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A study on optimization of a new PGPR formulation towards the growth of Brinjal plants and soil fertility

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Abstract
In the present study a new PGPR formulation was optimized for the suitable carrier, pH and weather conditions towards the growth response of brinjal plants at 30 days and improving soil fertility. The effect of inoculation of different carrier based PGPR formulations on soil available plant nutrients, population size of PGPR and plant growth promotion of brinjal seedlings at 30 days in green house trial was significant at $p<0.05$. Good result was observed with Humus based PGPR; 578.24 ± 1.14 $KgNha^{-1}$, 648.26 ± 1.24 $KgKha^{-1}$, 38.34 ± 1.32 $KgPha^{-1}$, 15 ± 2 × 10^6cfu/g soil Nfb, 16 ± 3.61 × 10^6cfu/g soil Psb, 15.67 ± 2.08 × 10^6cfu/g soil Ksb, 26.38 ± 0.56 shoot length, 8.22 ± 0.60 root length and 6.20 ± 1.50 number of leaves. H-PGPR was found to be effective on soil available plant nutrients; 578.28 ± 1.14 $KgNha^{-1}$, 38.40 ± 1.28 $KgPha^{-1}$, 648.32 ± 1.20 $KgKha^{-1}$, population size of PGPR; 15.67 ± 2.08 Nfb, 16.67 ± 3.22 Psb, 16.33 ± 2.52 Ksb (10^6cfu/g soil) and plant growth promotion; 26.62 ± 0.85 shoot length, 8.24 ± 0.68 root length and 6.60 ± 0.55 number of leaves at pH 7.0. Significant result was observed with H-PGPR on summer season when compared to winter and rainy season ($p<0.05$).From this work, it is concluded that PGPR grows and works better in summer season at pH 7.0 and humus as the carrier.

Keywords: carrier, pH, PGPR, Humus, biofertilizer

1. Introduction

Biofertilizers plays an important role in developing an integrated nutrient management system, sustaining agricultural productivity with low environmental impact [1, 2, 16]. By using biofertilizers, it is possible to obtain a crop productivity similar to that obtained with other fertilizers but with a significant reduction of their use.

Generally PGPR may face competitive conditions once introduced into the soil that significantly reduce their plant growth promoting traits [4]. Therefore the efficiency of a specific biofertilizer may differ greatly under different agricultural soils [7]. To avoid such problems, the farmers should consider some factors such as soil pH, season, carrier substrate etc. before using biofertilizers for getting improved crop productivity.

Any type of PGPR formulation, able to maintain its biological activity at an adequate level uptoatleast one season is the main factor assuring its efficiency [5]. Therefore formulation of an inocula with a particular carrier in order to protect the cells during storage and transport and enhancing the persistence of the inocula in soil, plays an important role in determining the efficiency of biofertilizer [17, 24]. Different carriers exhibits specific positive effects and drawbacks, thereby affecting the overall quality of the biofertilizers [16, 10, 11]. Carrier based biofertilizer technology makes use of a large number of carriers including charcoal, wheat bran, peat, press mud, lignite, vermiculite etc. [9, 13]. But still researches are going on to explore cheap, easily available and efficient carrier for a biofertilizer to perform successfully.

Soil pH has been reported to be the most important predictor of microbial population structure at the ecosystem in which higher density associated with neutral soils [22]. It affects the colonization potential and efficiency of all the microorganisms included in biofertilizers.

This research was conducted to determine the suitable carrier substrate, pH and weather condition for the proposed PGPR formulation towards the growth response of brinjal plants.

2. Methodology

The PGPR formulation was made with N fixing, P and K solubilizing strains of bacteria (*Azotobacterchroococcum*, *Pseudomonas putida*, *Burkholderia cenocepacia* and *Pseudomonas fluorescence*). These strains were isolated from various agricultural fields in Kerala by dilution plate technique in selective media [8, 20, 12] and identified by 16srRNA Sequencing. The given PGPR formulation was optimized for the suitable carrier substrate, pH and weather conditions. The growth response of brinjal plants, soil available plant nutrients and soil microbial load were monitored at 30 days of treatment.

2.1. The carrier substrate

The PGPR Mix prepared in different carriers were tested for their efficiency in promoting brinjal plant growth. The carrier materials used were talc, humus and coir pith. This experiment was conducted in summer season (April 2015) at a pH between 6.5-7.0. Six treatments (control, reference strain, humus based PGPR Mix, talc based PGPR Mix, coir pith based PGPR Mix and chemical fertilizer) were made with 5 replications. Medium size grow bags were filled with garden soil and planted with one week old brinjal seedlings. All the bags were applied with treatments and watered daily.

