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Phosphate Solubilizing Bacteria Enhancing Plant Growth

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Abstract

Sustainable agriculture is reliable, acceptable and trustworthy way to maintain a healthy soil and thus to produce good quality and quantity of food. To achieve this task there is often use of biofertilizers which contain nitrogen, phosphorous and potassium as major nutrient. Soil is living entity contain variety of Macronutrients and Micronutrients. Phosphate is one of the major nutrients required by plants but not easily available to them because of its insoluble nature. Phosphate solubilizing bacteria (PSB) make it soluble thus play important role in sustainable agriculture. The use of PSB as a biofertilizers has concurrently increase phosphorus uptake in plants and improve yields in several crop species. A laboratory study has conducted to isolate, identified and characterize the phosphate solubilizing bacteria from different soils of Vidarbha region, four isolates were obtained from four districts respectively. The four strains (S1, S2, S3 and S4) were screened by biochemical study and their phosphate solubilizing ability helps in plant growth.

Keywords: Characterization, identification, isolation, phosphate solubilizing bacteria, NPK

Introduction

To maintain the quantity of products farmers use fertilizers. Fertilizers are nutrients which are necessary for the growth of plants and thus for the productivity of cultivated plants. use of fertilizers for increasing productivity is one of the aspects of green revolution. Fertilizers are classified as inorganic (chemical) and organic (biological). Inorganic fertilizers are synthetic where mineral salts of NPK are mixed in definite proportion and then dusted in the field. Non judicious or excessive use of such fertilizers lead to pollution of soil air and groundwater, soils become acidic

Organic fertilizers are biological in origin and include farm yard manure (FYM), compost and green manure. Use of this fertilizers increases the fertility of soil. Now a days for better and sustainable agriculture production farmers use biofertilizers and practice organic farming. Biofertilizers are commercial preparation of ready to-use live bacterial or fungal formulations. Their application to plant soil or composting pits helps to enrich the soil fertility due to their biological activity. Use of biofertilizers is cost effective and eco-friendly. They play a vital role in maintaining a long-term soil fertility and sustainability.

Phosphorus (P) is the second most important nutrient for plant growth, accounting for 0.2% (w/w) of plant dry weight (Mahajan *et al*., 2018.) [14]. Phosphate plays an irreplaceable role in the ecosystem by the participating in most aspects of energy metabolism, Nucleic acid and protein synthesis, and kinase regulation. (Neseme *et al*., 2018) [15]. P affects root development, stalk and stem strength, crop maturity and nitrogen fixation in legumes (Khan *et al*., 2009) [10]. The average phosphate content in soil is. Nearly 0.05%. (w/w) with the main two forms being inorganic P (Pi) and organic P (Po). Nevertheless. Only 0.1%. of P can be utilized by plants, rendering available P a restrictive factor for plant growth (Lambers and Plaxton, 2018) [13].

The phosphate content in the soil. Can exist in calcium-, aluminium-or iron-complexed forms that are unavailable for plant use. As a result, mineral phosphorus. P2O5 is often used as a fertilizer to supplement the nutrients for crop growth. To reduce the addition of mineral phosphorus to agricultural soils, research in naturally occurring phosphate solubilizing microorganism has been conducted for decades (Sharon *et al*., 2016) [16]. Progressive depletion of major plant nutrients in soil due to intensive cultivation has necessitated the use of higher dose of chemical fertilizers, particularly in tropical soil where the organic matter content is very low (Kucey, Janzen, Legett 1989, 1983) [12, 11]. This huge drain on nutrients will continue to impoverish the soil unless these are replenished by natural means. Biological fertilizers are best remedy for this purpose. In agriculture systems, 'P' fertilizers are routinely applied to promote crop yield (Gaur, Gaind 1984). The P in these fertilizers is initially available to the plant but it rapidly reacts with soil and becomes progressively less available for plant uptake (as much as 90%). This is known as chemical fixation of phosphorous. Hence, the current trend throughout the world is to explore the possibility of using alternate nutrient sources for increasing the efficiency of chemical fertilizers since the since the available P from the rocks is very low or negligible. The phosphate solubilizing

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microorganisms dissolving interlock phosphates appear to have an important implication in Indian agriculture.

