International Journal of Engineering & Technology, 7 (4.14) (2018) 40-45

SPC

International Journal of Engineering & Technology

Website: www.sciencepubco.com/index.php/IJET

Research paper



Evaluation of Potential Native Phosphate Solubilizing Bacteria for the Agriculture Practice of okra *(Abelmoschus Esculentus)* with the Target to Replace Chemical Fertilizer - in a Field Study

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Abstract

Phosphorus (P) is a major growth limiting nutrient. Phosphate Solubilizing Bacteria (PSB) plays an important role in the development of agricultural industry. In this study, the native PSB were isolated from the okra plant root by using Pikovskaya's medium and potential strains were selected based on the phosphate solubilization efficiency. The potential strains in presence of Rock Phosphate (RP), Tea dust, Eggshells and Bone waste of animal (TEB) were studied as an alternative fertilizer instead of chemical fertilizer. The PSB consortia strains were prepared and inoculated to the plant root. Different growth parameters were studied to determine the effectiveness of strains. In all cases the difference was significant with $p<0.05$. A decreasing trend of results were recorded among the tested parameters from plants in PSB with RP, PSB with TEB, and PSB with TSP and followed by PSB without fertilizer. The highest results were recorded among plants in PSB with RP for mean shoot length (64cm), mean weight of fruits (29gm), mean length of fruits (23cm), mean number of leaves (13Nos) and chlorophyll content (5.1mg/g). Hence, the present study showed that the application of PSB with the cheap source of nutrients has shown the capability of providing similar or enhanced results based on the various characters expressed by the plant and thus reduce the reliance on synthetic chemical fertilizers.

Keywords: *Phosphate Solubilizing Bacteria (PSB), Triple Super Phosphate (TSP), Rock Phosphate (RP), Tea dust, Eggshells and Bone waste of animal (TEB).*

1. Introduction

Okra (*Abelmoschus esculentus*) is an essential vegetable crop which supplies greater nutrition consists of protein, fats, carbohydrates, vitamins and minerals [1]. Okra is known as a multipurpose vegetable crop as they have many uses from the leaves, flowers, buds, pods and seed. Immature okra pods are frequently consumed as a vegetable in Asia. The leaves, buds and flowers are also edible as well as the dried okra seeds which can also be used as an oil source [2]. Okra is a high yielding crop at which its morphological traits vary in the plant such as the height, degree of branching, a period of maturity, size and colour of the pods. The predominant producers of commercial okra are India and Nigeria due to its suitable hot air climate [3]. Okra contains myricetin, possess anti-diabetic properties, anti-oxidant, anti-cancer and even anti-inflammatory activities which is beneficial to human [4].

Phosphorus is a part of the complex nucleic acid structure of plants that regulates protein synthesis which is essential in cell division and proliferation of new tissue. Phosphorus is taken in several process and functions in the plant development and metabolism. Phosphorus is also vital for the reproductive purposes of the plant [5]. Moreover, phosphorus have vital role in stimulating the root growth. The poor availability of phosphorus for the plant may lead to stunted growth with an unusual dark-green colour, root structure, yield and seed development [6, 7]. Malaysia is one of the heavy rainfall countries with annual average of 2500mm and has average temperature of the 27°C report the year This phenomenon directly affects the soil nutrients which are more essential to the plants. Studies have proven that the soils in Malaysia are mostly deficient in P content. This may due to the drainage with the rainfall [8]. The usage of chemical fertilizer has caused the reduction of organic matter in the soil in addition to unsuitable management practices. This will have a negative effect to the physical, chemical, and biological properties of the soil and will eventually increase soil erosion [9].

Naturally Phosphate exists in inorganic form in environment. The examples of external source phosphate are eggshells, bones, Triple superphosphate (TSP) and rock phosphate (RP). Huge amounts of lime and inorganic P fertilizers, for instance, phosphate rocks and TSP are used to chelate aluminium and iron ions [10]. Bones are a by-product of the food industry which contains nitrogen, phosphorus, potassium and calcium. Phosphorus resources are limited and recycling phosphorus within the food industry is crucial for sustainable production. This sort of mineral fertilizer is known as meat and bone meal (MBM). MBM contains approximately 5% of phosphorus making it an important source for phosphorus recycling. The use of MBM as fertilizers has been tested to various crops such as, ryegrass, barley and oats, where there has been a marked increase in grain yields [11]. Besides MBM, eggshells are also known to be used as an external source of phosphorus in the agricultural practice [12].

