Available Online at http://www.recentscientificcom

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 15, Issue, 12, pp.5063-5066,December,2024

International Journal of Recent Scientific Research

ISSN: 0976-3031

RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF PLANT GROWTH-PROMOTING MICROBES, A STEP TOWARDS SUSTAINABLE AGRICULTURE

[1]Shaheer A. M., [2]Prabhu. V and [3]Dhinesh. V

[1]Nehru Memorial College (Autonomous), Puthanampatti – 621 007, Trichy District, India.
[2]Assistant Professor Department of Botany Nehru Memorial College (Autonomous), Puthanampatti – 621 007, Trichy District, India.
[3]Assistant Professor, Soil Science and Agricultural Chemistry, Kumaraguru Institute of Agriculture, Erode, Tamil Nadu, India.

DOI: http://dx.doi.org/10.24327/ijrsr.20241512.0959

ARTICLE INFO

Article History:

Received 14th October, 2024
Received in revised form 21st October, 2024
Accepted 16th November, 2024
Published online 28th December, 2024

Key words:

Microbiota, plant growth promoting soil microbes**,** *Arachis hypogaea* and *Sorghum bicolor*

ABSTRACT

All plants need the right amount of light, water, nutrients, and space in order to survive. Seed plants also need to be pollinated and produce seeds. The seeds must germinate at the right time in a place that will supply all their needs. More and more food is needed all over the world in order to meet rapidly growing population demands, while the modern agriculture on increasing output has already reached its limit. It is badly in need to cultivate new crop varieties for increase the yield and resistant to environmental stress and insects. However, crops are still need to provide the necessary fertilizer nutrients which is insufficient in soil. Recently, many evidences showed that soil microbes provide an opportunity for reducing agricultural demand in inorganic fertilizer. Microorganisms, due to their huge gene pool, are also used for a potential resource in biochemical reactions, which recycle nutrients for plant growth. Therefore, we need to modify and better use of soil microbiota to promote plant growth. The main aim of the study isolation of plant growth promoting microbes from *Arachis hypogaea* peanut (Fabaceae) and their effect on plants such as *Sorghum bicolor* solam (poales).

INTRODUCTION

Groundnut is an invaluable source of protein, calories, essential fatty acids, vitamins, and minerals for human nutrition (Willett *et al*., 2019). Groundnut consumption is reported to be associated with several health benefits (Kris-Etherton *et al*., 2008; Sabate *et al*., 2010 and Guasch-Ferre *et al*., 2017).

Groundnut is a rich source of dietary protein with ability to meet up to 46% of recommended daily allowance; essential vitamins especially E, energy from its oils and fats, and dietary fiber. It is also a rich source of minerals such as K, Na, Ca, Mn, Fe, and Zn among others and a rich source of biologically active compounds (arginine, resveratrol, phytosterols, and flavonoids). Zinc in particular, is one of the limiting micronutrients especially among rural households in Africa affecting especially infants and young persons (Wessells and Brown, 2012).

*Corresponding author: **Shaheer A. M**

Nehru Memorial College (Autonomous), Puthanampatti – 621 007, Trichy District, India

Two routes of Rhizobium infection have been described for root-nodule formation in legume roots: entry via roothairs and via cracks. Root-hair entry occurs in most legumes, e.g. soybean and common bean (*Phaseolus vulgaris*). Crack entry occurs in few legumes: peanut and *Sesbania*. In peanut, root nodules develop only at the sites of lateral-root emergence (Uheda *et al*., 2001), the epidermis and cortex of the parent root are broken by emergence of the lateral root (reviewed in Boogerd and van Rossum, 1997) Uheda *et al*., 2001) visually demonstrated Rhizobium infection into the root through the intercellular gap created by lateral-root emergence. Rhizobacteria that benefit plants by stimulating growth and suppressing disease are referred to as plant growth promoting rhizobacteria (Lugtenberg and Kamilova 2009).

Use of microbial inoculants or plant growth-promoting rhizobateria (PGPR) for the enhancement of sustainable agricultural production is becoming a more widely accepted practice in intensive agriculture in many parts of the world.

Majeed *et al*., 2015 Plant growth-promoting rhizobacteria are free-living soil bacteria that aggressively colonize the rhizosphere plant roots, and enhance the growth, and yield of plants when applied to seed or crops Kumar *et al*., 2014. The

plant growth promoting (PGP) effect of the PGPR is mostly explained by the release of metabolites directly stimulating growth. Several mechanisms have been postulated to explain how PGPR benefit the host plant. These include: (a) the ability to produce plant growth regulators or phytohormones such as indole acetic acid (IAA), cytokinins, and gibberellins Glick 1995 and Marques *et al*., 2010.