Many bacteria, fungi, actinomycetes and cyanobacteria are potential solubilizers of bound phosphates in soil (Bank and Dey, 1983 [3]; Illmer and Schinner, 1992 [8]; Sing and Kapoor, 1992 and Anusuya and Jayarajan, 1998) [1]. Phosphate solubilizing microorganisms are found in all soils but their numbers varies with soil climate as well as history (Gupta *et al*. 1996; Illmer and Schinner, 1992) [8]. Efficiency and economic uses of phosphate fertilizers could be achieved by using phosphate-solubilizing microorganisms in legumes, cereals and useful crops (Dadarwal *et al* 1997; Yadav and Dadarwal 1997) [17].

This study aims to investigate and characterize various species of phosphate solubilizing bacteria found in soil of Vidarbha region of Maharashtra state.

Identification of PSM

When classifying microorganisms, all known characteristics are taken into consideration, but certain characteristics are selected and used for the purpose of identification. Primary identification usually involves a few simple tests such as morphology (usually shown by Gram stain), growth in the presence or absence of air, growth on various types of culture media, catalase and oxidase tests. Using these few simple tests it is usually possible to place organisms, provisionally, in one of the main groups of agricultural importance

Materials and Methods

Collection of Representative Soil Samples: Representative soil samples were collected for the isolation of phosphate solubilizing bacteria at the rate 1 sample from each district and from most of the district of Vidarbha.

Screening of Soil Samples for Phosphate Solubilizing Microorganisms: Take 1gm of soil sample of each district, suspended in flask containing 50ml of Pikovskaya's broth, Phosphate solubilizing bacterial colonies were obtained by repeated streaking on Pikovskaya's agar and then repeated streaking for purity. Tri-calcium phosphate was used as P source in the medium. P-solubilization by the isolates was confirmed by the appearance of a transparent zone around a single colony (Photoplate 1 and 2).

Microbial Solubilization of Insoluble Phosphate in the Liquid Medium: Take 25ml of Pikovskaya's medium in 100ml of flask, inoculated it with PSB of each district respectively, flasks put on rotary shaker at 120 rpm at 28 ± 2^0c for 24, 36, 48, 60, 72 hrs time interval. Centrifuge it at 10,000 rpm for 15 min. supernatant used to estimate solubilized P by measuring intensity of blue colour at 600nm on spectrophotometer. The soluble P content in culture supernatant was determined by bromophenol blue (Sangeeta mehata, C. Nautiyal).

Identification of PSB: The isolated bacterial strains were identified using standared biochemical tests as listed in the Bergey's Manual of Bacteriology (Krieg & Dobereiner 1984).

Fig 1: Four way streaking of PSB

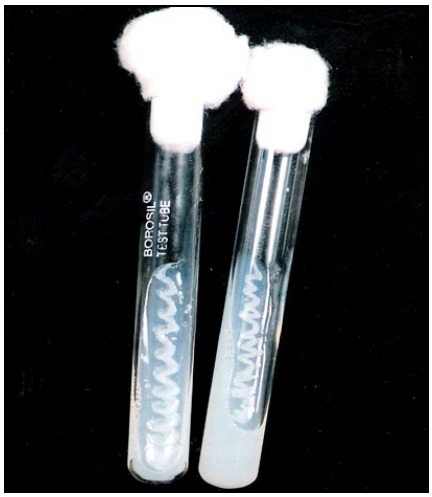


Fig 2: Pure culture of PSB

Pot Trial

Soybean seeds were surface sterilized. Seeds were coated with S1, S2, S3 and S4 formulation. The various combinations of these isolates were stated as follows for preparation of Consortia.