2.2. Seasons

Summer (April 2015), rainy (June2015) and winter (November 2015) seasons of Kerala were selected for optimization experiment. For each seasons, 4 treatments were made with 5 replications. The treatments were, control, reference strain, Humus based PGPR Mix and chemical fertilizer. Medium size grow bags were filled with garden soil and planted with brinjal seedlings. The treatments were applied to one week old brinjal seedlings and watered daily.

2.3. pH

The garden soil samples were filled in grow bags and the pH were adjusted to 5.5, 6.0, 6.5,7.0,7.5,8.0 and 8.5 by adding wood ash in the ratio of 10:1, 10:2, 10:3, 10:4, 10:5, 10:6, 10:6, 10:7 (soil : wood ash). For each pH conditions, 4 treatments were made with 5 replications as mentioned above. All the bags containing soil samples of different pH were planted with one week old brinjal seedlings and watered daily.
After 30 days, the seedlings were monitored for shoot length, root length and number of leaves. The soil samples were also tested for microbial load and available plant nutrients. The microbial population especially Nfb, Psb and Ksb was calculated by dilution plate techniqueusing selective media. The soil available plant nutrients such as N, P and K were determined by Alkaline permanganate method [25], Colorimetry [18] and Flame photometry [6] respectively.

2.4. Statistical Analysis

The results were biostatistically analysed using the software SPSS Version 16. A two way mixed, One Way, Welch and Kruskal Wallis ANOVA followed by multiple comparison (Post hoc) using Bonferroni correction, Games Howell and Mann whitney test respectively were performed to find out the effect of pH, season and carriers on soil available plant nutrients, microbial population size and plant growth parameters at $p<0.05$ level.

3. Results and discussion

The given treatments were applied to brinjal seedlings under greenhouse conditions. All the plants were harvested at 30 days and recorded the growth parameters such as shoot length, root length and number of leaves. The soil samples were also analysed to determine microbial population size and available plant nutrients like nitrogen, phosphorous and potassium.

3.1. The carrier substrate

The selection of a suitable carrier, capable of supporting high viable microbial population for a prolonged duration is very much important for a biofertilizer to work effectively in the soil system.
The inoculation of PGPR in the rhizosphere region of brinjal plant resulted in a significant increase in the soil available plant nutrients. The level of N, K and P in H-PGPR treatment were found to be 578.24 ± 1.14 $KgNha^{-1}$, 648.26 ± 1.24 $KgKha^{-1}$ and 38.34 ± 1.32 $KgPha^{-1}$respectively (Table 1).
The population of PGPR in the brinjal rhizosphere soil increased significantly. Among the treatments, H-PGPR recorded highest population density of Nfb, Psb and Ksb with 15 ± 2 × 10^6cfu/g soil, 16 ± 3.61 × 10^6cfu/g soil and 15.67 ± 2.08 × 10^6cfu/g soilrespectively (Figure 1).
H-PGPR showed significant stimulating effect on the growth of brinjal plants particularly in shoot length (26.38 ± 0.56), root length (8.22 ± 0.60) and number of leaves (6.20 ± 1.50). Figure 2 represents the results.

Table 1: Effect of different carrier based PGPR formulations on Soil available plant nutrients at 30 days of brinjal seedling growth

Treatment	Kg N ha^{-1}	Kg P ha^{-1}	Kg K ha^{-1}
C	247.86 ± 2.04^{a}	4.50 ± 0.47^{a}	178.28 ± 1.23^{a}
RS	407.42 ± 1.80^{b}	20.62 ± 0.46^{bd}	288.08 ± 1.82^{b}
H-P	578.24 ± 1.14^{f}	38.34 ±1.32^{f}	648.26 ± 1.24^{f}
CP-P	492.20 ± 3.94^{d}	22.38 ± 0.60^{cd}	449.48 ± 1.61^{d}
T-P	448.96 ± 0.61^{c}	19.86 ± 0.23^{b}	427.06 ± 1.23^{c}
CF	564.78 ± 1.06^{e}	28.92 ± 0.75^{e}	597.74 ± 1.43^{e}

Note: C-Control; RS-Reference Strain; H-P-Humus based PGPR; CP-P-Coir Pith based PGPR; T-P-Talc based PGPR; CF-Chemical Fertilizer

Values expressed as means ± SD of five replicates. Mean values not sharing the same alphabet differ significantly at $p < 0.05$.

