THINK INDIA JOURNAL

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

Effect of Pgpr Azospirillum Sp. on The Growth of Marigold Plant

Kaveri[1], Arun Karnwal[2]*

[1]Department of Microbiology, Bhojia Institute of Life Sciences, Budh, Baddi, H.P. India

[2*]Department of Microbiology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, India, arunkarnwal@gmail.com

Abstract

Rhizobacteria are referred to as Plant growth promoting rhizobacteria (PGPR) which exert useful effects upon plant development and growth. When inoculated by the PGPR, ornamentals, forest trees, vegetables and farm crops may lead to several positive results such as plant growth in early season, improvement in seedling germination, plant health and plant height, shooting weight, nutrient content of the shoot, early flowering, increase chlorophyll content and increased nodulation in legumes.These bacteria help to improve the fixation of N^2 in legumes, to encourage number of bacteria that fix nitrogen freely, to increase nutrient supply such as phosphor, sulfur, iron and copper, to produce plant hormones, to improve other beneficial bacteria or fungi in nearby area, and to control fungal, bacterial and insect phytopathogens. Calendula officinalis is considered a yearly flowering plant, especially in colder regions with poor winter survival. *Calendula officinalis*is often applied as a hepato protective agent, antiseptic and anti-inflammatory agent in the treatment of skin disorder and pain. The present study was carried out to determine the impact of PGPR Azospirillum sp on marigold growth.

Keywords: Marigold, Azospirillum, PGPR, Indole, Phosphate liquefaction.

Introduction

Calendulaofficinalis(family Asteraceae)is a valuable plant of the *Calendula* genus (marigolds) with a number of medicinal uses in India and around the world[1].The plant is a native of southern Europe but occurrence of this plant is everywhere and on commercial scale, it is cultivated in North America, Eastern Europe and Germany. The plant is commonly known as Garden-Marigold, Gold Bloom, Holligold, Marigold, Marybud, Pot-Marigold, and Zergul.It is a herb with a high stem ramifications and with yellow flowers[2], [3]. There are usually two genus to which the common name applies, marigolds viz. *Calendula*(Pot

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

marigolds) and *Tagetes* (African-marigolds and French marigolds). These are in various colours, the most famous being yellow and orange.The majority of marigolds have a powerful, potent scent that makes them useful in cosmetic care[4].

Plant growth promoting rhizobacteria (PGPR's) are the bacteria those reside under the neighborhood area of plant roots [5]–[7]. These bacteria are naturally free living, soil born bacteria, and primarly isolated from rhizospheric area of plant roots [6]. These PGPRs exert various effects on plant growth and assumed to provide enhanced plant development through various methods [8]–[11]. These bacteria are also reduce and decline the negative effect of phytopathogens on plant development [12].

In present agricultural system, primarly used fertilizers are chemical in nature and impact negatively on environment sustainability [13]. It is necessary to use other substitute of chemical fertilizers for sustaining the environment stability and reduction in chemical pollutants [14]. PGPRs are having various traits by them these bacteria exert incline development in plant growth and may be used as substitute of chemical fertilizers [12], [15] Various authors [16]–[18] reported the use of PGPRs enhanced plant growth *In-vitro* and *In-vivo* condition when applied on seeds and during cultivation [19]. In current study, bacterial isolate from rhizosphere was screened for their biochemical and plant growth promotion traits such as: Phosphate solubilization, Indole production etc. Further isolated bacteria strain evaluated for its PGP activity with marigold seeds in pot analysis upto 30 days.

Material and Methods

Sample collection

The rhizospheric soil specimen was obtained from the garden of the Bhojia Institute of Life Sciences (BILS), Budh. Baddi, Himachal Pradesh, from the rhizospheric area of plants.

Isolation of *Azospirillum sp.*

Serial dilution agar plate technique was adopted for the isolation of *Azospirillum* sp. from the rhizospheric soil[5], [20]–[22]. 10g of soil sample of rhizospheric region from healthy plant was taken and transferred into conical flask containing 90 ml sterilized distilled water and mixed properly. Six test tubes were taken and filled with 9 ml of sterilized distilled water. Then they were marked as I to VI. 1 ml of suspension from the conical flask was transferred to the test tube marked as I. Additional 1 ml of suspension from test tube I has now been transferred to the test tube II. Same procedure was repeated until 10^{-6}. For plating *Azospirillum* agar medium[23], [24](Glucose 20.0 gm/l, Agar 15.0 gm/l, K_2HPO_4 0.8 gm/l

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

$MgSO_4.7H_2O$ 0.5 gm/l, KH_2PO_4 0.2 gm/l, $Fecl_3.6H_2O$ 0.1 gm/l, Yeast extract 0.1 gm/l, $CaCl_2.2H_2O$ 0.02 gm/l, $Na_2MoO_4.2H_2O$ 0.02 gm/l) was used. 1ml of sample from the dilutions 10^{-4} and 10^{-5} was transferred on *Azospirillum* agar medium plates and incubated at 26˚C for 48 hours. Streak plate method and spread plate technique was used for the isolation of bacterial colonies.

Streak plating

Bacterial coloniesisolated, were screened on the criterion of colony elevation, size, colour, shape, margin and mucosity[25]. *Azospirillum* agar plates were prepared. Bacterial isolates were streaked on the agar plates with the red hot-flamed cooled inoculum loop and cultivated at 26±2 ℃ for 48 hours.

Characterization of isolate through biochemical assays[26]

Gram staining

Gram staining is preliminary used to differentiate bacterial spp. on the basis of their cell wall and cell membrane composition. In present study this procedure was used to determine the types of Bacterial isolates. One or two drops of 24 h old culture were evenly speeded on non waxy glass slide and culture was fixed by gentle heat.1-2 drops of primary stain i.e. crystal violet applied on bacterial smear for 1 min followed by gentle wash by sterilized water. Gram's iodine was applied and allowed to react with smear for 1 min. later the iodinated alcohol was applied and allowed to react for 1min. Finally treated with a counter strain stain i.e. safranin after 5 min washed with sterilized water and air dried. The smear was examined under oil emersion in light microscope the Gram +ve cell appears violet/purple and Gram -ve red/pink.

