International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 7; Issue 7(B); July 2018; Page No. 13986-13990
DOI: http://dx.doi.org/10.24327/ijcar.2018.13990.2519

Research Article

ECOFRIENDLYAPPLICATION OF PHOSPHATE SOLUBILIZING BACTERIA IN CHILLY PLANT (*Capsicum annuum* L.)

Jegatheeswari J., Subha.K* and Eugin Amala.V

Department of Microbiology, Idhaya College for Women, Kumbakonam-612001, Thanjavur Dt., Tamilnadu

A R T I C L E I N F O

Article History:

Received 16th April, 2018
Received in revised form 4th May, 2018 Accepted 12th June, 2018
Published online 28th July, 2018

Key words:

Phosphate Solubilizing Bacteria (PSB), FYM (Farm Yard Manure), PVK edium,*Capsicum annuum*L.*Pseudomona*sp., *Bacillus* sp.

A B S T R A C T

The aim of the study was to isolate phosphate solubilizing bacteria from soil and investigate their potential for plant growth promotion. Most of the bacteria were isolated from soil sample with pH values close to 6.8-7.2. There is a close relationship between the phosphate solubilizing activity and low pH levels growth medium. The bacterial isolates were identified as belonging to the following genus *Pseudomonas* sp., *Bacillus* sp., *Rhizobium* sp., *Azotobacter*sp. and named as PSB1, PSB2, PSB3 and PSB4. The pot culture experiment was conducted to study the effect of T1 – Control (with out inoculums), T2 – Control + PSB, T3 – Control + FYM, T4 – Control + PSB + FYM for the enhancement of growth of Chilly (*Capsicum annuum* L.) plant by recording plant height, shoot length, Rootlength, Leaf lengthand Dry Weight. In general, plant growth was better in T2 –PSB treated pot. The PSB treated *Capsicum annuum* L. plant were showed better growth than T3 & T4.

INTRODUCTION

Soil is a dynamic, living matrix that is an essential part of the terrestrial ecosystem.It is a crucial resource not only for agricultural production and food security but also towards maintenance of most life processes. Soil is considered as a storehouse of microbial activity, though the space occupied by living microorganisms is estimated to be less than five percent of the total space. The functions of soil microorganisms are central to the decomposition processes and nutrient cycling. They play an important role in soil processes that determine plant productivity. For successful functioning of introduced microbial bio-inoculants and their influence on soil health, efforts have been made to explore soil microbial diversity of indigenous community, there distribution and behaviour in soil habitats. “Soil bacteria are one of the major groups of microbes, which are abundant in rhizosphere soil ranging between 10-6 to 10-8 colony-forming units (CFU) per gram, and some of them have shown great potential for plant health promotion and as bio-control agents of nematodes”. These plant growth promoting rhizobacteria (PGPRs) are currently being exploited towards these ends. Improving soil fertility is one of the most common practices in agricultural production (Sarkar and Uppal, 1994).

Biofertilizers play a very significant role in improving soil fertility by fixing atmospheric Nin association with plant roots and without it, solubilise insoluble soil phosphates and produces plant growth substances in the soil (Khan *et al*.,2011).

Phosphorous is one of the major plant nutrients which are essential for growth of normal plants. As a component of every living cell, phosphorus controls all living processes including heredity and energy transport system (Donahue *et al.,* 1990).Phosphorus plays an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the living plant (Gyaneshwar*et al.,* 2002). The objective of this study is to isolate, identify and characterizethe phosphate solubilizing bacteria from soil sample at IdhayaCollege for Women, Kumbakonam.

MATERIALS AND METHODS

Collection of Soil Sample

The soil samples were collected from IdhayaCollege for women, Kumbakonam. Sakkottai, Thanjavur District located in Tamilnadu, India. The soil samples were collected from a depth of 10-20cm below the soil surface. The top soil (10 cm from the surface) was collected randomly from the study site using alcohol rinsed Peterson grab, then the samples were transferred to new polythene bags using sterile and transported to the laboratory for further examination. For the analysis of soil nutrients, l0 gram of soil separately collected in polythene bags from the station.