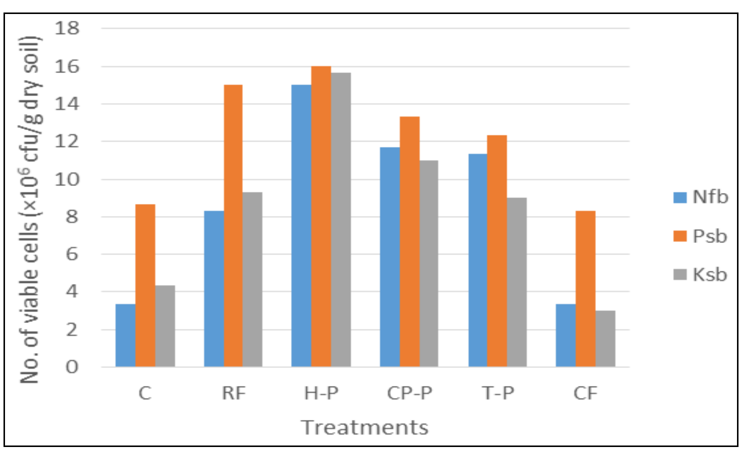

Fig 1: Effect of different carrier based PGPR formulations on the population rate of PGPR in the rhizosphere of brinjal seedlings at 30 days of growth

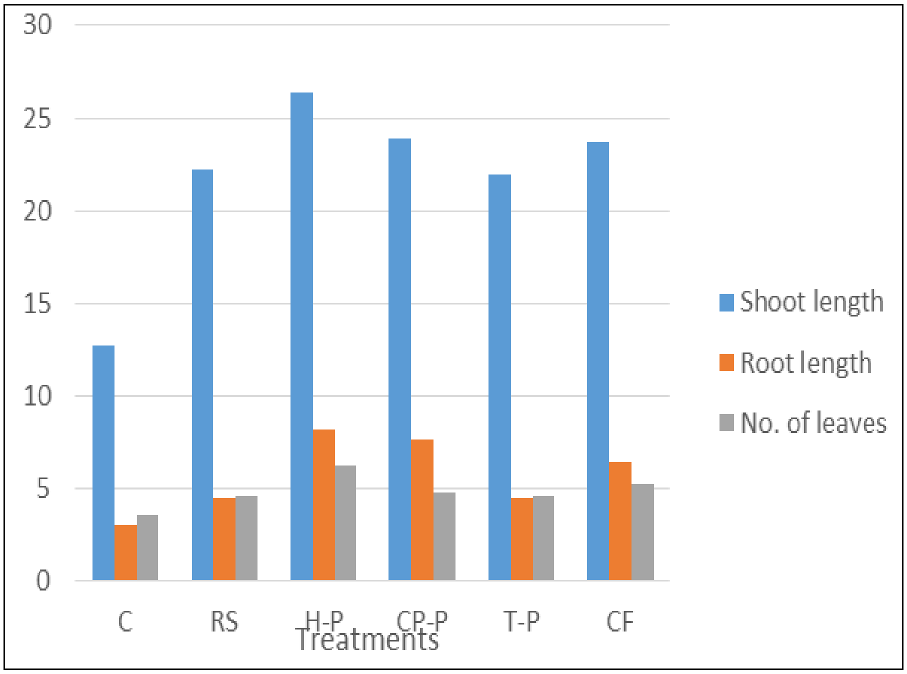

Fig 2: Effect of different carrier based PGPR formulations on the growth promotion of brinjal seedlings at 30 days of growth

3.2. Seasons

Significant result was observed with H-PGPR in summer season when compared to winter and rainy season at $p<0.05$. The N, P and K availability in the rhizosphere soil was found to be maximum with H-PGPR treatmentat summer season followed by winter and rainy season. The results obtained were 578.24 ± 1.14 Kg N ha^{-1}, 38.34 ± 1.32 Kg P ha^{-1} and 648.26 ± 1.24 Kg K ha^{-1} (Table 2).