Negative staining

Negative staining involves the application of acidic stain like nigrosin. A droplet of 7% nigrosin was placed on the slide. After that a small amount of bacterial culture was mixed into the droplet; air dried and examined the slide under a magnification of 1000x under microscope. Colourless microbial cells were observed against the dark field background.

Endospore staining

Bacterial smear flooded with 0.5 percent malachite green and remained for 5 minutes in a water bath.Prepared slide was thoroughly rinsed with water and further covered for 30 secondswith safranin. Slide was further rinsedwith water and observed under oil immersion objective of microscope. Spores were stained green, while the cytoplasm was stained red.

Acid-fast staining

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

Bacterial smear was flooded with carbol fuchsin and kept over a water bath for 5 minutes. Bacterial smear was rinsed with water till discoloration of effluent. HCL was applied for decolorization of smear and immediately washed with tap water. Now, malachite green as a counter satin was added for 20-30 seconds and further washed with water.

Metachromatic Granule staining

Bacterial smear was prepared on a glass slide. Culture was heat fixed by gentle heat. 1-2 drops of Loeffler's methylene blue was applied on fixed bacterial smear to react for about 10-30 seconds. After 10-30 seconds slide was washed with tap water. Lugal's iodine solution was then applied and allowed to react for 1 minute. After 1 minute slide was washed with tap water and air dried. The smear was examined under oil immersion in a light microscope.

Catalase test

Picked up bacterial colony from the plate and transfer the colony on glass slide in a drop of water place few drops of 3% H_2O_2 (dilute 30% commercial solution by 1:10) over the culture.

Indole production

The tryptone broth was inoculated with bacterial isolate and cultivated for 48 h at 28°C. kovac's reagent was added to each tube including control . Appearance of red coloured layer on the top of tube was taken as positive test for indole production

Sugar hydrolysis

Isolated bacterial culture were tested for their ability to hydrolyze different sugars i.e. glucose, sucrose, maltose, xylose and raffinose in broth. Took 4 test tubes for each sugar and 5 ml broth was poured into them. Each test tube was contained 1% of sugar along with sufficient amount of peptone and beef extract, add phenol as pH indicator for acid detection. The indicator was red initially but turns yellow at acidic pH. To determine the gas production inverted Durham tubes were placed in all test tubes. Sterilized at 121°C for15 min at 15 psi and allowed to cool at room temperature. Three test tubes were inoculated with the isolated strains and I test tube set as control for each sugar.

Citric acid utilization

Slants of Simon citrate agar were inoculated with test isolates and incubated at 24°C. Presence of growth at the end of inoculation and the resultant colour change from green to blue indicated a positive test.

Starch hydrolysis

Starch agar media was prepared and were poured into petri plates and allowed to solidify. Single colony was picked and were streaked on the plates and incubated at 37^oC for 24 hrs.

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

Gelatine liquefaction

Nutrient broth with gelatin was prepared and allowed to stand at 4^0C over night. Then broth was heated to dissolve the gelatin up to 50^0 C. The bacterial isolates were inoculated in sterile gelatin medium.

Urease test

The urea was sterilized by the filter sterilization. 2 %of urea was emended in nutrient broth to which 0.012% phenol red was added as indicator. These test tubes were incubated at 280C for 7days. Appearance of red colour turbidity showed urea hydrolysis.

Voges- proskauer test

Glucose broth was prepared containing sufficient amount of peptone and beef extract. Inoculate each bacterial isolate in test tubes containing sterile glucose broth. Test tubes were Incubated at 37^0C for 48 hrs. Two solutions A (potassium hydroxide 40%) B (5 %@ naphthol in absolute alcohol) were prepared and added to tests, appearance of pink colour in 3 to 5 min indicate the positive result.

Triple sugar iron (TSI) test

The test evaluates an organism's ability to utilise particular carbohydrate in a nutrient medium with or without gas formation and also the production of water to aid in the identification among the family Enterobacteriaceae . Streaked pure colony with an inoculation loop on TSI medium and incubated at 37^0C for 18-24 hrs

Test for plant growth promotion (PGP) activity [24], [27]–[29]

Nitrate reduction

Inoculated the test isolates in the broth containing peptone and potassium nitrate[30]. Then the test tubes were incubated at 37^0C for 96 hrs. Prepared two solutions A (*Sulfonic acid* in acetic acid) and B (α napthylamine in acetic acid). Mixed and added to the test. Formation of red colour indicates the nitrate reduction ability of organism.

Ammonia production

Peptone broth (4%) was prepared that contains an organic nitrogen substrate. Peptone broth was inoculated by isolated bacterial culture. For 24 hours, the broth was incubated and 0.5 g of soil was applied to each tube[31]. Further all tubes incubated at 30 °Cfor 7 days. Nesseler's reagent was mixed to the incubated tubes. A positive test resulted for ammonia processing was the conversion of brown to yellow color.

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

Phosphate solubilisation

Phosphate solubilization was performed by doing spot inoculation of test organism on Pikovskaya medium[32]. The bacterial inoculated plates were placed in incubator for incubation at 28 $^{\circ}$C for 4-5 days. Formation of transparent zone around the colony indicate the positive phosphate liquefaction property

Plantation of marigold plant

Earthen pots have been used in the research to perform the pot analysis. Such containers are filled with soil from the Institute garden. Further, these pots with soil were sterilized[15], [33]. Marigold seeds were sterilized in 1% $HgCl_2$ for 2 minutes and after that, the seeds are washed at least 10 times with sterilized distilled water to remove toxicity of$HgCl_2$. Marigold seeds are planted into sterilized pots after proper air drying (three seeds per pot). *Azospirillum* strain along with their respective non-bacterized seed, as control, were sown in following set of treatments:

Treatment 1- used soil and seeds both were sterilized (sterilized control).