Table 1: Pot trial

Serial no.	Various Combinations of Isolates	Serial no.	Various combinations of isolates
1	Control	8	S3+S4
2	S1	9	S4+S1
3	S2	10	S1+S2+S3
4	S3	11	S2+S3+S4
5	S4	12	S3+S4+S1
6	S1+S2	13	S4+S1+S2
7	S2+S3	14	S1+S2+S3+S4

Pots containing 5 kg of local field's soil were inoculated with S1, S2, S3 and S4 coated soyabean seeds (each pot containing 5 seeds). Seeds were irrigated every alternate day with 50ml of water and maintained at room temperature 28^0c. In order to check the influence of bacteria on percent germination, pot assay was performed using local field soil (pH 6.8). During pot assay various parameters such as shoot height, no of leaves, no. And length of roots, chlorophyll content was studied and results were recorded after 5, 10, 20, 30 and 40 days, after sowing respectively.

3.2.7-Seed Germination, and Chlorophyll Content and Root Ramification

1. **Germination:** Percent germination of soyabean seeds were recorded 5 days after sowing.
2. **Root Ramification:** It was recorded by cleaning and measuring the length and numbers of soyabean plantlet roots.
3. **Chlorophyll Content:** Chlorophyll content was determined according to (Arnon, 1949). Chlorophyll was extracted in ice cold 80% acetone under dim green light and amount of chlorophyll in the extract was estimated.

Results and Discussion

Isolation of phosphate solubilizing bacteria

- Four PSB isolates were obtained from four district's soil labeled as S1, S2, S3 and S4 respectively (table 4.1). The PSB isolates were distinct in their morphology; they produced distinct clearing zones of varying thickness in Pikovskaya's agar, which was an indication of their P-solubilization abilities (photoplates 3 and 4).
- The isolates strains formed white-colored colonies, which were also white on reversed side. The texture of the colonies was powdery, with entire margins.
- A clear halo zone was formed around the colonies within 1-5 days. The P solubilization by bacteria other than PSB was low, as distinct clearing zones were observed around their colonies in Pikovskaya's agar.

Table 2: Various isolates

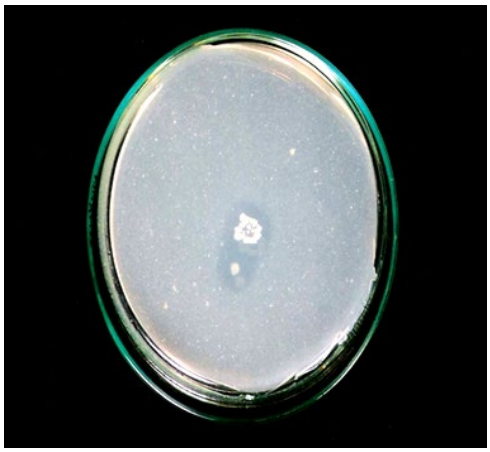
Name of District	Isolates
Amravati	S1 (I)
Bhandara	S2 (II)
Wardha	S3 (III)
Nagpur	S4 (IV)

Solubilizing Activity of PSB

The drop in pH and soluble content in the TCP-supplemented Pikovskaya's broth at different times was different depending upon PSB strains. The maximum amount of soluble P was found in Nagpur [S4] (at 48 h after incubation) district soil, followed by Buldhan [S3] (36 h after incubation) and Bhandara [S2] (table). The fastest rate of P solubilization was observed in S4 inoculated broth, i.e. maximum soluble P content at 48 h after incubation. Similarly, pH drop of the medium was maximum in S3 inoculated broth and least pH drop was recorded in P1 and P2 inoculated broth. The relation between pH drop and soluble P content in the Pikovskaya's broth was not statistically significant for all PSB strains; therefore, it appears that production of organic acids by the isolates was not the sole mechanism of solubilization of TCP. The proton associated with extracellular polysaccharides secreted by the microbes is also responsible for dissolution of TCP in the pikovskaya's broth (Illmer *et al.*). The P solubilization by the bacteria of other districts was low, as less distinct clearing zones were observed around their colonies in Pikovskaya's agar.

Table 3: Effect of pH and rate of phosphate solubilization using Pikovskaya's broth against time.

Isolates	Incubation (hour)	pH	Soluble P (ppm)
Initial value at the time of inoculation	0	6.90	6.3
S1 (Amravati)	48	4.9	39.2
S2 (Bhandara)	72	4.8	60.0
S3 (Wardha)	36	4.4	129.9
S4 (Nagpur)	48	4.6	200.0



Activity of isolate IV.