Figure 3 shows the effect of different seasons on the microbial population and was significant at the 0.05 probability level. PGPR treatment on brinjal plants showed maximum shoot length 26.38 ± 0.56 cm, root length 8.22 ± 0.6 cm and number of leaves 6.20 ± 0.84 in summer season. The results are presented in Figure 4.

Table 2: Effect of humus based PGPR formulation on soil available plant nutrients in different seasons at 30 days of brinjal seedlinggrowth

Season	Treatments	Kg N ha^{-1}	Kg P ha^{-1}	Kg K ha^{-1}
Summer	C	247.86 ± 2.04^{a}	04.50 ± 0.47^{a}	178.28 ± 1.23^{a}
	RS	407.42 ± 1.80^{b}	20.62 ± 0.46^{b}	288.08 ± 1.82^{b}
	H-P	578.24 ± 1.14^{c}	38.34 ± 1.32^{c}	648.26 ± 1.24^{c}
	CF	564.78 ± 1.06^{d}	28.92 ± 0.75^{b}	597.74 ± 1.43^{d}
Rainy	C	198.68 ± 0.99^{a}	03.18 ± 0.31^{a}	138.48 ± 1.41^{a}
	RS	348.46 ± 1.25^{b}	20.78 ± 0.81^{b}	608.22 ± 1.51^{b}
	HP	528.50 ± 1.14^{c}	28.46 ± 0.58^{c}	614.48 ± 0.44^{c}
	CF	518.88 ± 1.09^{d}	23.70 ± 0.88^{b}	549.30 ± 1.36^{d}
Winter	C	247.50 ± 2.13^{a}	04.44 ± 0.68^{a}	178.12 ± 1.52^{a}
	RS	403.24 ± 1.99^{b}	36.32 ± 1.09^{b}	597.98 ± 1.28^{b}
	HP	577.92 ± 0.97^{c}	38.08 ± 1.38^{c}	648.48 ± 1.34^{c}
	CF	564.68 ± 1.20^{d}	30.12 ± 1.37^{b}	604.10 ± 2.33^{d}

Note: C-Control; RS-Reference Strain; H-P-Humus based PGPR; CF-Chemical Fertilizer

Values expressed as means ± SD of five replicates. Mean values not sharing the same alphabet differ significantly at $p < 0.05$.

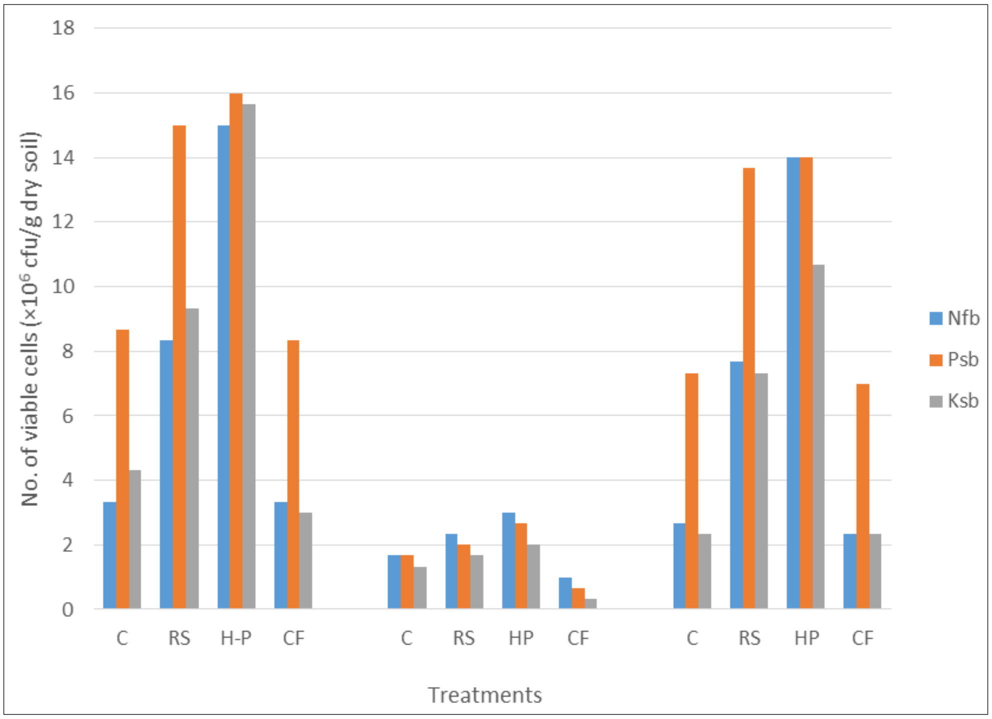

Fig 3: Effect of humus based PGPR formulation on the population rate of PGPR in the rhizosphere of brinjal seedlings in different seasons at 30 days of growth