Treatment 2- used soil and seeds both were not sterilized (non-sterilized control).

Treatment 3- used sterilized soil and seeds with bacterial surface coating (sterilized test).

Treatment 4- soil + seed coated with bacterial strain (non-sterilized test).

In the subsequent procedures, 1ml of log culture (10^8 cells) of bacterial isolate has been added as inoculum. Daily irrigation of sterilized and non-sterilized pots with sterilized water was carried out on regular basis. 2ml of microorganism inoculums are inoculated in the corresponding pots as booster dose after every 10 days of interval. Potts have been irrigated every day as best practice. Different plant parameters like number of leaves, shoot length, root length, root dry weight were measured.

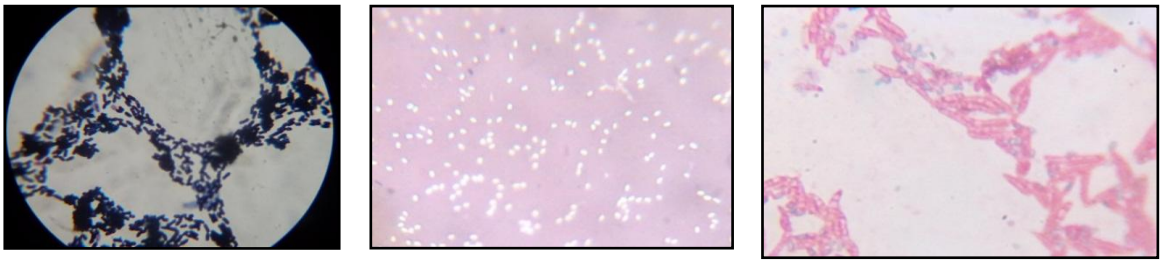
RESULT

PGPR strain was extracted from rhizospheric samples obtained from the BILS. The isolated strain was Gram +ve and rod form as shown in Fig 2 and documented in Table 1. Bacterial isolate was grown on the Azospirillum medium (Fig 1). The biochemical characteristics of the bacterial isolate were examined as outlined in Bergey's Manual of determinative Bacteriology[26].

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

(a) **(b)**

Figure 1: Colonies of isolated bacterial culture on *Azospirillum* agar plates



(a) **(b)** **(c)**

Figure 2: Staining results of isolated culture. (a) Gram's staining; (b) Negative staining; (c) Endospore staining

Table 1: Morphological characterization of isolated bacterial strain

Staining	Shape	Result
Gram's staining	Rod	Positive
Negative staining	Rod	Positive
Endospore staining	Rod	Positive
Metachromatic granule staining	Rod	Negative
Acid fast staining	rod	Negative

Biochemical tests

The isolated strain was found to be citric acid positive, TSI positive, urease positive, indole positive as shown in Fig. 3 and Table 2.

It was noted after studying the biochemical characteristics that the isolated strain shows biochemical characteristics associated with Azospirillum sp.[34]–[36].This isolated strain has been further examined for its plant growth promotion behaviour with inoculation in marigold seeds[28].

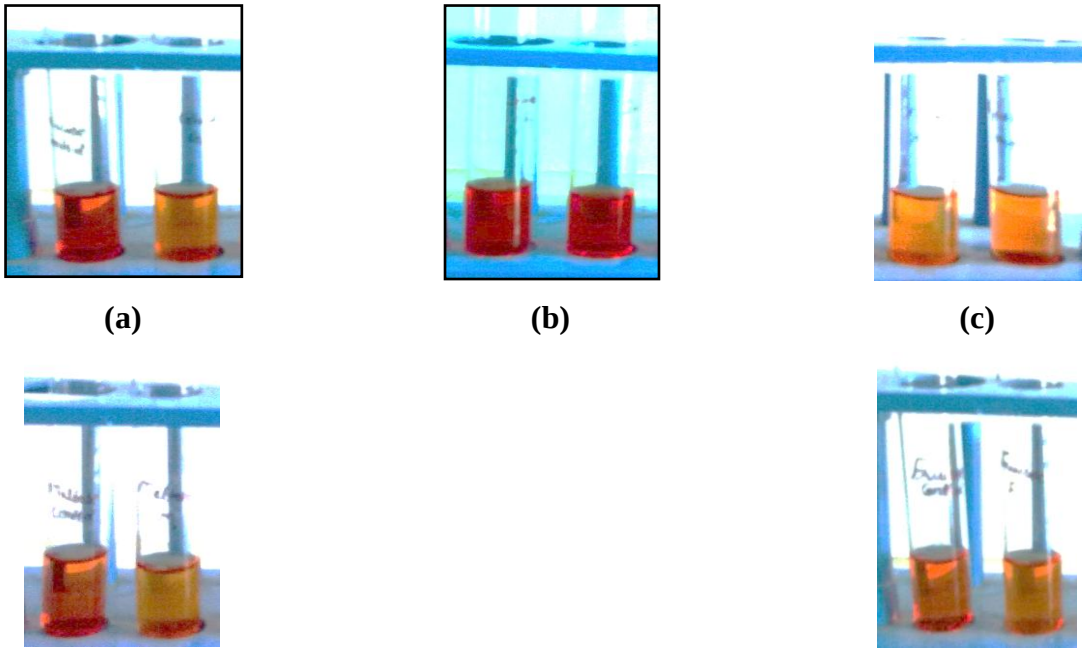
ISSN: 0971-1260
Vol-22-Issue-17-September-2019

Table 2: Biochemical properties of isolated bacterial strain

Test	Result
Indole production	Positive
Urease production	Positive
Citric acid production	Positive
Triple sugar iron test	Positive
Ammonia production	Negative
Starch hydrolysis	Negative
Phosphate solubilisation	Negative
Catalase activity	Negative
Methyl red test	Negative
Nitrate reduction	Negative
Voges-Proskauer test	Negative
Hydrogen sulphide production test	Negative
Gelatine agar test	Negative

Sugar fermentation

Nutrient broth with different carbon sources inoculated with bacterial isolate (Table 3). The isolated bacterial culture was glucose fermenter in which it fermented glucose partially (Fig 3). The isolate was sucrose negative as it was unable to ferment it (Fig 3b).