Fig 3: Phosphate solubilizing

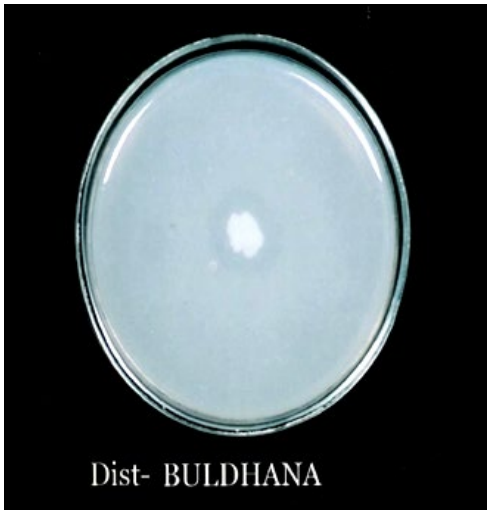


Fig 4: Phosphate solubilizing activity of isolate III

Identification

Four morphologically different aerobic as well as anaerobic bacterial colonies were isolated from soil sampled on selective Pikovskaya's agar after 72hrs of incubation at 30°C.all the four cultures were gram positive rod.

Isolate S3 were motile while isolate S2, S3 and S4 was non motile. Isolate S2 and S3 was found to produce endospores.

The isolates were identified using Bergey's manual of determinative bacteriology (Holt *et. al.* 1994). Isolates S3 and S2 were might be *Agromyces sp.* and *Bacillus sp* while Isolates S1 and S4 were might be *Arthrobacter sp.* and *Acetogenium sp.* respectively (Table).

Pot Trial Using Various Consortia

Fourteen pots were prepared of various combinations (shown in table 2.4), each containing 5.0 kg of local field soil. Each pot was inoculated with plain soybean seeds, seeds with S1, seeds with S2, seeds with S3, and seeds with S4, seeds with S1+S2, seeds with S2+S3, seeds with S3+S4 and seeds with S4+S1, seeds with S1+S2+S3, seeds with S2+S3+S4, seeds with S3+S4+S1 and seeds with S4+S1+S2, seeds with S1+S2+S3+S4 respectively. It was irrigated everyday and results were recorded after 5, 10, 20, 30 and 40 days respectively.