Phosphate solubilizing bacteria (PSB) has a beneficial effect such as the solubilization of the insoluble phosphate (P) compound. A plant interacts with these pathogens to create an infection process known as the rhizosphere effect [13]. PSB has the ability to release organic acids that produce hydroxyl and carboxyl groups that chelate the cation bound to phosphate. This causes the phos-

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phate to become soluble [5]. Different bacterial species are available to solubilize phosphate that exists in different forms such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate [14]. PSB has several mechanisms which are almost similar to those of plant growth promoting bacteria which augment the capacity of plants to obtain P in required amounts. Root colonization by potential PSB tend to improve existing root growth length or by auxin hormone production from indole-3-acetic-acid by the plant [15].

Hence this present study was aimed to isolate potential phosphate solubilizing bacteria (PSB) from rhizosphere and non- rhizosphere soil of okra as well as to evaluate the solubilization efficacy and the effectiveness of that strains in the presence of Tea dust, Eggshells, Bone waste of animal (ETB) and Rock Phosphate (RP) as an alternative fertilizer instead of chemical fertilizer with the reference of Triple Super Phosphate (TSP) as a control.

2. Methodology

2.1 Collection of rhizosphere and non-rhizosphere soil

Rhizospheric and non-rhizospheric soil of okra plants were collected from four different locations (MARDI Serdang, Sungai Buloh, Bandar Saujana Putra, and Puchong) and transfer into a sterile vial along with the root of the plant while the excess soils were removed through gentle shaking and agitation. The soil samples were processed on the same day in which a series of serial dilution was made.

2.2 Isolation of Phosphate Solubilizing Bacteria (PSB)

Rhizosphere and non-rhizosphere soil samples were collected and weighed. One gram of soil mixed with 100ml of sterile saline water in different conical flasks. These were kept on a shaker for 30 min for vigorous mixing at room temperature and serially diluted up to 10^{-6} fold with 9ml of sterile saline water in each test tube. 100 µL of diluted samples were spread on Pikovskaya agar media (PK) and incubated at 37°C and it was observed every 24 hours until a week [16].

2.3. Study of phosphate solubilzation efficiency and selection of psb

PSB colony formed a halo zone on the PK media The selected colony were streaked in PK media. The colony with the halo zone were noted and the the diameter of the colonies and halo zone was measured and recorded. The phosphate solubilizing efficiency were calculated based on the formula below.

$$\text{Solubilization efficiency} = \frac{\text{Diameter of halo zone (mm)}}{\text{Diameter of colony (mm)}} X100$$

Based on the highest phosphate solubilizing efficiency, the potential PSB strain were selected for the preparation of consortia as an inoculum for the plant. Then the selected colonies were quadrant streaked on the PK media to obtain a pure culture of PSB. Nutrient agar slants were used to maintain the pure culture. The selected PSB strains were stored in glycerol at -20°C for further study.

2.4 Phosphate solubilization estimation

A standard graph for phosphate solubilization estimation was done. Modified PK broth was used in this study. There were four sets of the conical flask with different composition of PK broth and addition of bone to two sets and eggshell to another two sets .Total volume per each conical flask was 100ml. The modified PK broth was prepared, 2 sets without tricalcium phosphate and 2 sets with tricalcium phosphate. Later 1g of bone and eggshell were added into the respective conical flask. It was then autoclaved. 200µl of selected PSB strain was inoculated into the broth and incubated at 37°C in a shaker over the period of five days. After incubation 1ml of the samples was pipetted out from each respective conical flask and transferred into the eppendorf tube every 12 hours until 108 hours. It was then centrifuged at 5000rpm for 10 minutes. The P content in the supernatant was estimate by ammonium molybdate method. The concentration of P was tabulated based on the standard graph and absorbance value obtained.

2.5 Preparation of the consortia for the plant inoculation

Seven potential strains of PSB were selected and inoculated into nutrient broth and incubated overnight at 37˚C. The selected strains were then mixed (consortia), 5ml each strain transferred into a 50ml centrifuge tube and centrifuged at 5000rpm for 15 minutes. Pellet was suspended with 270ml of tap water to obtain a total of 540ml of inoculum. The inoculums were inoculated to the plants at row E, F, G and H.

2.6 Agriculture field design

The total field length is 16m and total width is 6m. The field was divided equally for 8 sets of row(A, B, C, D, E, F, G and H). Each row consists of three segments(For eg. A1, A2, A3) which have 5 areas to plant each. Total of 15 seeds will be planted in a segment.

