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Replacing super phosphate with Tricalcium phosphate enhances Bhendi growth

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Abstract: The constant use of synthetic fertilizers in agriculture has lead to adverse environmental problems besides the increasing cost of production. Field experiments to enhance the available phosphorus, soil fertility and plant growth promotion have not been studied with vegetable crops. The impact of replacing super phosphate with tricalcium phosphate in the presence of phosphate solubilizing bacteria on the growth of bhendi plant was studied. *Bacillus* species isolated from the rhizosphere soil found to be good in releasing phosphate from tricalcium phosphate. Application of super phosphate and tricalcium phosphate at 1:3 ratios along with phosphate solubilising bacteria portrayed increase in the soil and leaf NPK, chlorophyll content, plant height, weight, number of leaves, acidic pH of soil and phosphatase activity which are discussed.

Keywords: Super phosphate; Tricalcium phosphate; Phosphatase; Phosphate solubilizing bacteria; Bhendi growth.

I. INTRODUCTION

Phosphorus (P) is an essential nutrient for the growth and reproduction of the plants. It is available in soil as organic and inorganic P which account for 0.05%. Nearly 0.1% of the total P is available as soluble inorganic phosphate ($H_2PO_4^-$ or HPO_4^{2-}) [1]. It has been reported by many researchers that P found to accumulate [2] either on the surface of soil particles or incorporated into soil particles [3]. Further reactions with soil constituents remove P from the solution, letting P unavailable and end-up with sedimentary cycle. Soil constituents and Fe/Al oxides provides surface areas for adsorption and it also gets absorbed to nanopores [4].

Ever increasing population creates tremendous pressure to produce food with the limited fertile land. Huge numbers of fertilizers are used to meet the substantial amount of yields [5]. About 32711 million tones of fertilizers are applied to the soil annually and surprisingly only about 1.0 mg kg^{-1} soil of the applied phosphate enters the plant system. Unfortunately, the available pool of soil phosphorus does not change after the addition of fertilizer for long periods. The frequent use of fertilizers cause detrimental effects to environmental, disturbing the ecological balance in soils, thus making the plants even more susceptible to pests. The exigency to enhance the efficiency of phosphorus uptake thereby minimizing environment pollution with the production of agricultural products at cheaper rate is candidly understood [6].

Many researchers have suggested that tricalcium phosphate (TCP) can be used in the place of synthetic fertilizer since they are natural and inexpensive [7]. However, tricalcium phosphate being the source for all phosphorus fertilizers and is a non-renewable resource increasingly scarce and expensive. Many researchers have stated that the reserve rock phosphate may be depleted in another 50 years [8]. Rodriguez and Fraga,[9] suggest that *Rhizobium, Pseudomonas* and *Bacillus* species are predominant species among phosphate solubilizers. It was also reported that tricalcium phosphate and hydroxyapatite appears to be more degradable substrates than rock phosphate. In this scenario**,** the use of PSB along with phosphate fertilizers could be an effective for enhancing P uptakes. Combined application of Phosphate solubilizing bacteria (PSB) along with fertilizers is known to be economically and environmentally safe to replenish and maintain soil fertility [10].

Several genera including, *Pseudomonas* sp., *Bacillus* sp., *Mesorhizobium* sp., *Rhizobium* sp., *Klebsiella* sp., *Acinetobacter* sp., *Enterobacter* sp., *Erwinia* sp., *Achromobacter* sp., *Micrococcus* sp., *Aerobacter* sp. and fungi belong

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to the genera *Penicillium* sp. and *Aspergillus* sp. possess the ability to convert unavailable P into available one. These organisms release phosphorus into the soil by secreting organic acids like formic, acetic, propionic, lactic, glucolic, fumaric and succinic which are dissociating the bound forms of phosphate [11].