(a) **(b)** **(c)**

(d) **(e)**

Figure 3: Sugar hydrolysis by isolated bacterium. (a): Glucose; (b): Sucrose; (c): Xylose;

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

(d): Maltose; (e): Fructose

Strain was xylose fermenter with gas production (Fig 3c). The strain was also maltose (Fig 3d) and fructose (Fig 3e) fermenter with the evolution of gas.

Table 3: Carbohydrate utilization by bacterial strain

Sugar	Result
Glucose	Positive
Sucrose	Negative
Xylose	Positive
Maltose	Positive
Fructose	Positive

On the basis of morphological, biochemical and sugar fermentation results, the isolated bacteria was identified as ***Azospirillum*** *brasilense* (upto 87% identical) with the help of an online bacterial identification software (ADVANCED BACTERIAL IDENTIFICATION SOFTWARE available at http://www.tgw1916.net/bacteria_logare_desktop.html).

Effect of temperature on bacterial growth

The temperature affecting bacterial enzymes function is among the key factors responsible for optimum growth of organism[21], [27]. Enzymes at the optimum temperature are most active and the rate of enzyme reaction is enhanced[37]–[39]. Enzymes are inactive below minimum and above maximum temperature. Therefore, the temperature to measure optimum bacterial growth requires to be optimized[22].

The broth has been prepared, inoculated and placed at different temperatures (26°C, 30°C, 35°C and 37°C) with bacterial isolate for about 24 hrs of incubation. The turbidity was measured with the help of a calorimeter. From the readings it was noted that bacterial culture show maximum growth at 26°C (Fig 4).

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

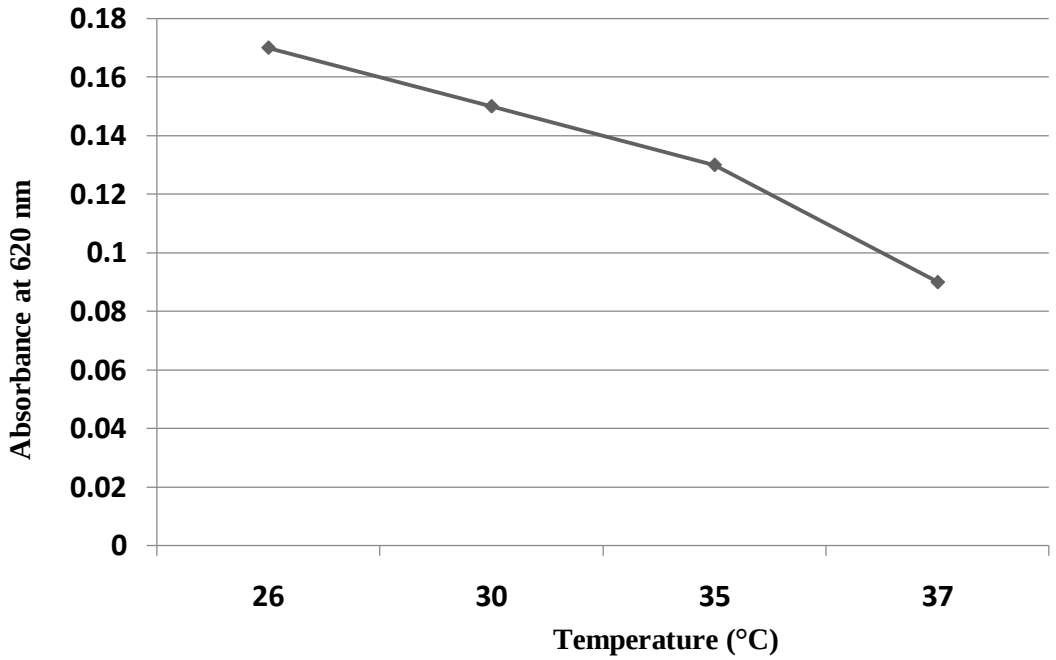


Fig 4: Effect of different temperatures on the growth of isolated bacterial strain

Effect of pH on bacterial growth

The amount of H+ in the environment of an organism has the greatest impact on growth[27]. This inhibits the production of the bacterial enzymes that make the new cytoplasmiccomponents [5], [22]. Each micro-organism, like the temperature, has an optimum pH. This only works if other factors stay constant. If other variables, such as the composition of media temperature or osmotic pressure are variable, pH is also change[40]–[42].

The broth was prepared of varying pH and was inoculated with bacterial culture in each tube and incubated at 26°C for 48 hrs. After incubation the turbidity was measured in a calorimeter at 620 nm. From the readings it was observed that the bacterial culture show maximum growth at pH 6 (Fig 5).