Table 1: Design of cultivation field

Without [d]PSB Consortia				**With PSB Consortia**			
Environmental plant (Uninoculated with PSB consortia)				Experimental plant (Inoculated with PSB consortia)			
A1	B1	C1	D1	E1	F1	G1	H1
A2	B2	C2	D2	E2	F2	G2	H2
A3	B3	C3	D3	E3	F3	G3	H3
Without fertilizer	[a]TSP	[b]RP	[c]TEB	Without fertilizer	TSP	RP	TEB

[a]Triple Super Phosphate, [b]Rock Phosphate, [c]Tea Eggshells dusts, Bone waste of animal, [d]Phosphate Solubilizing Bacteria.

2.7 Soil analysis

The soil sample from the cultivation land was collected and dried under the shadow part for two days. Later the soil was sent to MARDI Food & Agriculture Analysis Laboratory Serdang Selangor for soil analysis to show the suitability of the soil for cultivation and various physicochemical parameters such as carbon, nitrogen, calcium, magnesium, potassium, sodium, pH and soluble P were recorded.

2.8 Seed planting

The okra seeds were purchased from the Malaysian Agricultural Research and Development Institute (MARDI Serdang Selangor). Three seeds were planted for each segment.

2.9 Fertilizer preparation

Potash 120gm and 120g of urea was dissolved in 1200ml of tap water. 10ml of the mixture was poured to all the sets except for the

environmental control and experimental control set. For set B and F, 10g of triple superphosphate was dissolved in 100ml of tap water and 10ml of the mixture were poured to the plants. 10g of rock phosphate was dissolved in 100ml of tap water and 10ml of the mixture were poured to the plants in set C and G. Finally, the bone, eggshells and used tea dust were collected from few restaurants and were washed and dried. The tendons on the bone were also removed before drying. The tea dust also was dried under the sunlight. The dried bones and eggshells were crushed into a powder form using the mortar and pestle. 2gm of bones, 2gm eggshells and 1gm of tea dust were applied to each plant in set D and H.

2.10 Plant parameters

The shoot length, number of leaves, number of flowers, number of fruits, length of fruits, and weight of fruits were measured at a periodically (30, 50 and 70 days) and data were recorded.

2.11 Chlorophyll content estimation

Chlorophyll estimation was carried out according to Trivedy & Goel (1986) [17] protocol. Chlorophyll content of plant was estimated by using 0.5g of leaf from each set of plant and ground with acetone. Then, the grinded samples were transferred into 15ml centrifuge tube and added with 0.2 ml of $MgCO_3$ and the volume of acetone was made up to 10ml. Samples were then incubated for 4-6 hours at 4˚C and centrifuged at 3000rpm for 20 minutes. Absorbance reading was taken at 645nm and 663 nm using the Uv Line 94000 spectrophotometer. The test was done on 50th day after the application of bio fertilizer.

2.12 Statistical analysis

Statistical analysis was carried out using the statistical package SPSS windows version 23.The data were expressed as the mean ± standard error. All collected data were analyzed with independent t-test and accepted as statistically significant with $p<0.05$.

3. Results and Discussion

3.1 PSB isolated from rhizosphere and non-rhizosphere soils of okra plant

The rhizosphere effects of PSB of okra between non-rhizosphere and rhizosphere soils were identified as shown in (Table 2).

Table 2: Strains of PSB isolated from rhizosphere and non-rhizosphere soils of Okra plant.

Area	Serial number of soil samples	Phosphate Solubilizing Bacteria (PSB) (CFU/g)		No of isolate PSB
		Non-Rhizosphere	Rhizosphere	
M	M-01	1.4×10^4	1.4×10^5	M 01, 02, 03
	M-02	1.1×10^4	3.2×10^5	M 04, 05, 06
	M-03	1.0×10^5	3.3×10^7	M 07, 08
B	BS-01	2.4×10^6	3.3×10^7	BS 09, 10,11
	BS-02	2.3×10^6	3.7×10^6	BS 12, 13
	BS-03	2.0×10^5	3.2×10^6	BS 14, 15,16
S	S-01	3.2×10^4	2.8×10^5	S 17,18,19
	S-02	2.9×10^7	2.3×10^7	S 20,21
	S-03	3.2×10^7	2.9×10^8	S 21, 22
P	P-01	3.1×10^6	3.6×10^8	P 23, 24, 25
	P-02	2.5×10^6	4.0×10^7	P 26, 27, 28
	P-03	3.0×10^7	3.8×10^7	P 29,30

M=MARDI, B=Bandar Saujana Putra, S=Sungai Buloh, P=Puchong

3.2 Study of phosphate solubilization efficiency

The diameter of the colony and the halo zone were measured and tabulated in table 3. The phosphate solubilization efficiency was calculated. The solubilization efficiency greater than 50% were selected for this study, which includes strains, M03, M08, BS17, S22, S25, P27, P29. They had an efficiency of 90%, 50%, 71%, 75%, 115%, 52%and 57% respectively.