The beneficial rhizobacteria associated with cereals has increased recently and several studies clearly demonstrated the positive and beneficial effects of PGPR on growth and yield of different crops especially wheat at different environment under variable ecological conditions Ozturk *et al*., 2003 and Mehnaz *et al*., 2010.Use of microorganisms as environmental friendly strategies to support crop production has great potential. A lot of attention has been given to microorganisms that confer disease suppression Alexandre 2010 Certain organic compounds exuded act as chemical signals for microbes by stimulating colonization through chemotaxis Pacifici 2015. Roots support plants physically by anchoring and chemically through the acquisition of nutrients and water from soil. The root architecture adopts the most suitable structure depending on plant needs and is influenced by various environmental stimuli Lucy *et al*., 2004 Many rhizobacteria can thus associate with plants and ideally promote plant growth and fitness and are referred to as plant growth promoting rhizobacteria (PGPR) Pieterse *et al*., 2012. Referred to as priming of induced systemic resistance (ISR) Majeed *et al*., 2015. Chemical fertilizers are generally used to supply essential nutrients to the soil–plant system throughout the world. However, the prices, availability, and the environmental concerns of chemical fertilizers especially the N fertilizers are real issues of today's agriculture.

Use of microbial inoculants or plant growth- promoting rhizobateria (PGPR) for the enhancement of sustainable agricultural production is becoming a more widely accepted practice in intensive agriculture in many parts of the world Kumar *et al*., 2014. Plant growth-promoting rhizobacteria are free-living soil bac- teria that aggressively colonize the rhizosphere plant roots, and enhance the growth, and yield of plants when applied to seed or crops (Glick *et al*., 1995).

MATERIALS AND METHODS

Collection of seeds

The *Sorghum bicolor* and *Arachis hypogaea* seeds were collected from TNAU, ICAR Government Society, Manapparai Taluk, Trichy District.

Chemicals and reagents

Nutrient Agar medium, Nutrient broth, was purchased from Himedia, India. Whatman filter paper No. 1, Gentamicin antibiotic solution, test samples, test tubes, beakers conical flaks, spirit lamp, double distilled water and petri-plates.

Sample collection

Soil sample and *Arachis hypogaea* (groundnut) were collected from manikandam, Trichy. Tamil Nadu (Lattitude10.7379° N, and Longitude 78.6363° E).

Enumeration Soil sample and *Arachis hypogaea* (groundnut) root nodules

1g of test sample was dissolved in 10ml of sterile double distilled water and filtered through muslin cloth. The filtrate was used for the serial dilutions from 10^1 to 10^8. The 10^6 and 10^2dilution was used to bacterial isolation.

Nutrient Agar Medium

The medium was prepared by dissolving peptone- 0.25gm, NaCl- 0.25 gm, yeast extract- 0.1gm, beef extract- 0.05gm and agar powder- 0.875gm of the commercially available Nutrient Agar Medium (HiMedia) in 50ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten. The 10^8th and 10^2thdilution was plated on the nutrient agar medium by spread plate method and the plate was incubated at 37° C for 24 hrs. After incubation, bacterial colonies were isolated and plated in to a fresh plate.

Nutrient broth

Nutrient broth was prepared by dissolving peptone- 0.25gm, NaCl- 0.25 gm, yeast extract- 0.1gm, beef extract- 0.05gm and agar powder- 0.875gm of the commercially available Nutrient Agar Medium (HiMedia) in 50ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

Gram staining

A loop full of bacterial culture was spread in the glass slide. The slide was smeared in front of the flame. The slides were stained with crystal violet dye and kept it for 1 min and washed the slide in a distilled water. Gram's iodine was added and incubated for 1 minute, then rinsed with distilled water. The decolorizing agent was added and kept for 1 min and then safronin strain was added, after a minute it was washed using distilled water. The slides were observed under the Trinocular microscope the purple colors indicated gram positive bacteria and the pink color indicated gram negative organism.

Motility test - Hanging Drop Method

The motility test was performed by hanging drop method. The cover slip was taken where its edge was coated with Vaseline. The test samples were transferred to the cover slip which was placed over the cavity slide. The slide was viewed under 100X magnification and the organisms' characteristics being motile or non-motile were noted down.