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

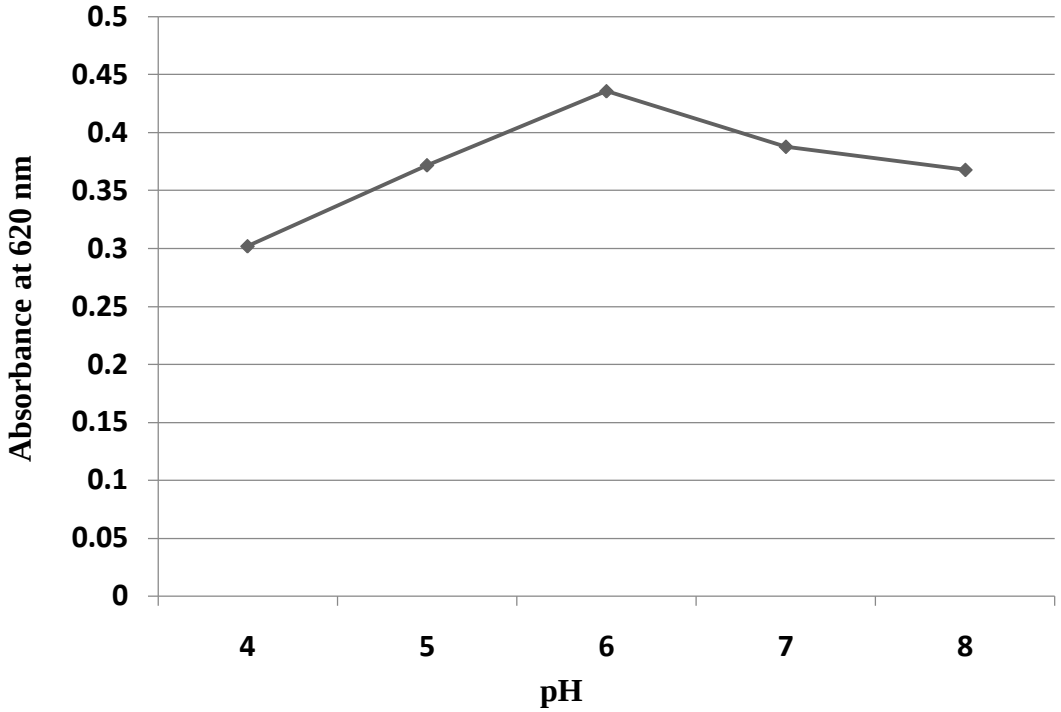


Fig 5: Impact of various pH on bacterial isolate

Report after 30 days of Marigold plant growth observed

In comparison to control, the isolated bacteria has a beneficial influence on plant development as shown in Table 4 (Fig 6).

Table 4: Effect of PGPR isolate on the growth of marigold plant after 30 days (mean values of three sets of test is given in table).

	Sterile Soil		**Non-Sterile Soil**	
	Control	Test	Control	Test
Shoot Height (in cm)	8.1	13.5	7.3	9
No. of Leaves	112	130	95	121
Root Length (in cm)	5.8	9.5	6.1	8.1
Root Weight (in gm)	0.1	0.19	0.07	0.16

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

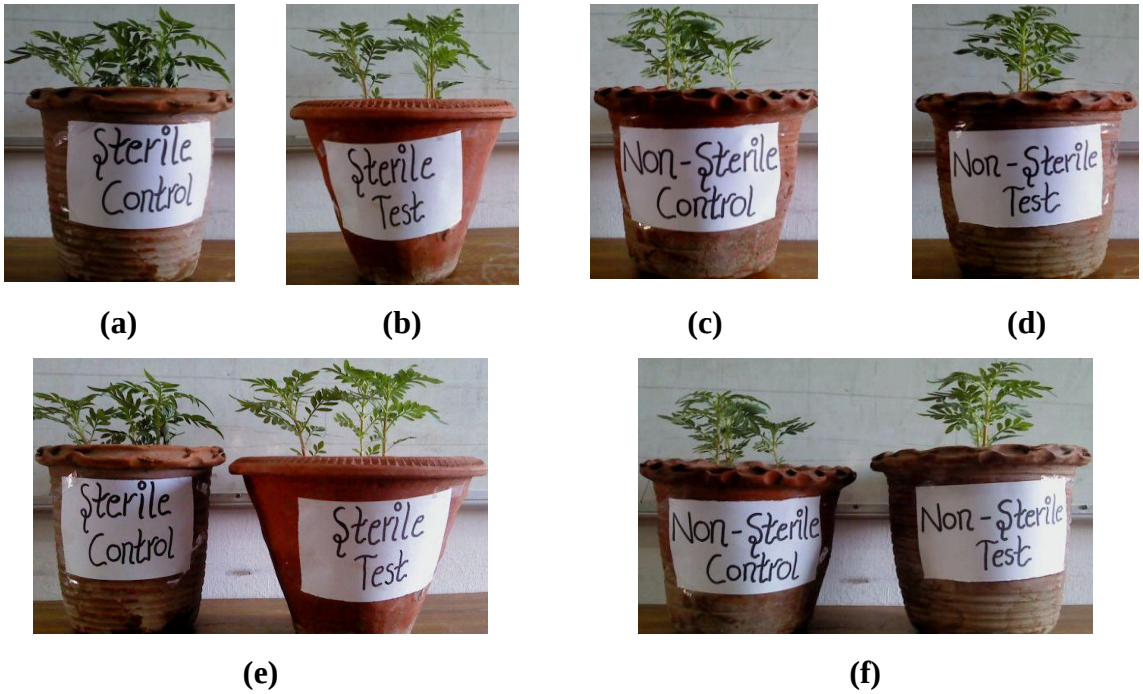


Figure 6: Effect of bacterial culture on plant growth in different treatments afte 30days

The strain also show positive effect by increasing the shoot length of the plant (Fig 7) and also the number of leaves was larger as compared to the control (Fig 8). Root weight (Fig 9) and root length (Fig 10) of plant inoculated by the isolated strain also show positive result by increasing the root length and root weight of test as compared to the control.

In present study it was found that the treatment in which we used the sterile soil (Test) show maximum growth as compared to the control and the treatment in which we employed the non sterile soil (Test) show less growth as compared to control.