Table 3: Phosphate solubilization efficiency of PSB strains

Strain name	Diameter of halo zone (mm)	Diameter of colony (mm)	Solubilization efficiency (%)
M01	2.0	2.3	87
M03	2.0	2.2	90
M08	1.0	2.0	50
BS 10	0.8	1.0	80
BS 13	1.8	2.5	72
BS 17	2.2	3.1	71
S20	1.9	2.0	95
S22	2.1	2.8	75
S25	2.3	2.0	115
P23	1.5	2.0	75
P27	1.2	2.3	52
P29	1.4	2.1	67

3.3 Phosphate solubilization estimation

PK= Pikovskaya media, TCP=Tri Calcium Phosphate

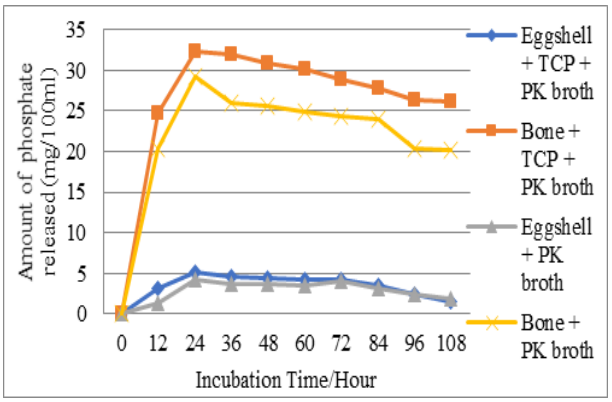

Fig.1: Phosphate released in modified PK broth with consortia

The amount of inorganic phosphate released from the bones and eggshells is being increased. The inoculated strain has digested and solubilises the organic P present in the bones and eggshells and released into the medium efficiently. The broth containing tricalcium phosphate and bones showing high concentration compared to egg shell with PK broth shown in figure 1. This indicates that the bacterial strain solubilised P efficiently with the presence of inorganic P.

3.4 Physicochemical parameters of soil

Table 4: Physicochemical parameters of soils used for cultivation

No.	Parameters	Results
1	Carbon	0.41 %
2	Nitrogen	0.07%
3	Calcium	3.24 meq/100gm
4	Magnesium	0.17 meq/100gm
5	Potassium	0.25 meq/100gm
6	Sodium	0.05 meq/100gm
7	pH	5.18 pH
8	Soluble P	1.00 ppm

The physicochemical properties of the soil collected from the experimental site shown in Table 4. The pH of the soil revealed that the soil was slightly acidic. The study shows PSB were able to grow in a range pH 5 to 9 [18].

3.5. Plant parameters

The cumulative result of plant parameters after application of fertilizer was recorded on 30th, 50th and 70th day.

3.5.1 Mean shoot length

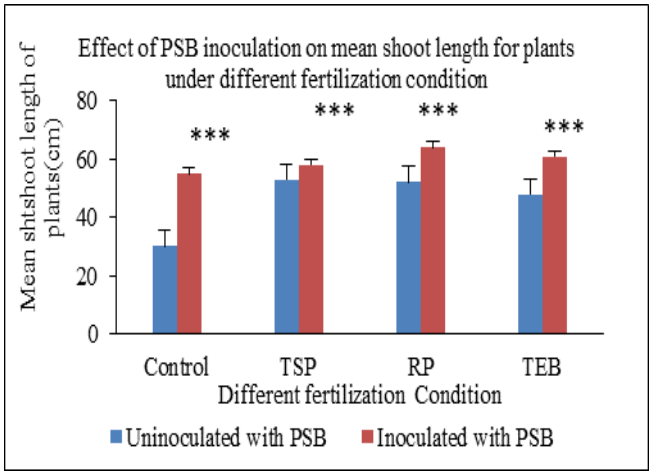

Fig.2: Effect of PSB inoculation on mean shoot length for plants under different fertilization condition expressed as mean ± standard error. Groups represented by *** indicates significant difference with p<0.05.