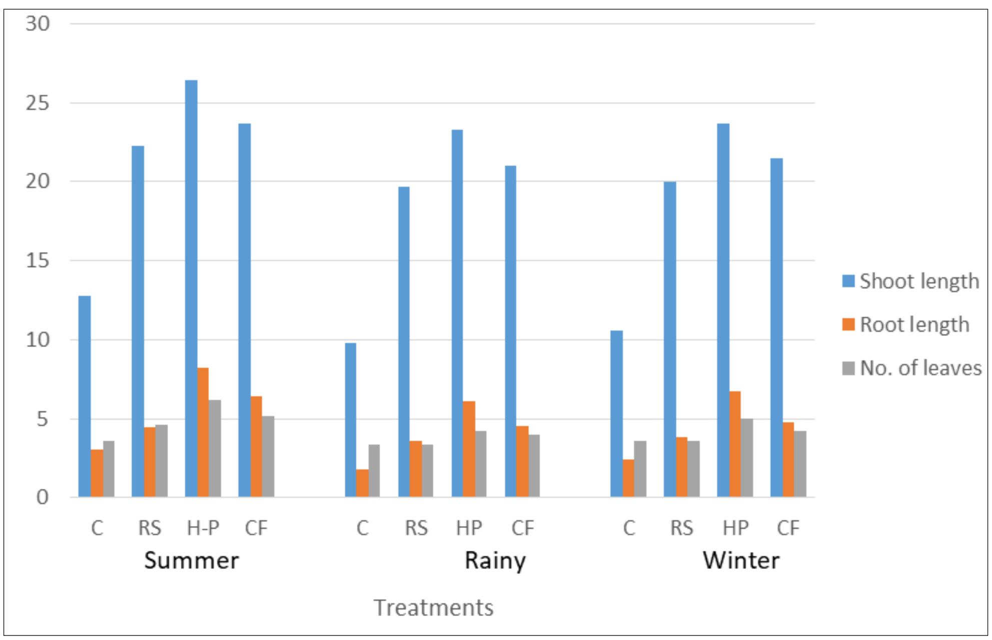

Fig 4: Effect of humus based PGPR formulation on growth promotion of brinjal seedlings in different seasons at 30 days of growth

3.3. pH

There was a significant increase in the soil available N, P and K with H-PGPR treatment at pH 7.0, which were 578.28 ± 1.14 Kg N ha^{-1}, 38.40 ± 1.28 Kg P ha^{-1} and 648.32 ± 1.20 Kg K ha^{-1} higher than other treatments respectively (Table 3).

The population size of PGPR in the brinjal rhizosphere soil were calculated as 15.67 ± 2.08 Nfb, 16.67 ± 3.22 Psb, 16.33 ± 2.52 Ksb $\times 10^6$cfu/g soil (Figure 5).

The growth of brinjal plant increased significantly with H-PGPR inoculation at pH 7.0 when compared to uninoculated plants. The maximum results observed as 26.62 ± 0.85 cm shoot length, 8.24 ± 0.68 cm root length and 6.60 ± 0.55 number of leaves (Figure 6).

Table 3: Effect of Humus based PGPR formulation on soil available plant nutrients in different pH at 30 days of brinjal seedling growth