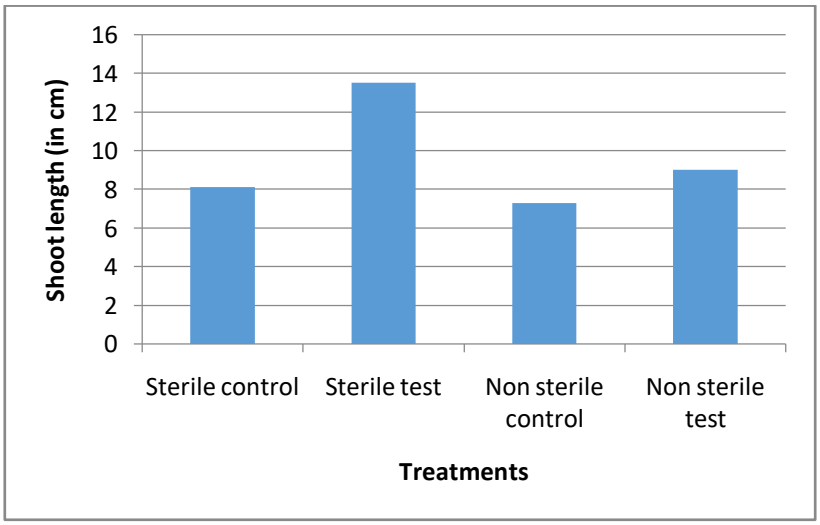


Fig 7: Effect of isolated PGPR bacterial strain on the plant growth

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

after 30 days

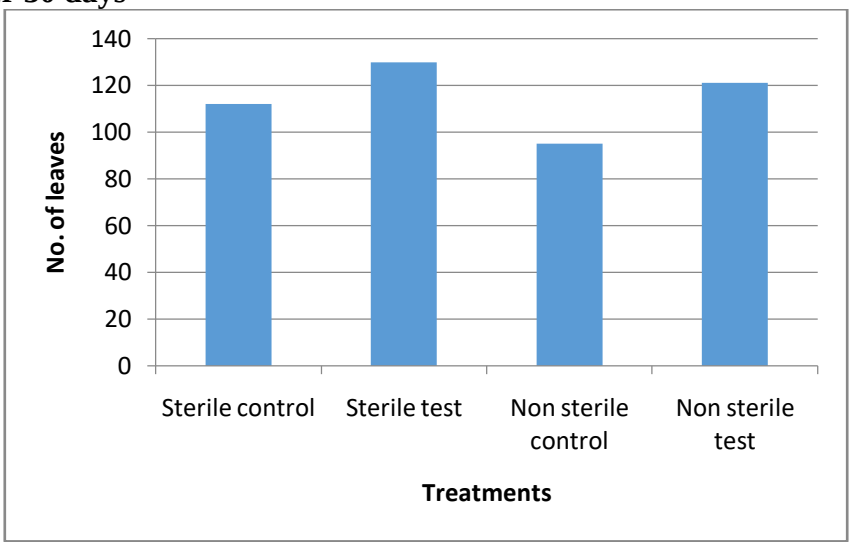


Fig 8: Effect of isolated PGPR strain on the number of leaves after 30 days.

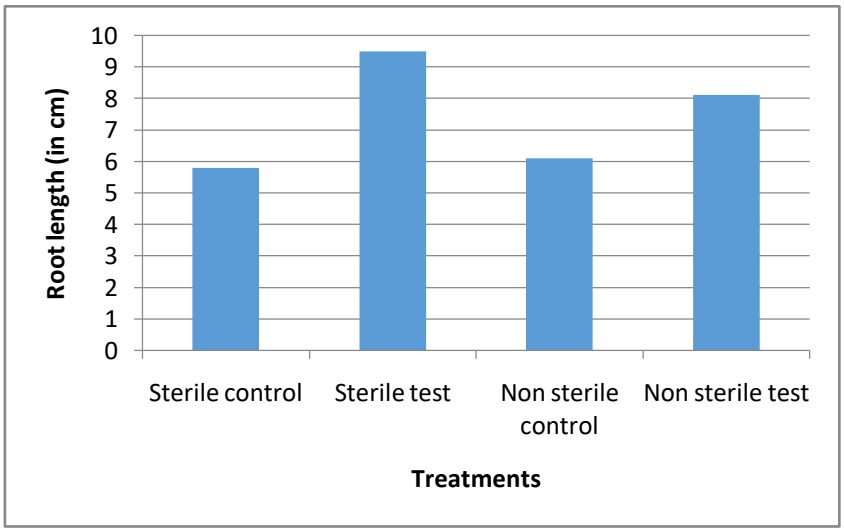


Fig 9: Effect of isolated PGPR strain on the root length after 30 days

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

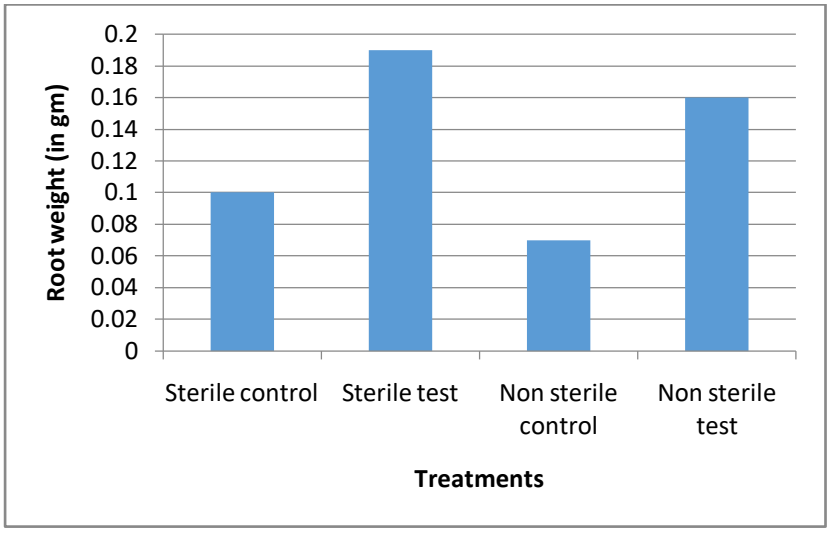


Fig 10: Effect of PGPR isolate on marigold root weight after 30 days of cultivation