There is no consistency was observed under field conditions. Lack of persistence and competitiveness of introduced microorganisms may be of the reasons for such inconsistency. A very poor understanding of plant microbial interaction of phosphate solubilizing bacteria with their plants needs to be sorted out [12]. According to many researchers the experiments with biofertilizers were successful only under the trials but not in the field trial. Hence, through present study extensive field trials with phosphatic fertilizers were taken up to understand the efficiency of replacing superphosphate (SP) with cheap sources of phosphate like TCP. An optimized application of TCP along with PSB is needed at this scenario for the sustainable agriculture in India. If it is resolved, we can improve productivity to meet ever increasing global population [13].

II. MATERIALS AND METHODS

II. A Sampling Area

To isolate efficient PSB, an agricultural field near Pottalpudur (Latitude: 8.81067 & Longitude: 77.41239), Tirunelveli district, Tamilnadu was selected for sampling. Rhizosphere soil samples were collected randomly using an auger (8.5 cm diameter) to attain six sub samples from the topsoil (7.5 cm depth). The collected samples were transported to the laboratory in a sterile polyethylene bag in an ice pack and preceded for microbial analysis within six hours of collection. Aseptic sampling and transfer procedures were duly followed at each step.

II. B Isolation of PSB

Isolation and enumeration of PSB, was carried out using the Pikovskayas medium. The inoculated plates were incubated at 28 ±2 °C for a period of 3-7 days. Positive PSBs were identified based on the zone of clearance around their colonies. Individual colonies were picked and restreaked on fresh hydroxyapatite medium for two to three times to get pure colonies. Identification of bacterial species was done on the basis of cultural, microscopic, and biochemical characteristics with reference to Bergey's manual of determinative bacteriology [14]. Similarly Total Viable Count (TVC) was estimated by the standard spread plate method using nutrient agar.

II. C Field Application of PSB

The field experimental design with three replicates in six fields (plots) each with the dimensions of 2m was marked by 1m. To evaluate the efficiency of P release from the TCP in the presence of SP, the following treatment setups were planned with control (without fertilizer & PSB), T1 (SP@25%), T2 (SP@50%), T3 (SP@75%), T4 (SP@100%), T5 (TCP@25%), T6 (TCP @50%), T7 (TCP @75%), T8 (TCP @100%), T9 (T5+PSB), T10 (T6+PSB), T11 (T7+PSB), T12 (T8+PSB), T13 (T1+T9), T14 (T1+T10), T15 (T1+T11) and T16 (T1+T12). 100 ml of the 48 hr old culture was used for inoculating the plots and inoculum was applied along with irrigation water. To avoid the leaching of fertilizers from one plot to another, a gap of one feet distance was maintained between each plot and plots were watered separately. Utmost care was taken to maintain an equal numbers of plants in each plot.

The various parameters like soil nitrogen, phosphorus, potassium, pH and plant morphological characters like leaf numbers, plant height, dry weight, and chlorophyll, NPK content of the plant leaves were analyzed at pre-flowering, flowering and final stages of plant growth. Ten samples from each plot were collected and respective parameters were analyzed.

II. D Estimation of NPK from soil

II. D.1. Soil Nitrogen

Soil nitrogen was estimated by the Macrokjeldhal method [15].

II. D.2. Soil Phosphorus

Phosphorus was estimated by the method of Olsen et al. [16].

II. D.3.. Soil Potassium

Potassium was estimated by flame photometry method of Jackson [17].

II. D.4. Soil pH

Using pH meter the soil pH was analyzed.

II. E.. Estimation of NPK and Chlorophyll from Plant Tissue

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II. E.1. Plant Nitrogen

Plant nitrogen was estimated by the Microkjeldhal method [18].

II. E.2. Plant Phosphorus

Phosphorus estimation was carried out in accordance with the method of Fiske and Subba Row [19].

II. E.3. Plant Potassium

Using flame photometry method estimated the potassium content as adopted by Jackson [18].

II. E.4. Chlorophyll

Chlorophyll estimation was carried out by Arnon's method [20].