The mean shoot lengths of inoculated and uninoculated plant were conditions compared in figure 2. The treatment plant (RP with PSB) significantly showed higher shoot length with an increase of 21% when compared with the plant uninoculated with PSB. Whereas, the lowest shoot length was obtained in control without fertilizer and experimental plant without fertilizer with a difference of 59% which was statistically significant (p<0.05).The treatment TEB with PSB recorded a 24% difference when compared to the uninoculated with PSB control plant which was also significant with (p<0.5). This is due to the low solubility of the fertilizer into the soil. Moreover, the mean shoot length of the uninoculated plant is maintained constantly whereas, the inoculated plant shows different values at different conditions. The inoculated plants showed the highest shoot length. So the presence of PSB and bio- fertilizer can increase the plant parameters. The present study was also consistent with a number of previous studies [19, 20, 21, & 22].

3.5.2. Mean number of leaves

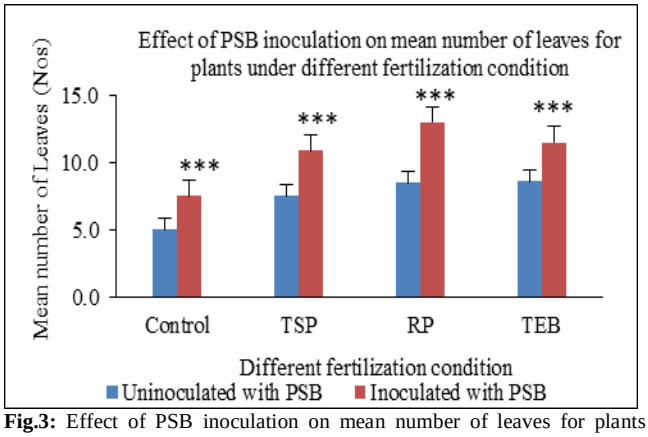

Fig.3: Effect of PSB inoculation on mean number of leaves for plants under different fertilization condition expressed as mean ± standard error. Groups represented by *** indicates significant difference with p<0.05.

Fig.3. shows the effect of all treatments on mean number of leaves until 70 days. The treatment (RP with PSB) recorded the highest number of leaves with a difference of 42% when compared to an uninoculated with PSB, followed by the treatment (TEB with PSB) increased the number of leaves by 29% when compared to uninoculated with PSB. The lowest mean number of leaves were obtained by the control without fertilizer and experimental plant without fertilizer which was the difference of 40%.In all cases, the increase was significant with p<0.05 Similar studies by Singh et al. (2013) [23] also showed inoculation with PSB significantly increased plant growth parameters compared to control plant.

3.5.3 Mean number of flowers

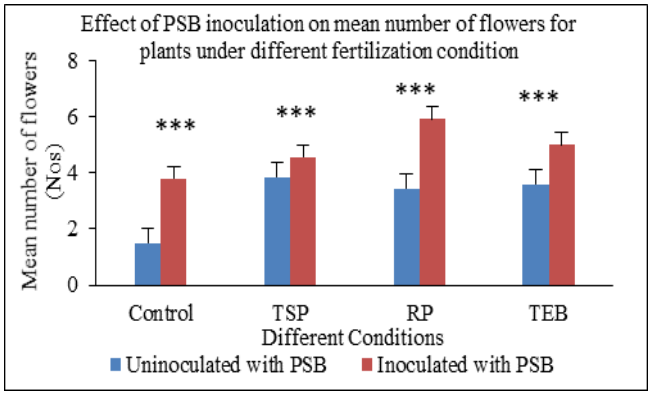

Fig.4: Effect of PSB inoculation on mean number of flowers for plants under different fertilization condition expressed as mean ± standard error. Groups represented by *** indicates significant difference with p<0.05.

The mean number of flowers counted up to 70 days in the different interval stated in Fig.4. The treatment RP with PSB significantly showed the higher number of flowers with an increase of 53% when compared with RP without PSB. TEB with PSB increased the number of flowers by 33% when compared with TEB without PSB. TSP with consortia recorded the moderately increases the number of flowers with a difference of 17% when compared to control. Lowest Mean number of flowers were obtained by the control without fertilizer and experimental plant without fertilizer with a difference of 86%.In all cases, the increase was significant with (p<0.05). Similar studies on plant growth promotion by phosphate solubilizing bacteria that have the capability to solubilize inorganic and/or organic P from soil after their application into the soil and it will be promoting plant growth and yield reported with some of the previous studies [24, 25, 23, & 26].