Screening and identification of microbes

The medium was prepared by dissolving peptone- 0.25gm, NaCl- 0.25 gm, yeast extract- 0.1gm, beef extract- 0.05gm and agar powder- 0.875gm of the commercially available Nutrient Agar Medium (Hi Media) in 50ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten. Five different colonies were isolated from $10^6$and 10^2 and dilution of test sample products. Five different colonies were streaked in a single petri plate.

Mass cultivation of microorganism

The nutrient broth was prepared by dissolving chemical composition of commercially available (Hi Media) in 100ml of distilled water. To identification of microbes 3 different colonies. Therefore, a sterilize inoculation loop and loop full amount of microbes inoculation with nutrient broth. To allow with bacteriological incubator at 24 hours.

Seed Germination

The seeds washed by submerging in water with a few drops of detergent in a beaker with vigorous shaking. The seeds were submerge in 70% alcohol for 40s, after which the alcohol was decanted. The seeds were transferred to a flask containing 20% commercial sodium hypochlorite solution and left there for 20 min for surface sterilization. Later they were rinsed thrice with sterile distilled water. 2-3 seeds were placed on the surface of wet cotton and treated with different concentration of SA and CH samples and incubated at 25^{o}C for 16 h photoperiod with 250 µE/m^{2}/ s light intensity for 2 weeks. Percentage of germination was observed regularly. If need be, transfer the individual plantlets to half MS medium.

Microorganism's treatment of seeds

Microorganisms treatment of seeds five different concentration (10ml, 5ml, 2,5 ml, 1 ml and 0.5 ml) and control. To soak a plant seeds (10) and 3 different microorganisms of treatment.

RESULTS AND DISCUSSION

At a global scale, the effects of continuous agricultural practices such as fertilization can cause serious damage to the environment. Inoculation is one of the most important sustainable practices in agriculture, because microorganisms establish associations with plants and promote plant growth by means of several beneficial characteristics. Endophytes are suitable for inoculation, reflecting the ability of these organisms for plant colonization, and several studies have demonstrated the specific and intrinsic communication among bacteria and plant hosts of different species and genotypes.

Plant growth-promoting bacteria (PGPB) are bacteria that can enhance plant growth and protect plants from disease and abiotic stresses through a wide variety of mechanisms; those that establish close associations with plants, such as the endophytes, could be more successful in plant growth promotion.

The microbes were isolated from the root nodules of *Arachis hypogaea* by micro dilution method and spread-plate method. The isolated microorganisms (S2-1, S6-3 and S6-5) were identified and characterized by gram staining method and hanging drop method. The identified microbes were inoculated into the selected seeds of *Sorghum bicolor.* The plant growth was observed after 15 days. There is an increase in plant growth after treatment of *Bacillus cereus, Enterobacter aerogenes, Enterobacter cloacae, Monocytogenes, Clostridium difficile,* and *Clostridium botulinum* in seeds.

CONCLUSION

The combination of different methodologies with the microbes, such as identification of plant growth-promoting characteristics, the identification of bacterial strains, as well as assays of seed inoculation in laboratory conditions and cultivation experiments in the field, are part of the search for new technologies for agricultural crops. Thus, when this search shows a potential bacterial inoculants, adequate for reintroduction in the environment, many genera such as *Bacillus cereus,* Enterobacter aerogenes, Enterobacter cloacae, Monocytogenes, Clostridium difficile, and *Clostridium botulinum.*

Finally, the search for beneficial bacteria is important for the development of new and efficient inoculants for agriculture. Also important are investments in technologies that can contribute to increase the inoculum efficiency and the survival rate of bacteria adherent to the seeds, which are other essential factors for successful inoculation. Thus, the introduction of beneficial bacteria in the soil tends to be less aggressive and cause less impact to the environment than chemical fertilization, which makes it a sustainable agronomic practice and a way of reducing the production costs.

Conflict of Interest

There is no conflict of interest with regard to this Manu-script.

Acknowledgements

Figure 1 *Arachis hypogaea* plant

Figure 2: *Arachis hypogaea* root nodules

Figure 3 Root nodules powder

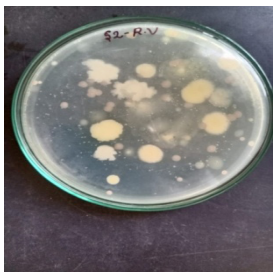

Figure 4 Spread plate (S2)

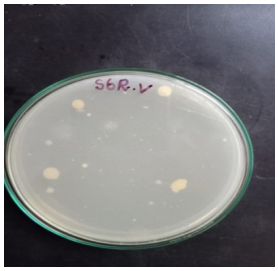

Figure 5 Spread plate (S6)