III. RESULTS

III.A. Enumeration of total viable count and PSB

Thirty eight different bacterial isolates were obtained and identified as *Alkaligens* Sp., *Bacillus* Sp., *Flavobacterium* Sp., *Brevibacterium* Sp., *Aeromonas* Sp., *Enterobacter* Sp., *Pseudomonas* Sp., and *Vibrio* Sp.,. Among the bacterial species, *Bacillus* Sp (37.8%) and *Pseudomonas* (18.9%) were found to be predominant, while *Brevibacterium* Sp. exhibits the lowest percentage (4.5%). Among these, *Bacillus* Sp. was found to be most efficient in solubilizing the insoluble phosphate to a soluble state which producing a wider zone of clearance around the colonies (Figure 1).

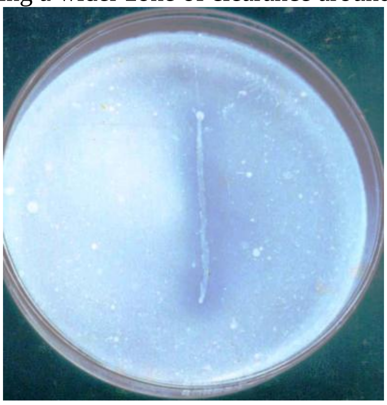

Fig. 1 GROWTH OF PSB ON PIKOVSKAYA'S MEDIUM

III.B. Field application of PSB

The properties like low solubility, low mobility, and high fixation by the soil matrix, the availability of P to plants is dominantly controlled by various factors including biological processes in the rhizosphere region [21]. Therefore, releasing more soluble form of phosphate using an efficient PSB was chosen as the candidate for the field application.

III.C. Soil Analysis

PSB counts as well as total viable in the plots treated with various combinations of SP and TCP along with PSB treatments varied with respect pre-flowering, flowering and final stages of the plant growth. It was noticed that the overall population decreased during the final stages of plant growth (data not shown). Among the different concentrations, plots treated with 25% of SP + 75% of TCP with PSB showed the maximum TVC (9.5×10^{7} CFU g^{-1}) during the flowering stage than the other plots tested. It was noticed that both TVC and PSB increased significantly during flowering stage and later got reduced gradually. Compared with the SP, TCP at 100% with PSB, showed 7.0 $\times 10^{4}$ CFU g^{-1} PSB count. Plot seeded with PSB is known to increase the number in the presence of TCP than the SP (data not shown).

III.D. Effect in the Soil NPK

Phosphate solubilization is depending on various factors like nutritional, physiological and growth conditions of the PSB. The total nitrogen (N) content of the soil in different plots analyzed was found to be 246.8 kg ha^{-1} in plot treated with 25% of SP + 75% of TCP along with PSB and while it was 210.5 kg ha^{-1} in control plot. Plot treated with 50% TCP along with PSB showed appreciable amount of nitrogen (245.0 kg ha^{-1}) when compared with plot amended with 100% of SP which showed a slight increase in N content (238.0 kg ha^{-1}) than TCP added at 100% (231.0 kg ha^{-1}).

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The phosphorus content of the soil in different plots ranged from 110.8 kg ha^{-1} to 143.0 kg ha^{-1}. The highest value was observed in the plot treated with 25% of SP + 100% of TCP along with PSB. PSB offers an alternative, which is capable of solubilizing native as well as added insoluble phosphate, making it available to the plants. It was also seen that the bacterial supplementation showed better results than single fertilizer. The phosphorus content in the soil was found to be very less during the final stages of plant growth than initial stages. PSB is known to render more phosphorus into the soil during the flowering stage. Slightly increased amount of P (128.0 kg ha^{-1}) was observed in the plot treated with 75% SP than the plot amended with 75% TCP (116.2 kg ha^{-1}). The release of soluble form of phosphorus from the SP was found to be more significant than TCP. However, the phosphorus released from TCP was high when it was applied along with PSB.