Analysis of Physico- chemical character

Organic carbon content was determined by adapting chromic acid wet digestion method as described by Walkley and Black,

Corresponding author:* **Subha.K
Department of Microbiology, Idhaya College for Women, Kumbakonam-612001, Thanjavur Dt., Tamilnadu

(1934), and available phosphorus by Brayl method described by Bray and Katz, (1945). Calcium (Neutral 1N NH_4 OAC extracted 1:5) was extracted with neutral 1N ammonium acetate and the available calcium in the extract was determined by verse ate method (Jackson, 1973). Available micro nutrients such as Zn, Cu and Mn determined in the diethylenetriaminepenta acetic extract of soil (Lindsay and Norvell, 1978). Magnesium, sodium, calcium, potassium, and available iron were analysed by APHA method (1989).

Isolation of Phosphate Solubilizing Bacteria (Goenadi, 2006)

Microbial population was estimated by plate count method. Ten gram soil was suspended in 90ml sterile distilled water in Erlemeyer flask and mixed thoroughly for 30 minutes using a mechanical shaker at 110 rpm. Then 1ml an aliquot transferred with sterile pipettes to 9ml sterile distilled water in test tube. This suspension was stirred for 10 seconds. A subsequent serial dilution was prepared as above to 10^{-1}-10^{-9}. From each serial dilution, 0.1ml of aliquot was transferred to sterile petriplate and over poured, dispersed swirling with on Pikovskaya's agar medium (PVK) containing insoluble Tricalcium phosphate and incubated at 27-30ºC for 7 days. Colonies showing halo zones were and purified by 5 times subculture method on Pikovskaya's (PVK) agar medium for studying colony morphology.

Isolation of Pure cultures (Goenadi, 2006).

The best zone formed colonize of Pikovskaya's agar medium were picked and inoculated into the following specific medium MacConkey agar medium, PYD medium, YEMA medium, Waksman Base -77 medium by this best 4 specific PSB were isolated.

Identification of the PSB (Holt and Krieg, 1994)

The different phosphate solubilising bacterial colonies were identified based upon their morphological characteristics like shape, size, structure, texture, appearance and elevation. Colours and by various biochemical test according to Bergey's manual for systematic bacteriology methods systematic and further identification was done on the basis of staining. Differential staining i.e. Gram staining and biochemical test was performed.

Screening test for PSB (Qualitative method)

All the suspected colonies were screened for phosphate solubilization on Pikovskaya's medium. Isolates showing phosphate solubilizing ability were inoculated at the centre Pikovskaya's plate and incubated at30ºC. Diameter of clearance zone was measured successively after 24 hours, upto 7 days. The Phosphate Solubilization Efficiency (PSE) is the ratio of total diameter .i.e. clearance zone including bacterial growth and the colony diameter (Singh *et al.,* 2011).

$$PSI = \frac{\text{Colony diameter + Halozone diameter}}{\text{Colony diameter}}$$

All the observations were recorded in triplicate. Strains developing clear zones around their colonies could easily identify as PSB.

Production of phosphate Solubilizing Biofertilizer
Inoculums preparation

The broth cultures of selected organism were prepared for the biofertilizer production.

Carrier material

Well decomposed FYM (Farm Yard Manure) was collected from Andimadam, AriyalurDt, Tamilnadu, India. The FYM sterilized under sunlight.

Biofertilizer formulation

The broth culture of selected organism and carrier material were mixed in 1:2 Proportions. Then it was packed in polythene bags and stored at 4ºC temperature.

Application of biofertilizer on Chilly (Capsicum annuum L.) plant

The pot culture experiment conducted for brinjal plant with the following treatments.

TC	-	Control Soil
T1	-	Control Soil+ PSB
T2	-	Control Soil+FYM
T3	-	Control Soil+FYM+PSB

After 15 days it was transplanted to the field with above treatment.The effects of these treatments on plant productivity were observed for 15, 25 & 35 days for root, shoot and leaf length.

RESULTS AND DISCUSSION

Physico-chemical characteristics

In the present study of Physico-chemical characteristics of the soil sample were collected from Idhaya College for Women, Kumbakonam, Thanjaur District, located in Tamilnadu, India revealed the following features. The temperature was recorded to be 27°C - 30°C and the pH which varied between 7.2 and observed in an optimum range. The data were summarized in the table-1.Jha*et al*., (1992) found that biological activity and composition of soil microbes are generally affected by many factors including physico-chemical properties of soil, temperature and vegetation totally, 36 isolates obtained from 18 soil samples collected randomly, and the samples were tested for phosphate solubilizing property, of these 22 were found to be PSB. Upon screening, 12 isolates showed varying levels of phosphate solubilizing activity from 18 regions of Visakhapatnam and aruku valley.