pH	Treatments	Kg N ha^{-1}	Kg P ha^{-1}	Kg K ha^{-1}
pH 5.5	C	218.98 ± 0.94^{a}	03.16 ± 0.29^{a}	147.52 ± 1.93^{a}
	RS	398.20 ± 1.92^{b}	12.50 ± 0.60^{b}	233.04 ± 1.94^{b}
	H-P	498.98 ± 0.82^{c}	28.96 ± 0.96^{d}	588.26 ± 1.25^{d}
	CF	497.98 ± 1.72^{c}	24.62 ± 0.67^{c}	528.46 ± 0.91^{c}
pH 6.0	C	246.24 ± 2.20^{a}	04.34 ± 0.47^{a}	174.18 ± 0.58^{a}
	RS	466.88 ± 1.97^{b}	14.76 ± 0.40^{b}	281.42 ± 1.25^{b}
	HP	541.32 ± 2.29^{d}	36.00 ± 1.18^{d}	631.48 ± 2.06^{d}
	CF	534.32 ± 0.84^{c}	29.80 ± 0.59^{c}	574.24 ± 0.53^{c}
pH6.5	C	247.86 ± 2.04^{a}	04.50 ± 0.47^{a}	178.28 ± 1.23^{a}
	RS	407.42 ± 1.80^{b}	20.62± 0.46^{b}	288.08 ± 1.82^{b}
	HP	578.24 ± 1.14^{d}	38.34 ± 1.32^{d}	648.26 ± 1.24^{d}
	CF	564.78 ± 1.06^{c}	28.92 ± 0.75^{c}	597.74 ± 1.43^{c}
pH7.0	C	247.98 ± 2.08^{a}	04.56 ± 0.53^{a}	178.56 ± 1.43^{a}
	RS	407.52 ± 1.82^{b}	20.70 ± 0.49^{b}	288.30 ± 2.14^{b}
	HP	578.28 ± 1.14^{d}	38.40 ± 1.28^{d}	648.32 ± 1.20^{d}
	CF	564.90 ± 1.01^{c}	29.06 ± 0.82^{c}	597.80 ± 1.40^{c}
pH7.5	C	243.98 ± 0.86^{a}	04.48 ± 0.43^{a}	178.40 ± 1.17^{a}
	RS	398.50 ± 1.06^{b}	17.90 ± 1.25^{b}	288.28 ± 1.07^{b}
	HP	558.50 ± 0.90^{d}	37.66 ± 1.33^{d}	638.00 ± 1.51^{d}
	CF	538.24 ± 1.40^{c}	28.58 ± 0.79^{c}	578.72 ± 1.13^{c}
pH8.0	C	109.08 ± 0.55^{a}	03.72 ± 0.41^{a}	157.54 ± 1.42^{a}
	RS	247.88 ± 1.29^{b}	06.68 ± 1.09^{b}	258.56 ± 1.04^{b}
	HP	272.30 ± 2.08^{d}	08.34 ± 0.50^{c}	588.80 ± 1.06^{d}
	CF	267.78 ± 1.68^{c}	08.14 ± 0.54^{c}	529.12 ± 0.60^{c}
pH8.5	C	97.82 ± 1.39^{a}	02.68 ± 0.38^{a}	108.86 ± 0.80^{a}
	RS	217.72 ± 1.47^{b}	05.44 ± 0.48^{b}	127.74 ± 1.31^{b}
	HP	247.72 ± 1.40^{d}	06.98 ± 0.60^{c}	244.42 ± 0.77^{d}
	CF	243.66 ± 0.91^{c}	06.78 ± 0.53^{c}	228.22 ± 1.02^{c}

Note: C-Control; RS-Reference Strain; H-P-Humus based PGPR; CF-Chemical Fertilizer
Values expressed as means ± SD of five replicates. Mean values not sharing the same alphabet differ significantly at $p < 0.05$.