The influence of PSB application with phosphate fertilizer on the soil potassium was found to be significant. It was observed that the plot treated with 25% of SP+100% of TCP along with PSB showing 34.6 kg ha^{-1}. It was slightly high in the plot treated with 100% of SP (26.2 $kgha^{-1}$) than TCP amended soil with 100% (25.0 kg ha^{-1}) concentration. In the present study, soil analysis of treated plots showed higher concentrations of nitrogen, phosphorus and potassium in the flowering stage over control, which is an indication of a better globalization and in turn better uptake by the plant for growth (Table 1).

Table – 1 EFFECT OF TRICALCIUM PHOSPHATE SUPPLEMENTATION ON SOIL AND LEAF NPK

Treatment	Soil Nutrients (kg $^{-ha}$)				Leaf Nutrients (mg $^{-g}$ of leaf)		
	N	P	K	PSB count (CFU $^{-g}$)	N	P	K
Control	210.5	110.8	24.0	3.9×10^4	2.5	0.0065	30
T1- (SP*@25%)	228.7	114.2	24.2	3.2×10^4	3.0	0.0481	31
T2- (SP@50%)	229.0	115.0	26.1	3.3×10^4	3.2	0.0503	34
T3- (SP@75%)	235.1	116.2	26.0	6.3×10^4	3.4	0.0620	35
T4- (SP@100%)	238.0	118.0	26.2	7.0×10^4	3.2	0.0640	35
T5 - (TCP**@25%)	231.9	124.9	24.5	3.9×10^4	3.5	0.0520	32
T6 - (TCP@50%)	230.0	126.0	24.8	6.0×10^4	3.6	0.0534	33
T7 - (TCP@75%)	232.0	128.0	25.0	7.2×10^4	3.8	0.0550	35
T8 - (TCP@100%)	231.0	125.0	25.0	6.9×10^4	4.0	0.0560	37
T9- T5 + (PSB***)	234.0	127.7	28.8	7.5×10^4	4.2	0.0720	38
T10- T6 + (PSB)	235.0	130.0	29.0	7.9×10^4	4.4	0.0740	40
T11- T7 + (PSB)	236.0	135.0	29.4	8.3×10^4	4.6	0.0745	41
T12- T8 + (PSB)	237.0	140.0	29.6	8.5×10^4	5.0	0.0760	42
T13 –T1+T9	240.6	136.5	29.7	9.2×10^4	5.2	0.0780	40
T14 –T1+T10	245.0	141.0	30.0	9.3×10^4	5.4	0.0801	45
T15 –T1+T11	246.0	141.5	31.2	9.5×10^4	5.5	0.0870	46
T16 –T1+T12	243.8	143.0	34.6	9.4×10^4	5.7	0.0945	48

* SP- Super Phosphate, **TCP – Tricalcium Phosphate, *** PSB – Phosphate Solubilizing Bacteria

III.E. Influence on leaf NPK:

The influence of PSB application with SP and TCP was significant in the leaf NPK content. Plot inoculated with 25% of SP + 100 % of TCP with PSB showed a maximum level of NPK than the control. Maximum NPK was 5.7, 0.0945 and 48 kg ha^{-1} respectively when the plot was amended with 25% of SP + 100% of TCP with PSB, while with other treatments it was found to be moderate, Plot inoculated with 25% of SP + 25% of TCP with PSB also showed comparatively a good response of N, P and K content as 5.2, 0.0780 and 40 kg ha^{-1} respectively (Table 1).

Total nitrogen in the leaf collected from different plots showed a maximum value during the pre-flowering stage of observation (5%) and then reduced to 4.7% followed by a farther reduction to 1.9 % during the final stages. P content in the plant leaves among different plots treated with SP and TCP showed varying results. The plot treated with 100% of SP showed better results than the plot treated with 100% TCP. Leaf potassium in the plot applied with 100% of TCP

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and PSB was found to be more significant (42 kg ha^{-1}) than other concentrations of TCP and SP used either with or without PSB. However, the maximum K was observed in the plot treated with 25% of SP+100% of TCP with PSB. The maximum amounts of K observed also showed the presence of more TVC and PSB found in the plot applied with 25% of SP+100% of TCP with PSB (Table 1).