3.5.4 Mean length of fruits

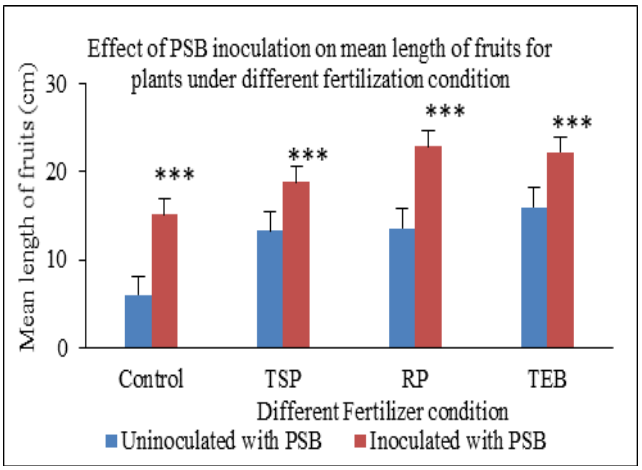

Fig.5: Effect of PSB inoculation on mean length of fruits for plants under different fertiliza6tion condition expressed as mean ± standard error. Groups represented by *** indicates significant difference with p<0.05.

The results in Fig.5.also showed the same trend. PSB consortia inoculated plants. For the PSB uninoculated plants, the values were remaining constant whereas, for PSB inoculated plants the parameters were increasing The treatment with RP and PSB recorded the highest length of fruits with the difference of 49% when compared to RP without PSB control which was statistically significant ($p<0.05$), followed by the treatment TEB with PSB increased the length of fruits by 32% when compared with TEB without PSB. The treatment TSP with PSB recorded 38% difference when compared to TSP without PSB. Lowest mean number of fruits length was obtained by the control plant without fertilizer and experimental plant without fertilizer which was also significant with $p<0.05$. A similar study conducted by Sreedevi Sarsan (2016) [27] showed significant plant yield parameters in tomato with PSB stain.

3.5.5 Mean weight of fruits

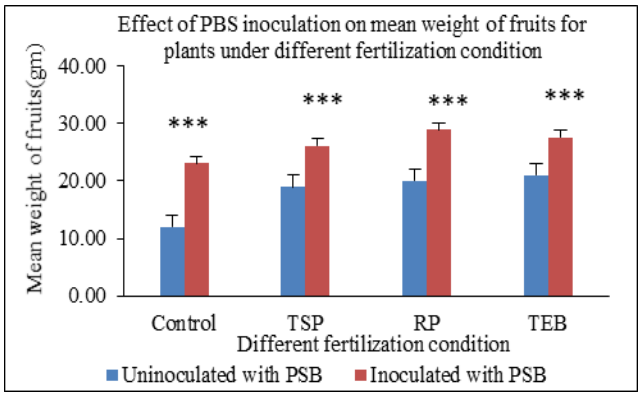

Fig.6: Effect of PSB inoculation on mean weight of fruits for plants under different fertilization condition expressed as mean ± standard error. Groups represented by *** indicates significant difference with $p<0.05$.

Comparison of the mean weight of fruits obtained from okra plant, whereby the mean weight of fruits obtained from inoculated plants is higher compared to uninoculated plants shown in figure 6. Significant ($p<0.05$) difference in the weight of fruits were observed in PSB inoculated plant with RP with a difference of 37%, followed by consortia with TEB 27%, consortia with TSP 32%, and low weight of fruits was observed in control without fertilizer and experimental plant without fertilizer. The difference was significant with $p<0.05$. PSB help to increase the nutrient uptake of the plant, providing a healthier growth for plants to produce quality fruits that have higher dry weight [28].

3.5.6 Total chlorophyll content

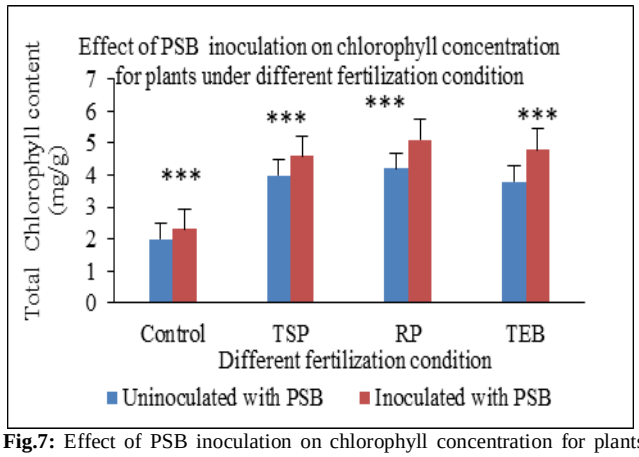

Fig.7: Effect of PSB inoculation on chlorophyll concentration for plants under different fertilization condition expressed as mean ± standard error. Groups represented by *** indicates significant difference with $p<0.05$.

