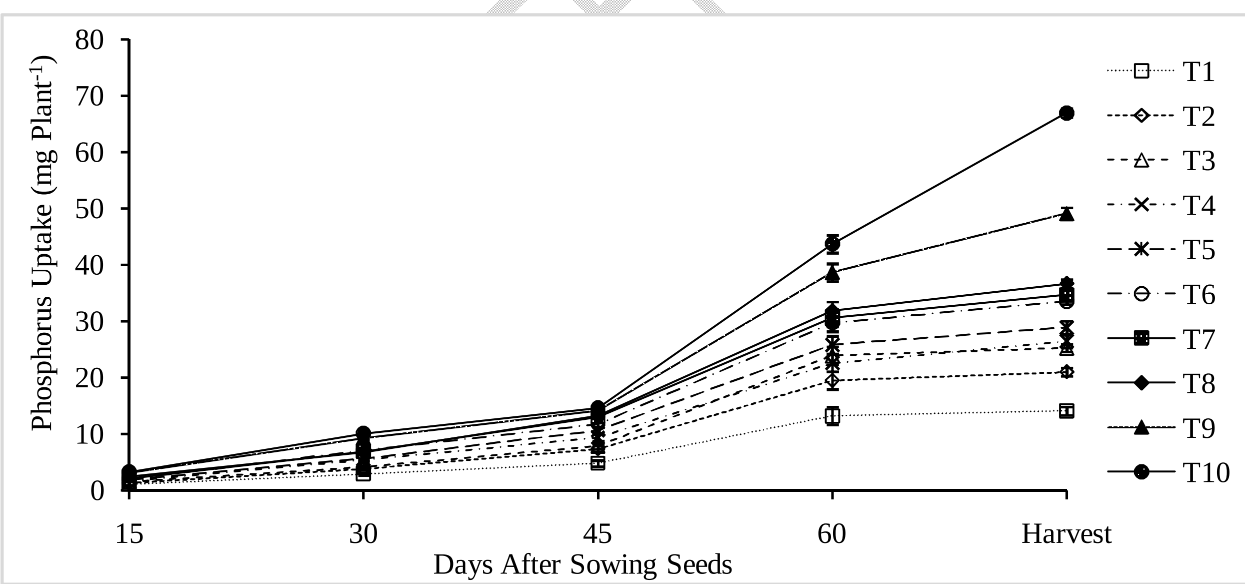


Figure 6: Phosphorus Uptake (mg Plant^{-1}) by green gram grown in rock phosphate fertilized soil applied with phosphorus solubilizing bacteria and lime (error bar shows the standard error of mean)

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 (figure 7). The result revealed that statistically higher yield was recorded in green gram grown in P treated systems as compared to control. This is at par with the records of [54, 33, 55, 15, 35]. Statistically 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 higher economic yield than the unlimed soils.

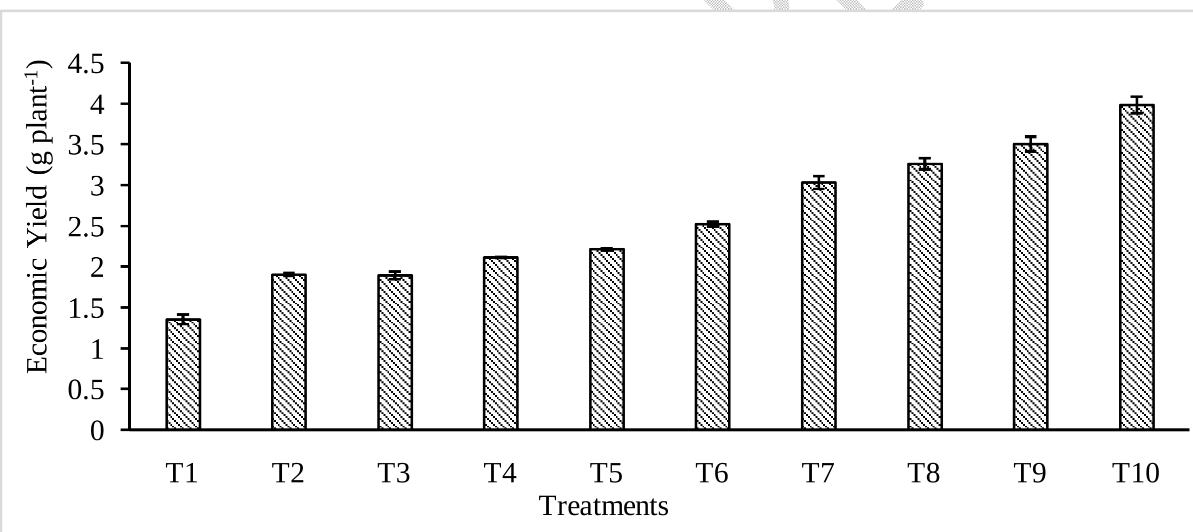


Figure 7: Economic Yield (g Plant^{-1}) of green gram grown in rock phosphate fertilized soil applied with phosphorus solubilizing bacteria and lime (error bar shows the standard error of mean)

4. Conclusion

Irrespective of 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 control whereas total P and Organic P reduces. The treatment which includes the combination of RP+PSB+lime showed statistically more accumulation of available P, P uptake, and economic yield of green gram. 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 yield of green gram are significantly affected by the application of RP either singly or in combination with PSB and lime.

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