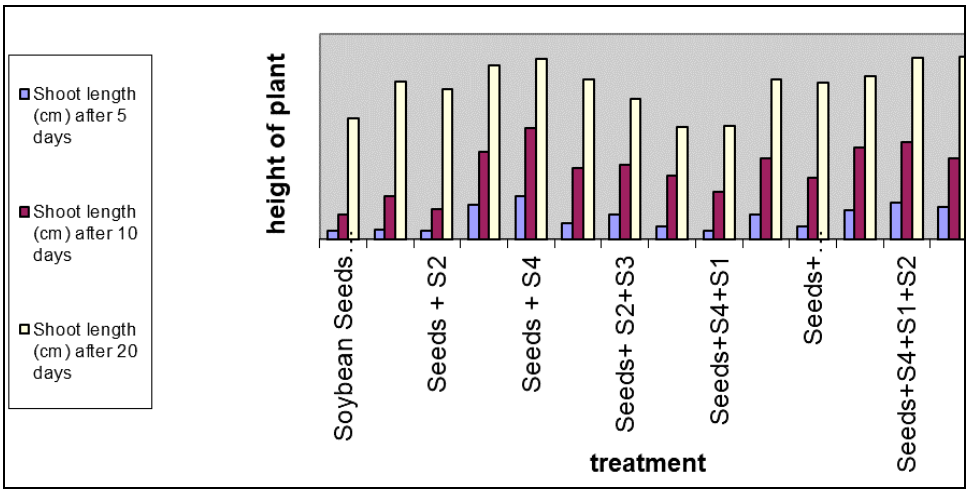


Fig 5: Analysis of plant growth promotion

Comparative Analysis of Various Combinations

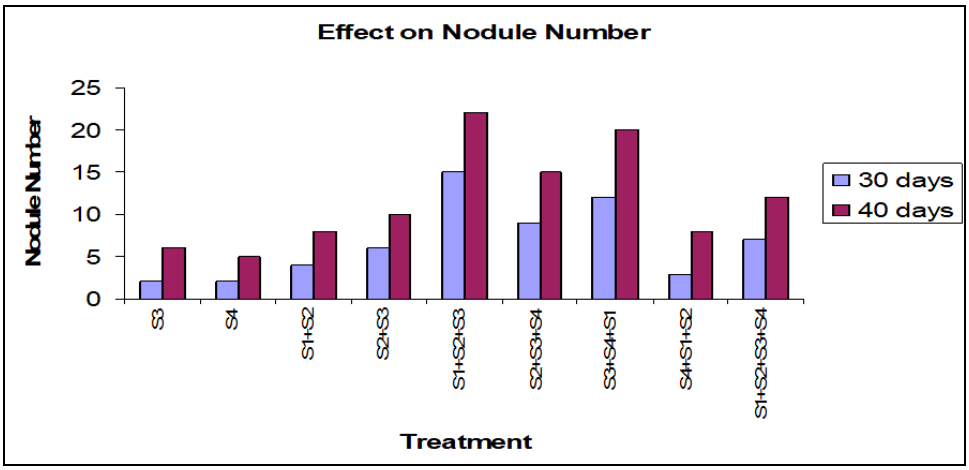


Fig 6: Comparative study of nodule numbers

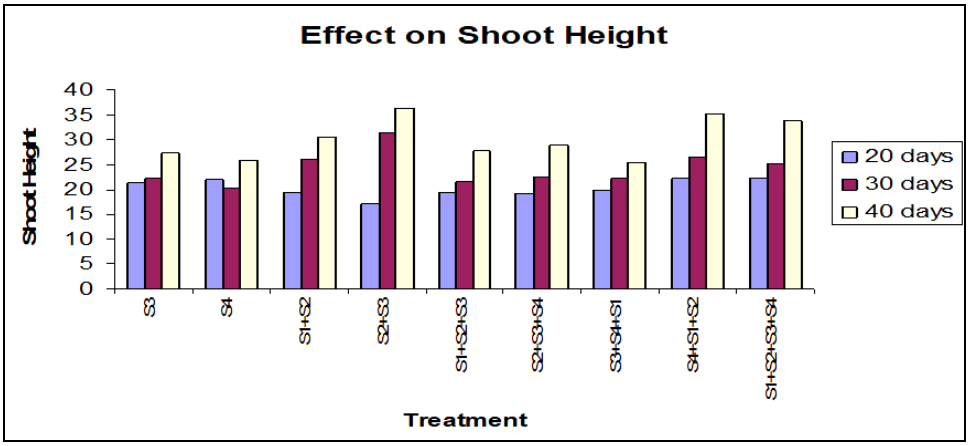


Fig 7: Comparative study of shoot height (cm).