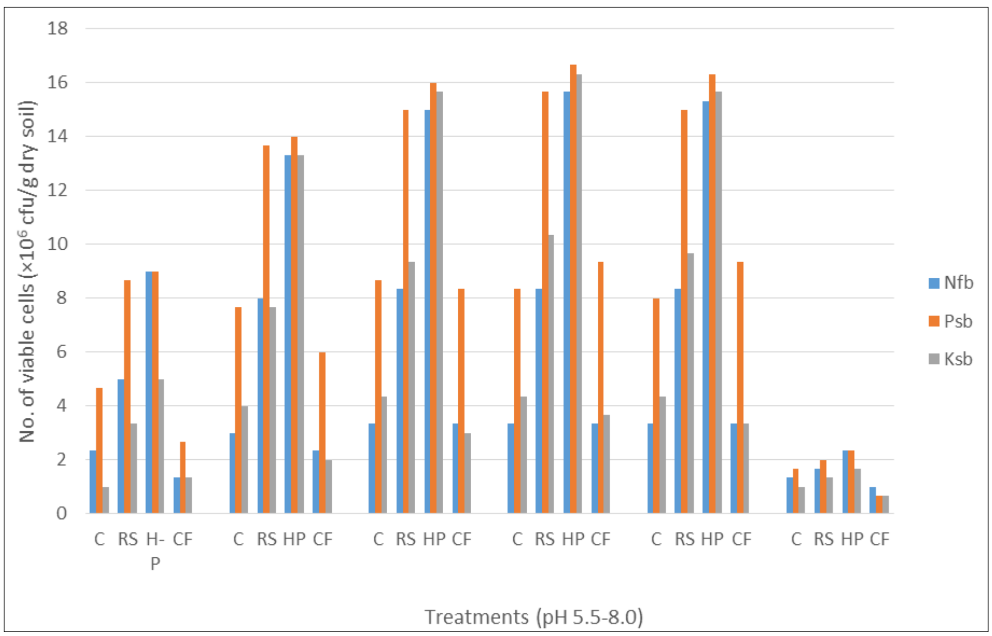

Fig 5: Effect of humus based PGPR formulation on the population rate of PGPR in the rhizosphere of brinjal seedlings in different pH at 30 days of growth

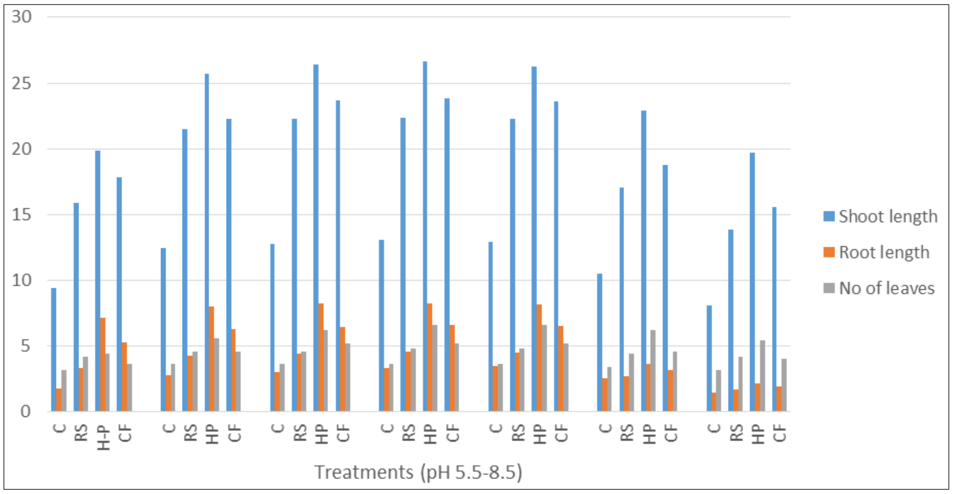

Fig 6: Effect of humus based PGPR formulation on growth promotion of brinjal seedlings in different pH at 30 days of growth

A combination of 4 PGPR, isolated from rhizosphere soil were used for the optimization study to find out the best carrier, pH and season for the biofertilizer to perform well in the soil system. The commercial application of PGPR as biofertilizer and biocontrol agent has been questioned because of their inconsistent behaviour in different fields due to variations in climate and soil [19]. Most of the PGPR exhibited multiple plant growth promoting traits in vitro and such character has been reported to be the major factor responsible for the plant growth promotion and biocontrol activity [3].
Application of PGPR had supported the maximum growth of bacterial community in the rhizosphere. There was a reduction in the microbial population densities at pH below 6 and above 8. Changes in the soil pH might created unfavourable environment for the multiplication of the microorganisms. Rainy season was observed with low microbial load and this may be due to washing out of bacteria by continuous rain. Unlike other carriers, humus supported maximum microbial population size by providing nutrients for the growth of microbes. Because of its negative charge humus holds many of the plant nutrients such as ammonium, calcium, magnesium, phosphorous etc. and these nutrients will be easily accessed by the plant roots when contact with it [21]. It has been reported by some researchers that the bioavailability of N, P and K in the soil with PGPR inoculation is increased [23, 14].

4. Conclusion
The application of PGPR as fertilizer showed a stimulating effect on growth rate of brinjal plants and improvement of soil fertility through a greenhouse trial. Analysis of variance shows that the carriers, pH and seasons influenced all the treatments significantly at $p<0.05$ and multiple comparison of data mean values shows that best result was obtained with humus as the carrier in summer season at pH 7.0. Further studies are needed to improve the activity of PGPR in different pH and climatic conditions. Research could also be conducted to examine their effect on other plants under field conditions. Application of humus based PGPR is an alternative for chemical fertilizer that favours longterm soil fertility without damaging the soil ecosystem.