Table 1 Physico-chemical parameter

S.NO	Name of the parameter	Results
1.	pH	7.3
2.	Temperature	27-30°C
3.	Salinity (ppt)	-
4.	Electrical conductivity (dsm^{-1})	0.22
5.	Organic carbon (%)	0.36
6.	Organic matter (%)	0.36
7.	Available phosphorus (mg/kg)	5.0
8.	Available potassium (mg/kg)	12
9.	Available zinc (ppm)	1.23
10.	Available copper (ppm)	1.21
11.	Available iron (ppm)	8.64
12.	Available manganese (ppm)	3.21
13.	Cations Exchange Capacity (C. Mole $Proton^+$ /kg)	21.2
	Exchangeable Bases (C. Mole $Proton^+$/kg)	
14.	Calcium	11.6
15.	Magnesium	9.3
16.	Sodium	1.33
17.	Potassium	0.22

Microbial count

Totally, 13 microbial species isolates obtained from soil sample collected randomly, and the samples were tested for phosphate solubilizing property, of these 4 were found to be PSP. Upon screening isolates showed varying levels of phosphate solubilizing activity PVK agar plate. Isolates are showed in the plate-1.

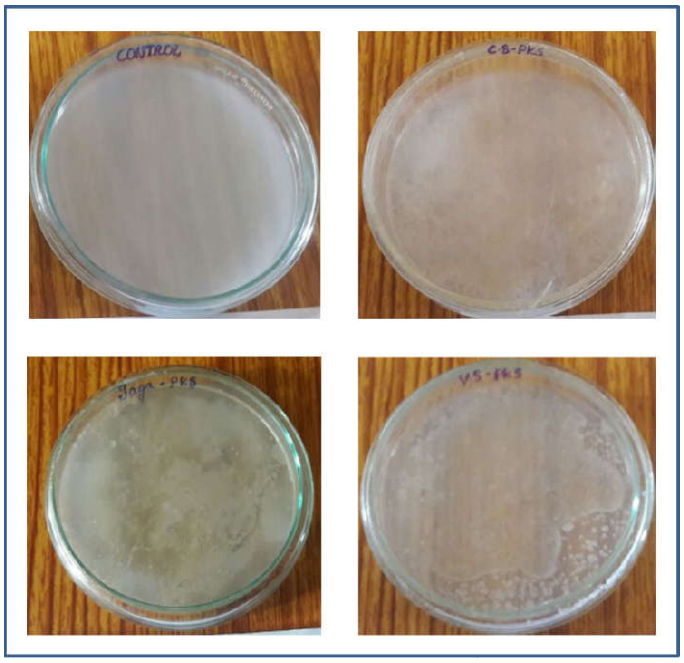

Plate 1 Isolation of PSB from soil

Identification of bacterial isolates through biochemical test

The isolates were gram positive rods and gram negative rods shaped and motile organisms. By biochemical characterization, the isolates was identified as belonging to the following genus *Bacillus* sp., *Pseudomonas* sp., *Rhizobium* sp., *Azotobacter* sp., and named as PSB-1, PSB-2, PSB-3 and PSB-4. Morphology and Biochemical test is determine the physiology of the isolated strain, a series of biochemical test were performed in the plate-1 and table-2 and 3.

In the previous investigation, Anjum*et al*., (2007) isolated total 15 phosphate solubilizing plant growth promoting bacteria belonging to genus *Pseudomonas* sp. were isolated from healthy and sick sites of apple tree on kings be medium from shimla and kulluDt of Himachal Pradesh. Preliminary selection as phosphate solubilize was done on Pikovskaya's agar medium. The yellow colored colonies transparent halo was selected and purified a same medium. They all belongs to *Pseudomonas* sp*.,* on basis of morphological and bio chemical characterization *Pseudomonas* sp.

Table 2 Morphological characterization of phosphate solubilizing bacteria

S.No	Morphological characterization	PSB1	PSB2	PSB3	PSB4
1.	Size	Small	Small	Large	Small
2.	Shape	Rod	Rod	Rod	Rod
3.	Margins	Entire	Entire	Entire	Entire
4.	Surface Texture	Smooth	Smooth	Smooth	Smooth
5.	Consistency	Moist/ Butyrous	Moist/ Butyrous	Moist/ Butyros	Moist/ Butyrous
6.	Pigmentation	White	Pale yellow	White	White

A well-known plant-associated bacteria and effective root colonizer detected in this study as abundant members of the cultivable P-solubilizing PGPR Community. This most likely due to the microbial community compossion of the stud sites.