Conclusion

In this study we investigated the effectiveness of PGPR isolate whether it could increase the seed germination rate as well as growth of seedlings. The isolate significantly increased plant height in Test plant (with sterile and non-sterile soil). Number of leaves also increased in sterile and non-sterile soil treatments. Seed germination has also increased when seeds were pre-treated with PGPR isolate.These results suggest that the increased growth of marigold seedlings by application of PGPR is probably due to PGP traits those bacterial isolate carry and induce during plant growth, thereby improving the growth of plants. Our study conclude that the use of PGPR as inoculants is an efficient approach to replace chemical fertilizers and pesticides for sustainable agriculture system. Further, investigation, including efficiency test under greenhouse and field conditions, are needed to clarify the role of PGPR as biofertilizers on plant growth and development.

References

[1] A. Ait Kaki *et al.*, "Biocontrol and Plant Growth Promotion Characterization of Bacillus Species Isolated from Calendula officinalis Rhizosphere," *Indian J. Microbiol.*, 2013.

[2] R. Gupta, A. Yadav, and V. K. Garg, "Influence of vermicompost application in potting media on growth and flowering of marigold crop," *Int. J. Recycl. Org. Waste Agric.*, 2014.

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

[3] P. R. Warman and M. J. AngLopez, "Vermicompost derived from different feedstocks as a plant growth medium," *Bioresour. Technol.*, 2010.

[4] N. Q. Arancon, C. A. Edwards, S. Lee, and R. Byrne, "Effects of humic acids from vermicomposts on plant growth," *Eur. J. Soil Biol.*, 2006.

[5] M. Ahemad and M. S. Khan, "Alleviation of fungicide-induced phytotoxicity in greengram [Vigna radiata (L.) Wilczek] using fungicide-tolerant and plant growth promoting Pseudomonas strain," *Saudi J Biol Sci*, vol. 19, no. 4, pp. 451–459, 2012.

[6] T. Naqqash, S. Hameed, A. Imran, M. K. Hanif, A. Majeed, and J. D. van Elsas, "Differential Response of Potato Toward Inoculation with Taxonomically Diverse Plant Growth Promoting Rhizobacteria," *Front Plant Sci*, vol. 7, p. 144, 2016.

[7] S. K. Upadhyay and D. P. Singh, "Effect of salt-tolerant plant growth-promoting rhizobacteria on wheat plants and soil health in a saline environment," *Plant Biol*, vol. 17, no. 1, pp. 288–293, 2015.

[8] D. K. Maheshwari, *Bacteria in agrobiology : plant probiotics*. Heidelberg ; New York: Springer Verlag, 2012.

[9] W. H. James, "Polyglycerol polyricinoleate (PGPR) is a compound used in the baking industry and in the manufacture of chocolate," *Food Chem Toxicol*, vol. 37, no. 2–3, pp. 268–269, 1999.

[10] R. Dixit *et al.*, "Paenibacillus lentimorbus induces autophagy for protecting tomato from Sclerotium rolfsii infection," *Microbiol. Res.*, 2018.

[11] B. Arun, B. Gopinath, and S. Sharma, "Plant growth promoting potential of bacteria isolated on N free media from rhizosphere of Cassia occidentalis," *World J Microbiol Biotechnol*, vol. 28, no. 9, pp. 2849–2857, 2012.

[12] J. W. Kloepper, R. Lifshitz, and R. M. Zablotowicz, "Free-living bacterial inocula for enhancing crop productivity," *Trends Biotechnol.*, vol. 7, pp. 39–44, 1989.

[13] V. Menon, G. Prakash, A. Prabhune, and M. Rao, "Biocatalytic approach for the utilization of hemicellulose for ethanol production from agricultural residue using

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

thermostable xylanase and thermotolerant yeast," *Bioresour Technol*, vol. 101, no. 14, pp. 5366–5373, 2010.

[14] M. L. Reed, B. G. Warner, and B. R. Glick, "Plant growth-promoting bacteria facilitate the growth of the common reed Phragmites australisin the presence of copper or polycyclic aromatic hydrocarbons," *Curr Microbiol*, vol. 51, no. 6, pp. 425–429, 2005.

[15] A. Karnwal, "Isolation and identification of plant growth promoting rhizobacteria from maize (Zea mays L.) rhizosphere and their plant growth promoting effect on rice (Oryza sativa L.)," *J. Plant Prot. Res.*, 2017.

[16] A. Karnwal, "Production of indole acetic acid by fluorescent Pseudomonas in the presence of L-tryptophan and rice root exudates," *J. Plant Pathol.*, vol. 91, no. 1, 2009.

[17] F. J. Tapia, C. A. Lopez, M. J. Galan, and E. Rubio, "Bayesian model for optimization adaptive e-learning process," *Int. J. Emerg. Technol. Learn.*, 2008.

[18] R. Porcel, A. M. Zamarreno, J. M. Garcia-Mina, and R. Aroca, "Involvement of plant endogenous ABA in Bacillus megaterium PGPR activity in tomato plants," *BMC Plant Biol*, vol. 14, p. 36, 2014.

[19] M. A. Jorquera, B. Shaharoona, S. M. Nadeem, M. de la Luz Mora, and D. E. Crowley, "Plant growth-promoting rhizobacteria associated with ancient clones of creosote bush (Larrea tridentata)," *Microb Ecol*, vol. 64, no. 4, pp. 1008–1017, 2012.

[20] A. K. Chauhan, D. K. Maheshwari, K. Kim, and V. K. Bajpai, "Termitarium-inhabiting Bacillus endophyticus TSH42 and Bacillus cereus TSH77 colonizing Curcuma longa L.: isolation, characterization, and evaluation of their biocontrol and plant-growth-promoting activities," *Can J Microbiol*, vol. 62, no. 10, pp. 880–892, 2016.