III. F. Influence on the plant growth:

Application of PSB with SP and TCP on the plant growth was found to be more significant than control plot. Among various ratios of the SP and TCP used, the plot treated with 25% of SP + 100% of TCP with PSB showed maximum plant growth in terms of plant height, total weight, chlorophyll content and number of leaves. The maximum value of plant height, wet weight, chlorophyll content and total leaf numbers recorded were 154cm, 25g, 8.2mg g^{-1} and 18 respectively. Plant growth was slightly different when 25% TCP concentration was used. Interestingly there was a significant increase in the plant height (144 cm) when TCP was used at 100% than the use of SP at 100% which showed only 138cm. Of these, 25% of SP + 50% of TCP with PSB showed maximum plant weight as 26g while other treatments showed, moderate plant growth over control. Similar trend was observed with chlorophyll content. The maximum amount of chlorophyll (8.2 mg^{-g} of leaf) was recorded in the plot inoculated with 25% of SP+100% of TCP with PSB, while this was 7.0 mg g^{-1} when only 25% of TCP alone used. The plot inoculated with 25% of SP + 100% of TCP with PSB showed a significantly higher leaf number (19) than other treatment methods tested. The above results showed that when more of TCP was added it was found to get mobilizing in the field and absorbed by the plant system. As a result, the plot applied with more of TCP has resulted in higher chlorophyll content than other concentrations of TCP (Table 2).

Table – 2 SUPPLEMENTATION OF TRICALCIUM PHOSPHATE PLANT GROWTH IN THE PRESENCE OF PSB

Treatment	Plant growth			
	Height (cm)	Weight (g)	Chlorophyll (mg)	No. of leaves
Control	092	06	7.0	07
T1- (SP@ 25%)	125	12	7.2	15
T2- (SP@ 50%)	129	14	7.4	16
T3- (SP@ 75%)	134	18	7.6	15
T4- (SP@ 100%)	138	20	7.7	15
T5 - (TCP@25%)	130	11	7.0	13
T6 - (TCP@ 50%)	135	13	7.2	14
T7 - (TCP@75%)	141	14	7.3	13
T8 - (TCP@ 100%)	144	15	7.4	12
T9- T5 + (PSB)	140	20	7.6	14
T10- T6 + (PSB)	146	22	7.8	15
T11- T7 + (PSB)	150	23	7.9	16
T12- T8 + (PSB)	152	22	7.7	15
T13 –T1+T9	148	24	7.9	16
T14 –T1+T10	153	26	8.0	17
T15 –T1+T11	154	25	8.2	18
T16 –T1+T12	154	24	8.0	16

III. G. Impact of TCP on Phosphatase activity:

Plot treated with various combinations of TCP and SP along with PSB showed different ranges of phosphatase activity. The enzyme activity found to be significant with plot treated with 50% of TCP (412 EU mL^{-1}) than any other treatment. However, increasing the TCP concentration beyond 50% has not increased the enzyme activity. However, the 348.6 EU mL^{-1} was recorded as maximum with SP at 25% U mL^{-1}, and other concentrations were found to be lower (Table 3).

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Table – 3 EFFECT OF TRICALCIUM PHOSPHATE ON THE PHOSPHATASE ACTIVITY PIKOVSKAYA'S MEDIUM.

Treatment	Phosphatase activity (EU mL^{-1})
Control	160.8
T1- TCP @ 25% + PSB	361.0
T2 - TCP @ 50% + PSB	412.0
T3 - TCP @ 75% + PSB	397.1
T4 - TCP @ 100% + PSB	386.0
T5 –T1+SP @25%	348.6
T6 –T2+SP @ 25%	331.0
T7 –T3+SP@ 25%	346.0
T8 –T4+SP@25%	342.0

IV. DISCUSSIONS

Tricalcium phosphate is mineral apatite contains insoluble phosphate. Few experimental studies have been conducted in India to prove positive implication of PSB and plant growth. Several studies conducted with various crops have showed that fertilizing soil with PSB increased levels of available P. Sundara et al. [22] reported that sustaining long term productivity of sugarcane increased with RP and PSB inoculation. Going astutely through several results of the agricultural arena composing the chemical fertilizers, the PSB inoculants remains an open field wherein the combined use of bacterial fertilizer with tricalcium phosphate along with super phosphate to achieve higher vegetable crop yield was reported in the present study.