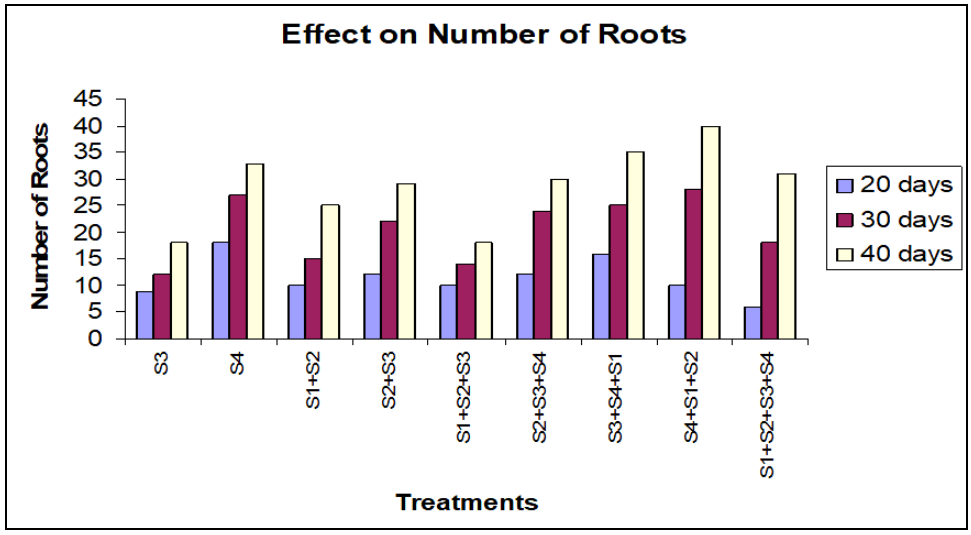


Fig 8: Comparative study of root numbers

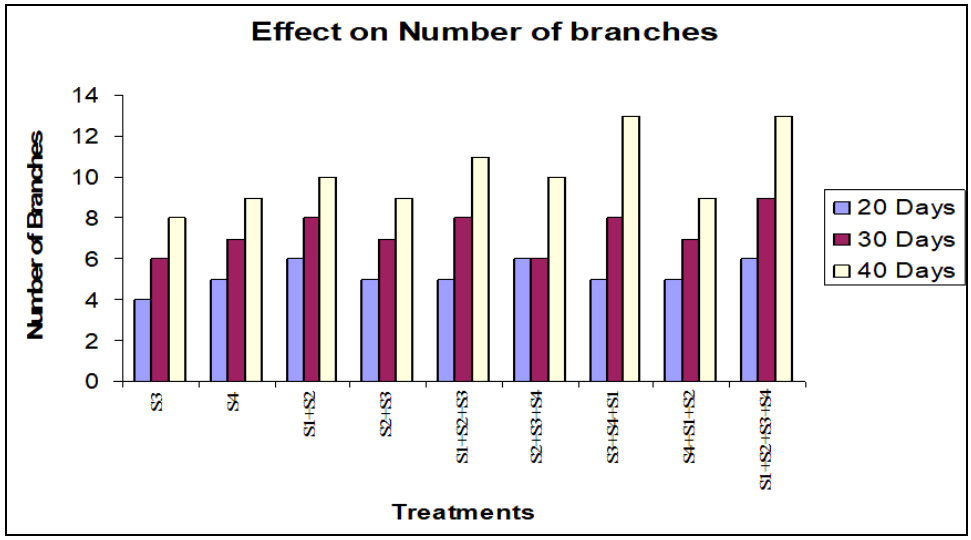


Fig 9: Comparative study of number of branches

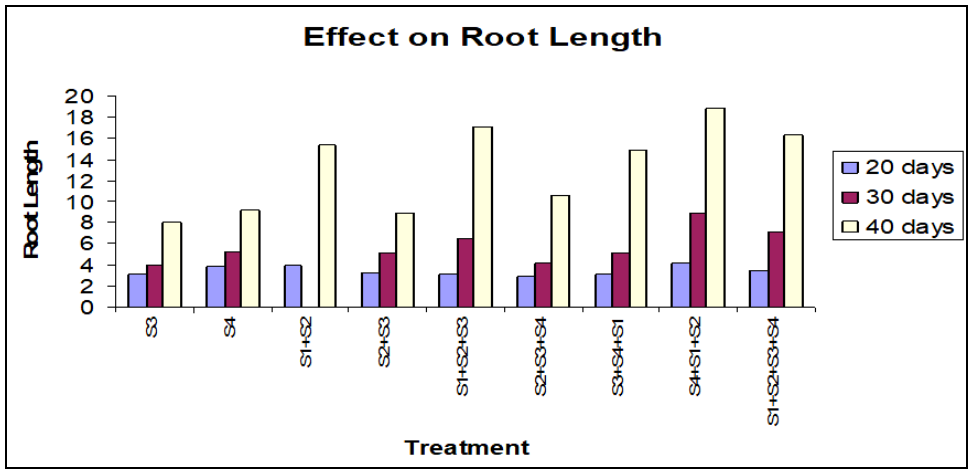


Fig 10: Comparative study of root length (cm).