5. References
1. Adesemoye AO, Kloepper JW. Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl. Microbiol Biotechnol, 2009; 85:1-12.
2. Adesemoye AO, Torbert HA, Kloepper JW. Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol., 2009; 58:921-929.
3. Antoun H, Prevost D. Ecology of plant growth promoting rhizobacteria In: Siddiqui ZA editor, PGPR: Biocontrol and biofertilization. Dordrecht: Springer, 2005, 1-38.
4. Bashan Y. Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv., 1998; 16:729-770.
5. Bashan Y, de-Bashan LE, Prabhu SR, Hernandez JP. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998-2013). Plant Soil, 2014; 378:1-33.
6. Black CA. Methods of soil analysis Part I Am. Soc. Agron. Inc. Publi. Madison Wisconsin USA. 1965.
7. Cummings SP. The application of plant growth promoting rhizobacteria PGPR in low input and organic cultivation of graminaceous crops; potential and problems. Environ Biotechnol, 2009; 5:43-50.
8. Dworkin M, Foster J. Experiments with some microorganisms which utilize ethane and hydrogen. J. Bacteriology. 1958; 75:572-601.
9. Gaind S, Gaur AC. Shelf life of phosphate solubilizing inoculants as influenced by type of carrier, high temperature and low moisture. Can. J Microbiol, 1990; 36:846-849.
10. Herrmann L, Lesueur D. Challenges of formulation and quality of biofertilizers for successful inoculation. Appl. Microbiol Biotechnol, 2013; 97:8859-8873.
11. Herridge DF. Inoculation technology for legumes. In: Dilworth MJ, James EK, Sprent JI, Newton WE eds Nitrogen-fi xing leguminous symbioses. Springer, Dordrecht. 2008, 77-115.
12. Hu XF, Chen I, Gvo JF. 2 Phosphate/Potassium solubilizing bacteria isolated for Tiannumountain, zhejieng, China. World J Micro. Biotech, 2006; 22:983-

990.
13. Jauhri KS, Philip K. Pressmud, a potential carrier for *Rhizobium* and *Azotobacter*. ZblMikrobiol, 1984; 139:97-107.
14. Lin QM, Rao ZH, Sun YX, Yao J, Xing LJ. Identification and practical application of silicate dissolving bacteria. Agri. Sci. in China, 2002; 1:81-85.
15. Malusà E, SasPaszt L. The development of innovative technologies and products for organic fruit production. An integrated project. In: Proceedings of international plant nutrition Coll. XVI. Sacramento USA. Paper 1359. http://repositories.cdlib.org/ipnc/ xvi/1359. 2009; 1-4.
16. Malusà E, Sas-Paszt L, TrzcinskiP, Górska A. Influences of different organic fertilizers and amendments on nematode trophic groups and soil microbial communities during strawberry growth. ActaHortic ISHS, 2012; 933:253-260.
17. Manikandan R, Saravanakumar D, Rajendran L, Raguchander T, Samiyappan R. Standardization of liquid formulation of *Pseudomonas fluorescens* Pf1 for its efficacy against Fusarium wilt of tomato. Biol Control, 2010; 54:83-89.
18. Olsen SR, Cole CV, Watanable FS, Dean LA. Estimation of available phosphorous in soils by extraction with sodium bicarbonate. Cir. US Department Agric, 1954; 939:1-19.
19. Ownley BH, Duffy BK, Weller DM. Identification and manipulation of soil properties to improve the biological control performance of phenazine producing *Pseudomonas fluorescens*. Appl. Environ. Microbiol, 2003; 69:3333-43.
20. Pikovskaya RI. Mobilization of phosphates in soil in connection with the vital activities of some microbial species, Microbial, 1948; 17:362-370.
21. Pavlis R. What is Humus. Garden myths- Book-1, 2013.
22. Rousk J, Baath E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG *et al.* Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J. 2010; 4:1340-1351.
23. Schilling G, Gransee A, Deubel A, Lezovic G, Ruppel S. Phosphorus availability, root exudates and microbial activity in the rhizosphere. Z. Pflanzenernahr. Bodenkd. 1998; 161:465-478.
24. Schoebitz M, Simonin H, Poncelet D. Starch filler and osmoprotectants improve the survival of rhizobacteria in dried alginate beads. J Microencapsul, 2012; 29:532-538.
25. Subbaih BV, Asija GL. A rapid procedure for the estimation of nitrogen in soils. Curr. Sci., 1956; 25:259-260.