The need of the hour is to identify methods to reduce the addition of super phosphate with an effective combination of PSB. Hence this finding will helps us to disarm the continued use of the synthetic fertilizer that has been affecting ecological balance, micro-biota and physico-chemical parameters of the earth crest. Application of SP and TCP along with PSB is known to increase the bacterial count and promote the flagella development in the rhizosphere region [23]. A good number of plants exudates get released during the flowering stages contributing to a high PSB and TVC among all the treated plots. Exudates leached from the plant roots also increases bacterial counts during the flowering stage [24]. The plant keeps excreting and sloughing off lower quantities of organic substances which favor the increasing bacterial counts around the rhizosphere area.

Phosphate solubilizing microorganisms produce wide range of phosphatases and able to use P from various forms P present in soil. It reported that higher in P-deficient soils showing increased phosphatase activity. Phosphatases being inducible enzymes, the intensity of their requirement determined this enzyme for orthophosphate [25] and which is also affected by soil pH. Therefore, enzyme activity differed with reference to availability of P. Depletion of soil organic P significantly increases in the activity of both mono- and diester phosphatases [26]. However, microorganisms present in the rhizosphere can directly solubilize P to meet their own requirements and produce appreciable amount of phosphatase. Soil extracts treated with phosphatase enzyme releases good amounts of orthophosphate. Measuring phosphatases activity implies rate of phosphate transformation in the microenvironment and determine microbial dynamics [27].

These organisms convert the soluble forms by releasing low molecular weight organic acids, hydroxyl and carboxyl groups of the organic acids, chelate with the cations bound to phosphate and release orthophosphate. Amino acids, carbohydrates, nucleic acid derivatives, growth factors, enzymes and other factors influence the abundant existence of bacterial colonies are high in the rhizosphere area during flowering stage [28].

Similar observations by Gopalakrishnamurthy et al. [29], showed that establishment of increased bacterial population in the rhizosphere could be a reason for the reduced fungal population in that region. Though antagonism is a factual phenomenon, it cannot be taken for granted as a reason for the decreased bacterial count at final stage. In turn,

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microbial activity releases plant nutrients around the root system. Total microorganisms of the biosphere soil play a key role in the availability of nutrients to plants [30]. PSB counts are directly influenced by soil properties especially the quantity of phosphorus present and the cultivation patterns. PSB establishes a synergistic interaction with the plants by providing them soluble phosphates and taking carbon compounds in return [31].

Bacillus species solubilize phosphorus by secreting phosphatase and are known to acidify external medium which lower the pH thereby dissociating the bound forms of phosphate and releasing it as orthophosphate into the soil. The change in soil pH from 6.8 to 5.8 at flowering stage plays a very significant role in mobilizing phosphate. TCP get well soluble when a drop in pH (to 4.9 and 6.0) from an initial pH of 6.8 - 7.0 [26]. The previous reports to fortify those calcium phosphates get dissolved by acidification [32]. Different studies have implicated that the inoculation of *Bacillus sp.* as biofertilizers play a major role in the releasing of orthophosphate from unavailable forms of phosphate and with the reduction of disease concomitantly. Saharan and Nehra, [33] also, reported that *Bacillus* sp., isolated from rhizosphere soil was able to soluble phosphate and influence the plant growth.