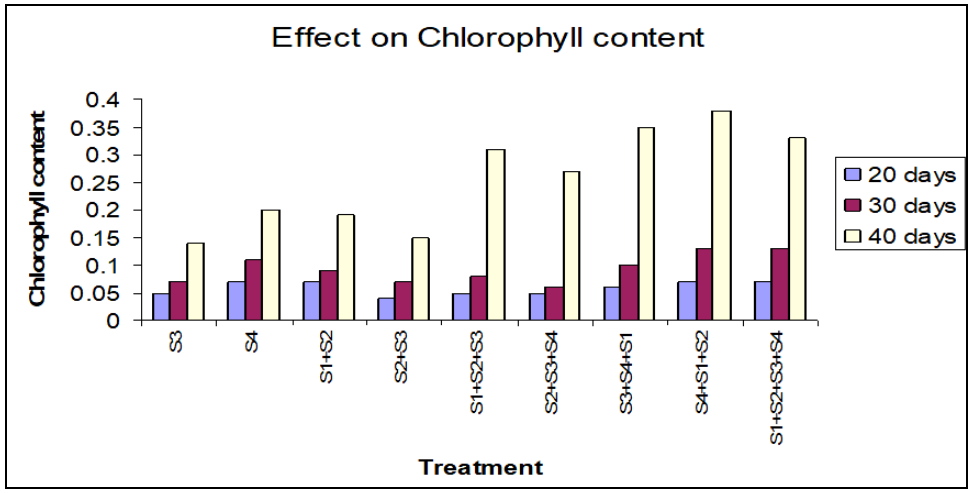


Fig 11: Comparative study of chlorophyll contents (mg/g)

Discussion

In this study, a total four PSBs were screened from the Vidarbha region soils. These PSB isolates may possess the potential to be applied in improving soil recovery and crop production, A higher P-solubility capacity of S4 was observed compared to others. The PSB strains were selected based on the P solubilization zone. Gaind (1987) reported that the PSB strains were isolated using the Pikovskaya's medium based on the formation of halo zone around these microorganisms. Screening of PSB clearly indicated that there was wide variation the PSB strains in solubilization zone formation, pH change, P solubilization in the liquid medium, phosphatase activity and organic acid production.

The pH of the culture medium turned to acidic was indicated that production of organic acids by PSB, which facilitate the solubilization of phosphate (Gaur & Sacher 1980) [6]. The maximum decline in pH was recorded with S4-up to 4.6. A fall in pH accompanied phosphate solubilization due to the production of organic acids.

The PSB strains were identified upto species level by studing the bacterial cultures morphology, cultural, physiological and biochemical characteristics using the manual of microbiological methods and identified using Bergey's manual of Determinative Bacteriology.

We found that the maximum shoot height is in the combination of Seeds+S4+S1+S2 (35.1cms), and Seeds+S2+S3 (36.3cms). The maximum number of branches is in Seeds+S3+S4+S1 (13 nos), and Seeds+S1+S2+S3+S4 (13 nos) combination. Whereas maximum root length is in Seeds+S4+S1+S2 (18.8 cms) combination, which in favor of the yield improvement. The effective nodulation is observed in Seeds+S1+S2+S3 (22 nos) and Seeds+S3+S4+S1 (20 nos) combination. High nodule number is an indication of efficient nitrogen fixation and ultimately the improvement in the yield. The high chlorophyll content is observed in Seeds+S4+S1+S2 (0.38 mg/g) combination. Higher the chlorophyll content better is the growth and high productivity.

In spite of increased dosage of chemical fertilizers the stagnation in food productivity made us to think over a technology which would benefit the society to overcome malnutrition. The world will not be able to produce the food needed by the use of Chemical Fertilizers to feed the projected population of about 8.3billion in 2025 (Borlaug 2001). Transformation of fixed Phosphate through microbes only appears to have an implication for alimenting the yield of crops.

Insoluble Phosphate dissolution by the isolates may not necessarily be the primary mechanism of plant growth promotion. Alternately, it is observed that the efficiency of the isolates to dissolve insoluble Phosphate in the field is more even in presence of complex microbial community (Richardson, 2001). This is an eco-friendly technology. We would like to make this technology available to the farmers as an economical package, so that the productivity is increase.

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