The plant treated with RP and PSB recorded the highest concentration of chlorophyll content with the difference of 19% when compared to RP without PSB control which was statistically significant ($p<0.05$), followed by the treatment TEB with PSB increased the chlorophyll content of leaves by 23% when compared with TEB without PSB. The treatment TSP with PSB recorded 14% difference when compared to TSP without PSB. The lowest amount of chlorophyll of leaves was obtained by the control plant without fertilizer and experimental plant without fertilizer which was also significant with ($p<0.05$) as shown in figure 7. The chlorophyll content was lower due to there are no any additional nutrients applied to, so the available nutrients in the soil will be absorbed by the plant in the early growing stage. Eventually, when the time passed, the availability of the nutrient in environmental control plants will be limited, subsequently limit the absorption of nutrient, next the formation of chlorophyll was disturbed. Overall, the treatment plants with PSB consortia are showing higher total chlorophyll content if compared with the treatment plants without consortia. According to Singh et al (2013), the enriched plant growth and yield can be because of improved photosynthesis to the proficiency of the plants [23].

4. Conclusion

Thus, the present study showed that PSB consortia were capable of solubilizing phosphate sources which contribute to promote the growth of Okra (*Abelmoschus esculentus*), there was a significant increase in the growth parameters compared to control treatment without consortia. Further, the large level pilot study can be conducted to find the significance of the effect of PSB consortia to promote the growth and yield parameters of the plant. The application of PSB microbial consortia with the cheap source of nutrients has shown the capabilities of providing similar or enhanced results based on various characters expressed by the plant and thus reduces the reliance on synthetic chemical fertilizer

Acknowledgements

The authors would like to express great appreciation to MAHSA University for providing facilities for carrying out the work. We thank our colleagues for their helpful support on research. Besides that I would also like to show gratitude Mr.Mahendran Director MAHSA technologies and team for helping to arrange the land for cultivation.

References

[1] Mal B, Mahapatra. P and Mohanty S (2014), "Effect of Diazotrophs and Chemical Fertilizers on Production and Economics of Okra (Abelmoschus esculentus, L.) Cultivars," American Journal of Plant Sciences, Vol. 5 No. 1, pp. 168-174.

[2] Binalfew T and Alemu Y (2016), Characterization of Okra (Abelmoschus esculentus (L.) Moench) Germplasms Collected from Western Ethiopia, International Journal of Research in Agriculture and Forestry, 3(2).

[3] Jonathan Eshiet, A. and Andi Brisibe, E. (2015), Morphological Characterization and Yield Traits Analysis in Some Selected Varieties of Okra (Abelmoschus Esculentus L. Moench), Advances in Crop Science and Technology, 04(01).

[4] Semwal DK, Semwal RB, Combrinck S, and Viljoen A, Myricetin: A Dietary Molecule with Diverse Biological Activities. Nutrients, [e-journal] 8(2), 90, (2016), available online: https://dx.doi.org/10.3390/nu8020090, last visit: 19.08.18.

[5] Karpagam T and Nagalakshmi P (2014), Isolation and characterization of Phosphate Solubilizing Microbes from Agricultural soil, International Journal of Current Microbiology and Applied Sciences, 3(3), pp.601-614.

[6] Wang T. Camps-Arbestain M, Hedley M and Bishop P (2012), Predicting phosphorus bioavailability from high-ash biochars. Plant and Soil, 357(1-2), 173-187.