Combine use of phosphate biofertilizers along with PSB are known to increase the availability of phosphorus, plant growth promoting substances, N, Fe, Zn etc., through production of various organic acids. Abou-Aly et al. [34] conducted a field trial using rock phosphate with PSB and showed that it increased the sugarcane yield and improved juice quality, and improved fruit quality of squash and wheat have been documented by El-Yazeid and Aly, [35]. PSB inoculums along with SP and TCP increase the biomass, plant height, pod yield, plant leaves and NPK-uptake in the plant.

Our results are very much confirmatory to the reports of El-Yazeid and Aly, [35]. Application of super phosphate decreases the phosphatase activity to a minimum than those treated with tricalcium phosphate. However, many of the earlier reports showed that super phosphate is better absorbed than the tricalcium phosphate [36]. Plot treated with PSB along with either TCP or SP showed better result than the plot treated only with SP or TCP. This shows that substantial amount of SP or TCP could be saved when bacterial inoculums are used. Earlier findings also show that PSB and phosphate fertilizers together could reduce phosphate fertilizer application by 50 % without affecting the crop yield.Tricalcium phosphate inoculated with PSB produced higher yields than other treatments. Similar results have been reported by Mohamed and Ibrahim [37].

Leaf NPK analyzed found to increase during flowering stage than the other stages analyzed. High TVC and PSB population resulted from the release of good quantity of plant exudates released during the flowering stage might be the reason for an increased NPK content. Plant exudates containing organic acids like glutamic acid, succinic acid, lactic acid, oxalic glyoxalic, maleic and fumaric acid forms complex with cations such as Ca^{2+} and Fe^{2+} which results in the release of orthophosphate into the rhizosphere regions [38].

The above results may be attributed to the increased uptake of phosphorus and potassium by the plant resulting in dry matter yields, fruits and plants. Similar findings of Gopalakrishnamurthy et al. [29] also revealed that the effects of various treatments of the seed on height, dry weight, nitrogen and phosphorus contents of plant increased which corroborate with our work. Findings of Chandrasekhar and Shetty [39] also imply that phosphorus content of the leaf is increased when combined inoculation with chemical fertilizer.

Higher plant growth and yield in the phosphate along with PSB inoculated field showed that the increased rhizosphere population produces fungistatic and growth promoting substances which influences plant growth. Our results like plant height, dry weight, chlorophyll content, leaf number and yield showed very similar trend with works of Sarkar et al. [40], showing that inoculation have positive yield response in various crops such as rice, wheat, barley, tobacco, sugar cane, potato and various vegetables and forage crops. Available phosphorus strongly influences leaf growth and chlorophyll content of plants. This was reported by Colomb et al. [41] that chlorophyll content was higher during the flowering stage and later reduces gradually.

Bouton and Zuberer [42] reported that overall increase in plant height, number of tillers, dry matters and grain yield could be attributed not only to nitrogen fixer, but also the growth promoting substances produced by this bacterium.

This clearly shows that increased bacterial population during the flowering stage produces more of plant growth promoting substance that increases overall plant growth. In addition, the increased PSB count also contributes to a higher phosphate solubilization thereby a higher uptake of phosphorus by the plant reflecting in a better growth. It is evidenced from our study that TCP could be used as a cheap source of phosphorus especially when it is mixed with 25% of superphosphate and PSB inoculation. The use of PSB would certainly alleviate the available phosphorus in soil and minimize the application of P fertilizer which in turn would reduce the problem of environmental pollution.

V.CONCLUSION

Field experiments to enhance the available phosphorus found to be a limiting factor for the sustainable agriculture production. It's envisaged from our results that the phosphate-solubilizing bacteria applied in the agriculture field would reduce P fertilizer application. Replacing superphosphate with tricalcium phosphate in the presence of PSB was shown to be efficient at 25 % of SP and 75 % of TCP along with PSB. These concentrations portrayed an increase in the soil and leaf NPK, chlorophyll content, plant height, weight and number of leaves in bhendi. It's learned that soil microorganisms are important for cycling of P in soil and it will provide basic information towards development of more P-efficient and sustainable agricultural systems.

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