Table 3 Identification of bacterial isolates through biochemical test

Morphological test	PSB-1	PSB-2	PSB-3	PSB-4
Gram staining	Gram (-)	Gram (+)	Gram (+)	Gram (-)
Shape	Rod	Rod	Rod	Rod
Motility test	Motile	Motile	Motile	Motile
Biochemical tests				
Indole	-	-	+	+
MR	-	+	+	-
VP	-	+	+	-
Catalase	+	+	+	+
Oxidase	+	-	-	-
Citrate	+	+	+	-
Urease	-	-	+	+
Carbohydrate fermentation	-	-	+	+
Nitrate reduction	+	-	+	-
TSI agar	-	+	+	-

Positive (+) ; Negative (-)

Screening test for PSB (Qualitative Analysis)

The result analysis of the concentration of solubilize phosphate which was determined using Fiske and Subbarow method and also indirect measurement of phosphate solubilization by plate assay were the halo and colony diameter of the incubated agar plates were reported in fig-1. From the bacterial isolates PSB2 (16mm) is high phosphate solubilization activity followed by PSB4(12mm), PSB3(8mm) and PSB1(5mm) respectively.

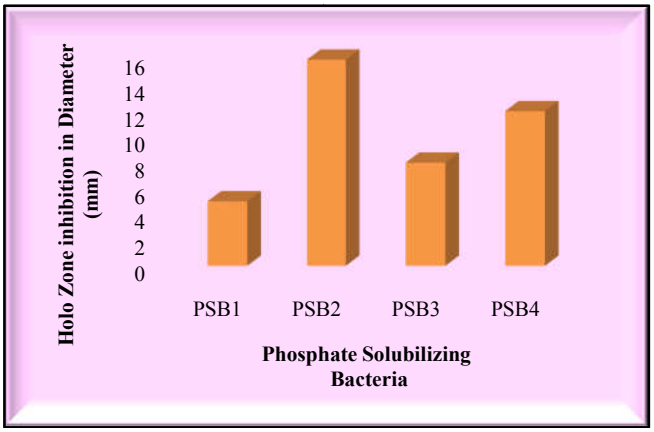

Fig 1 Screening test for PSB (Qualitative Analysis)

Evaluating the potential of PSB as fertilizer (Pot culture Technique)

First has been taken four pots with 50 chilly seed taken. The pot was marked (TC, T1, T2, and T3). After completing the seed inoculation incubate the pot in direct sunlight, water, Co_2 and other component used to enhance the plant growth. Few days later seeds were start to germinate. In First pot (TC) 36 seedsand (T1), 45 seeds (T2), 39 seeds and (T3) 40 seeds were germinated. In this method was determining PSB is best biofertilizer for plant growth (Plate-3 & Fig.-2).

The pot culture experiment was conducted to study the effect of T1-control (without inoculums), T2-control+PSP, T3-control+FYM, T4-control+PSB+FYM, for the enhancement of growth and yield parameters of chilly (*Capsicum annuum* .L) plant by recording plant,shoot, root and leaf height, and also

Dry weight. In general, plant growth was better in PSB treated pot. The PSB treated *Capsicum annuum*.L plants were showed better growth than control and Control+FYM. Several studies have reported that seed or soil inoculation with phosphate solubilizing bacteria such *Bacillus* sp. can solubilize fixed soil P and applied phosphate, resulting in higher crop yields (Yadav and Dadarwal 1997; Sindhu 2002).