[7] Anand K, Kumari B and Mallick M (2016), Phosphate Solubilizing Microbes: An Effective and Alternative Approach as Biofertilizers. International Journal of Pharmacy and Pharmaceutical Science, 8(2), pp.37-40.
[8] Huey T & Ibrahim A, Statistical analysis of annual rainfall patterns in peninsular Malaysia using TRIMM algorithm, The 33rd Asian conference and remote sensing, Thailand,(2012), https://www.researchgate.net/publication/260593838, last visit: 16.08.18.
[9] Mekki BE (2016). Effect of bio-organic, chemical fer-tilizers and their combination on growth, yield and some macro and micronu-trients contents of faba bean (Vicia faba L.), Bioscience Research, 13, pp.08-14.
[10] Ch'ng H, Ahmed O, and Majid N (2014), Improving Phosphorus Availability in an Acid Soil Using Organic Amendments Produced from Agroindustrial Wastes, The Scientific World Journal, 2014, pp.1-6.
[11] Kivela J (2015), Effects of meat bone meal as fertilizer on leaf ni-trogen status in sugar beet and on soil nitrate concentrations, Acta fytotechnica et zootechnica, 18(Special Issue), pp.71-73.
[12] Farahan R, Supri A, and Teh P (2015), Tensile and Water Absorp-tion Properties of Eggshell Powder Filled Recycled High-Density Polyethylene/Ethylene Vinyl Acetate Composites: Effect of 3-Aminopropyltriethoxysilane, Journal of Advanced Research in Ma-terials Science, 5(1), pp.1-9.
[13] Ahamed M and Kibret M (2014), Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Journal of King Saud University - Science, 26(1), pp.1-20.
[14] Goldstein AH (1986) Bacterial solubilization of mineral phosphates: Historical perspective and future prospects. American Journal of Alternative Agriculture 1:51-7.
[15] Richardson A and Simpson R (2011), Soil Microorganisms Mediat-ing Phosphorus Availability, American Society of Plant Biologists, Plant Physiology, 156, 989–996.
[16] Pikovskaya, R.I (1948) Mobilization of P in soil in connection with the vital activity of some microbial species, Microbiologiya 17: 362-370
[17] Trivedy PK, Goel PK, Chemical and biological methods for water pollution studies, Series in Methodology, Environmental Publica-tions, Karad, (1986), pp.126-127.
[18] Liba C M Ferrara F I S Manfio G P Fantinatti-Garboggini F Alburquerque R C Pavan C Ramos P L Moreira-Filho C A and Barbosa H R 2006 Journal of Applied Microbiology 101, 1076-1086.
[19] Sharma K, Dak G, Agrawal A, Bhatnagar M, and Sharma R (2007), Effect of phosphate solubilizing bacteria on the germination of Cicer arietinum seeds and seedling growth, Journal of Herbal Med-icine and Toxicology, 1, 61-63.
[20] Zaidi A, and Khan M S (2006), Co-inoculation effects of phosphate solubilizing microorganisms and Glomus fasciculatumon on green gram - Bradyrhizobium symbiosis, Turkish Journal of Agriculture and Forestry, 30,223-230.
[21] Gusain Y S, Kamal R, Mehta C M, Singh U S, Sharma A K (2015), Phosphate solubilizing and indole-3-acetic acid producing bacteria from the soil of Garhwal Himalaya aimed to improve the growth of rice, Journal of Environmental Biology, 36, 301–307.
[22] Hussain M I, Asghar H N, Akhtar M J, and Arshad (2013), Impact of phosphate solubilizing bacteria on growth and yield of maize. Plant Soil and Environment, 32(1), 71-78.
[23] Singh A V, Chandra R, and Reeta G(2013), Phosphate solubilization by Chryseobacterium sp. and their combined effect with N and P fertilizers on plant growth promotion, Archives of Agronomy and Soil Science, 59(5), 641– 651.
[24] Ajay Veer Singh, Birendra Prasad and Reeta Goel (2018), Plant Growth Promoting Efficiency of Phosphate Solubilizing Chryseobacterium sp. PSR 10 with Different Doses of N and P Fer-tilizers on Lentil (Lens culinaris var. PL-5) Growth and Yield, In-ternational journal of current microbiology and applied science, 7(5), 2280-2289
[25] Singh A V and Prasad B (2014), Enhancement of plant growth, nodulation and seed yield through Plant Growth Promoting Rhizobacteria in Lentil (Lens culinaris Medik cv. VL125), Interna-tional journal of current microbiology and applied science,3(6), 614-622
[26] Wang M, Zheng Q, Shen Q and Guo S (2013), the critical role of potassium in plant stress response, International journal of molecu-lar science, 14, 7370–7390.
[27] Sreedevi Sarsan. 2016. Effect of Phosphate Solubilising Bacteria Bacillus PSB24 on Growth of Tomato Plants. Int.J.Curr.Microbiol.App.Sci. 5(7): 310-320.
[28] Meena R, Kumar S, Maji S, Kumar D and Kumar M (2014), Effect of organic manures and biofertilizers on growth, flowering, yield and quality of tomato cv. Pusa Sheetal, International Journal of Ag-ricultural Sciences, 10(1), pp.329-332.