[21] A. Zaheer *et al.*, "Association of plant growth-promoting Serratia spp. with the root nodules of chickpea," *Res Microbiol*, vol. 167, no. 6, pp. 510–520, 2016.

[22] L. Praburaman *et al.*, "Significance of diazotrophic plant growth-promoting Herbaspirillum sp. GW103 on phytoextraction of Pband Zn by Zea mays L," *Env. Sci Pollut Res Int*, 2016.

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

[23] H. H. Youssef *et al.*, "Plant-based culture media: Efficiently support culturing rhizobacteria and correctly mirror their in-situ diversity," *J. Adv. Res.*, 2016.

[24] M. J. Mehdipour-Moghaddam, G. Emtiazi, M. Bouzari, and Z. Salehi, "Novel phytase and cellulase activities in endophytic azospirilla," *World Appl. Sci. J.*, 2010.

[25] M. Vinas, J. Sabate, M. J. Espuny, and A. M. Solanas, "Bacterial community dynamics and polycyclic aromatic hydrocarbon degradation during bioremediation of heavily creosote-contaminated soil," *Appl Env. Microbiol*, vol. 71, no. 11, pp. 7008–7018, 2005.

[26] J. G. Holt, N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams, *Bergey's Manual of Determinative Bacteriology*, 9th ed. Baltimore: Williams & Wilkins, 1994.

[27] P. R. Vaikuntapu, S. Dutta, R. B. Samudrala, V. R. Rao, S. Kalam, and A. R. Podile, "Preferential Promotion of Lycopersicon esculentum (Tomato) Growth by Plant Growth Promoting Bacteria Associated with Tomato," *Indian J Microbiol*, vol. 54, no. 4, pp. 403–412, 2014.

[28] U. Hildebrandt, K. Janetta, and H. Bothe, "Towards growth of arbuscular mycorrhizal fungi independent of a plant host," *Appl. Environ. Microbiol.*, 2002.

[29] M. Del Gallo, M. Negi, and C. A. Neyra, "Calcofluor- and lectin-binding exocellular polysaccharides of Azospirillum brasilense and Azospirillum lipoferum.," *J. Bacteriol.*, 1989.

[30] R. R. Saikia, S. Deka, M. Deka, and H. Sarma, "Optimization of environmental factors for improved production of rhamnolipid biosurfactant by Pseudomonas aeruginosa RS29 on glycerol," *J. Basic Microbiol.*, 2012.

[31] M. R. Islam, T. Sultana, M. M. Joe, W. Yim, J. C. Cho, and T. Sa, "Nitrogen-fixing bacteria with multiple plant growth-promoting activities enhance growth of tomato and red pepper," *J Basic Microbiol*, vol. 53, no. 12, pp. 1004–1015, 2013.

[32] M. S. Anwar *et al.*, "Multitrait plant growth promoting (PGP) rhizobacterial isolates from Brassica juncea rhizosphere : Keratin degradation and growth promotion," *Commun Integr Biol*, vol. 7, no. 1, p. e27683, 2014.

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

[33] R. Muthezhilan, B. S. Sindhuja, A. J. Hussain, and M. Jayaprakashvel, "Efficiency of plant growth promoting rhizobacteria isolated from sand dunes of Chennai coastal area," *Pak J Biol Sci*, vol. 15, no. 16, pp. 795–799, 2012.

[34] S. Nezarat and A. Gholami, "Screening plant growth promoting rhizobacteria for improving seed germination, seedling growth and yield of maize," *Pak J Biol Sci*, vol. 12, no. 1, pp. 26–32, 2009.

[35] N. De La Torre-Ruiz *et al.*, "Effect of plant growth-promoting bacteria on the growth and fructan production of Agave americana L," *Braz J Microbiol*, vol. 47, no. 3, pp. 587–596, 2016.

[36] Y. Herschkovitz *et al.*, "Inoculation with the plant-growth-promoting rhizobacterium Azospirillum brasilense causes little disturbance in the rhizosphere and rhizoplane of maize (Zea mays)," *Microb Ecol*, vol. 50, no. 2, pp. 277–288, 2005.

[37] K. Karmakar, A. Rana, A. Rajwar, M. Sahgal, and B. N. Johri, "Legume-rhizobia symbiosis under stress," in *Plant Microbes Symbiosis: Applied Facets*, 2015.

[38] Y. Kang, M. Shen, H. Wang, and Q. Zhao, "A possible mechanism of action of plant growth-promoting rhizobacteria (PGPR) strain Bacillus pumilus WP8 via regulation of soil bacterial community structure," *J Gen Appl Microbiol*, vol. 59, no. 4, pp. 267–277, 2013.

[39] M. S. Akhtar and J. Panwar, "Efficacy of root-associated fungi and PGPR on the growth of Pisum sativum (cv. Arkil) and reproduction of the root-knot nematode Meloidogyne incognita," *J Basic Microbiol*, vol. 53, no. 4, pp. 318–326, 2013.

[40] M. Ahmad, Z. A. Zahir, H. N. Asghar, and M. Asghar, "Inducing salt tolerance in mung bean through coinoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase," *Can J Microbiol*, vol. 57, no. 7, pp. 578–589, 2011.

[41] L. Mwita *et al.*, "Gene expression regulation in the plant growth promoting Bacillus atrophaeus UCMB-5137 stimulated by maize root exudates," *Gene*, vol. 590, no. 1, pp. 18–28, 2016.

ISSN: 0971-1260
Vol-22-Issue-17-September-2019

[42] P. K. Mishra, S. C. Bisht, J. K. Bisht, and J. C. Bhatt, "Cold-tolerant PGPRs as bioinoculants for stress management," in *Bacteria in Agrobiology: Stress Management*, 2012.