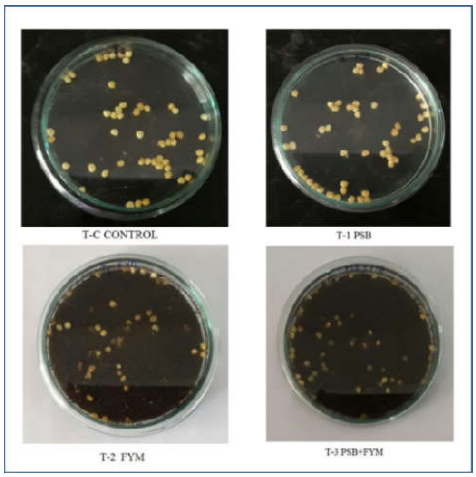

Plate 3 Seed Inoculum Preparation

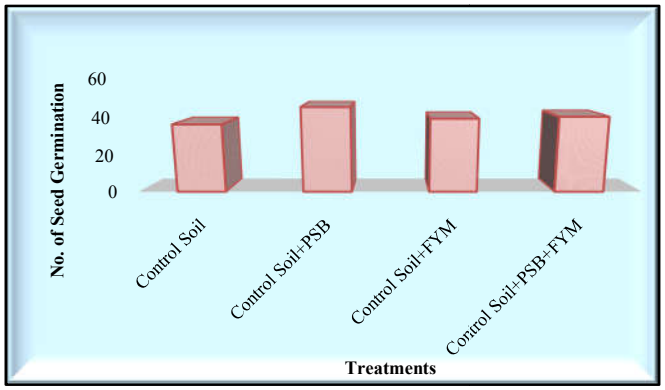

Fig 2 Measurement of the seed germination

Measurement of plant growth

The root length 3.5 was gradually increased in treated plants (T4) and the minimum level 2 and 1.2 was observed in T2 and T3. The shoot, Root and leaf length and also Dry weight was recorded in Plate: 4 &5; Table- 5 to 7.

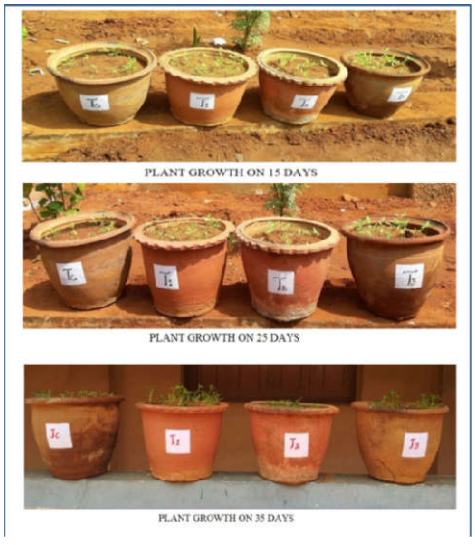

Plate 4 Pot culture Technique

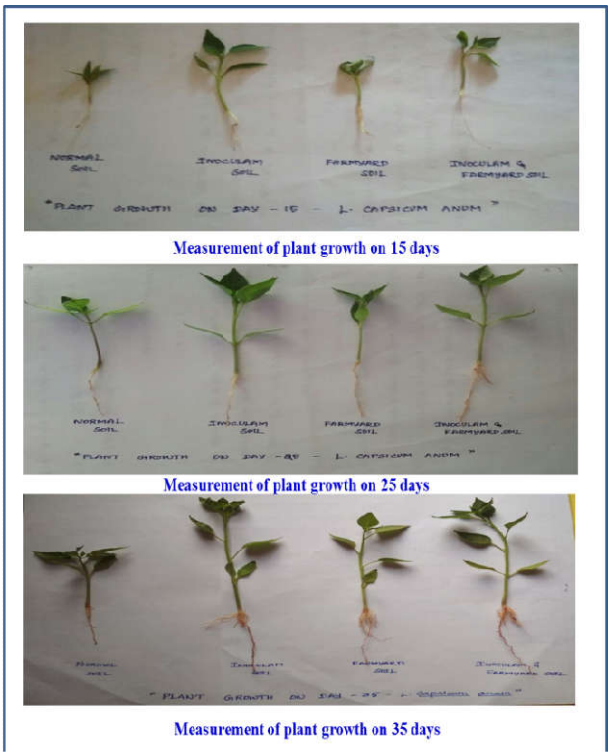

Plate 5 Measurement of plant growth on different days

Table 5 Measurement of plant growth on 15 days

S.No	Treatment	Plant height (cm)	Shoot (cm)	Root (cm)	Leaf (cm)
1	Control Soil	5.3	2.2	1	1
2	Control Soil+PSB	8.7	5	2	2.2
3	Control Soil+FYM	6	4	1.2	1.5
4	Control Soil+PSB+FYM	8	3	3.5	1.4

Table 6 Measurement of plant growth in 25 days

S.No	Treatment	Plant height (cm)	Shoot (cm)	Root (cm)	Leaf (cm)
1	Control Soil	10.9	4	4	2
2	Control Soil+PSB	15	6.5	5	3.5
3	Control Soil+FYM	12	5	5.3	2.5
4	Control Soil+PSB+FYM	13.8	6	4.9	2

Table 7 Measurement of plant growth on 35 days

S.No	Treatment	Plant height (cm)	Shoot (cm)	Root (cm)	Leaf (cm)
1	Control Soil	15	8	7	2
2	Control Soil+PSB	24	12	9.5	3.5
3	Control Soil+FYM	18	10	10	2.5
4	Control Soil +PSB+FYM	21.3	11	9	2

CONCLUSION

In this study concluded that PSB exhibited a soils collection. *Bacillus* sp., had shown highest solubilizing capacity. Relationship between plant and phosphate solubilizing bacteria(PSB), in which bacteria provide soluble phosphate and plants supply root borne carbon compounds (mainly sugars), that can be metabolized fort bacterial growth, hence for biofertilization the use of PSB improves the crop yield. In short, results from all these and other experiments suggest that co-inoculation of Chilly plant with different beneficial properties should be the future trends of biofertilizer application of sustainable crop production. Soil fertility management by biofertilizers (PSB) are one of the basic components of sustainable agriculture.

Acknowledgement

Theauthors are thankful to the Principal and Department of Microbiology, Idhaya College for Women, Kumbakonam, for providing the lab facilities to carry out this study.

References

Anjum, M. A., Sajjad, M. R., Akhtar, n., Qureshi, M. A., Iqbal, A., Jami. And Hasan, M. (2007).Response of cotten to plant growth promoting rhizobacteria (PGPR) inoculation under deferent levels of nitrogen.*Agri. Res*, 45; 135-143.

APHA.(1989). American public health association, standard method for examination of water and waste water APHA, AWWA, WPCF, Newyork, 17thEdn.

Bray, R. and Kortz, L. T. (1945). Determination of total organism and available forms of phosphors in Soil. *Soil sci,*59: 39-46.

Donahue, R.L., Miller, R.W.s, and Shickluna, J.C., (1990). An Introduction to Soil and Plant Growth. *Pren.Hall. Pvt Ltd.,* New Delhi, pp: 222.

Goenadi, (2006).Exploitation of soil microbial activities and use of less expensive through extracellular polysaccharides.

Gyaneshwar, P., Kumar, G., Parekh, L., and Poole, P.(2002).Role of Microorganism in improving P nutrient of plants. *Plant soil.,* 245,83-93.

Holt,J. G. and Krieg, N. R. (1994). "Bergey's manual of determinative bacteriology".

Jackon, M. L. (1973). Soil chemical Analysis Prentice Hall of India pvt.Ltd. New Delhi. 498.

Jha, D. K., Sharma,G.D. and Mishara.R.R.(1992). Ecology of soil micro-flora and mycorrhizalsymbionts. *Bio.Fertiile.Soil.*12:272-278.

Khan, T. A., Mazid, M., and Mohammand, F., (2011). A review of ascorbic acid potentialities against oxidative stress induced in plants. *J.Agrobiol.,*28(2): 97-111.

Lindsay, W. L. and Norwell, W. A. (1978). Development of DTPA of soil test for Zn, Fe, Mn and cu. *J. Ame.Soil.Scie,* 42:421-428.

Sarkar, M.C., and Uppal, K.S., (1994). Phosphorus research in India.Potash and phosphate sinstitute of Canada.India programme, Gurgaon, Hariyana, India.

Sindhu, (2002). Phosphate solubilization bacteria. *Microbiology Journal* vol-1(5):154-175.

Walkley,A. and 1. Black, A. (1934). An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method soil, *Soil sci*. 37:29-37.

Yadav, K.S. and Dadarwal, K.R (1997). Phosphate solubilization and mobilization through soil microorganisms. In: *SciPublis Jodhpur. Biot.Appr. Soil Micr. Sust. Crop Prod*., 293-308.

How to cite this article:

Jegatheeswari J *et al* (2018) 'Ecofriendly application of Phosphate Solubilizing Bacteria in Chilly Plant (Capsicum annuum L.)', *International Journal of Current Advanced Research,* 07(7), pp. 13986-13990.
DOI: http://dx.doi.org/10.24327/ijcar.2018.13990.2519

****