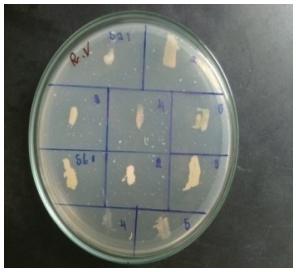

Figure 6 Isolation of microbes

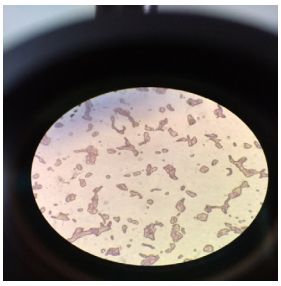

Figure 7 Gram staining of S6

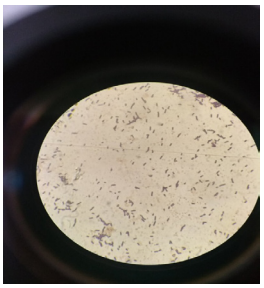

Figure 8 Gram staining of S2

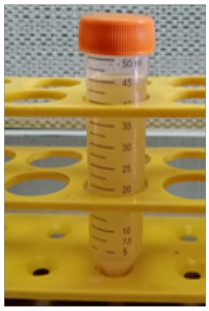

Figure 9 Mass cultrue of microbes

Figure 10 OD of microbes

Table 1 Identification of Microbes by Gram staining method

SAMPLE	GRAM STAINING	SHAPE	MOTILE OR NON-MOTILE	NAME OF THE ORGANISM
S2-1	Gram (+)	Rod	Motile	Bacillus cereus
S2-2	Gram(-)	Cocci chain	Motile	Campylobacter jejuni
S2-3	Gram (+)	Rod chain	Non-motile	Bacillus anthracis,- Clostridium perfingens
S2-4	Gram (-)	Cocci chain	Motile	Campylobacter jejuni
S2-5	Gram(-)	Cocci chain	Motile	Alcaligenes faecalis sub-sp.faecalis
S6-1	Gram (-)	Rod chain	Motile	Citrobacter freundii, Aeromonas hydrophila, E.coli
S6-2	Gram (+)	Rod chain	Motile	Bacillus subtilis
S6-3	Gram (+)	Rod	Motile	Monocytogenes, clostridium difficile, Clostridium botulinum
S6-4	Gram(-)	Rod chain	Non- motile	Actinomyces israilii, Aeromonas caviae
S6-5	Gram(-)	Rod	Motile	Enterobacter aerogenes, Enterobacter cloacae

Figure 11 Microorganism's treatment for seeds (S2 – 1 *Bacillus cereus*)

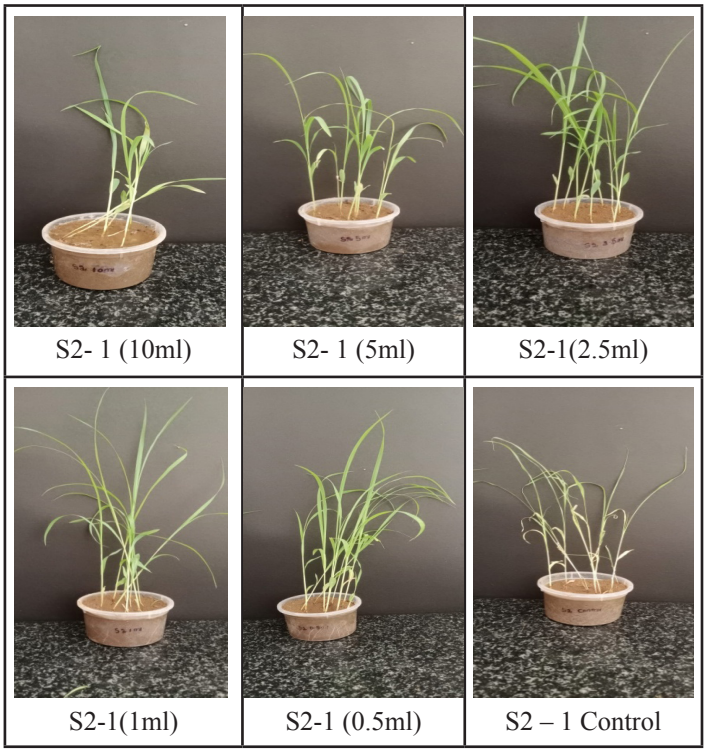

Figure 12 Microorganism's treatment for seeds

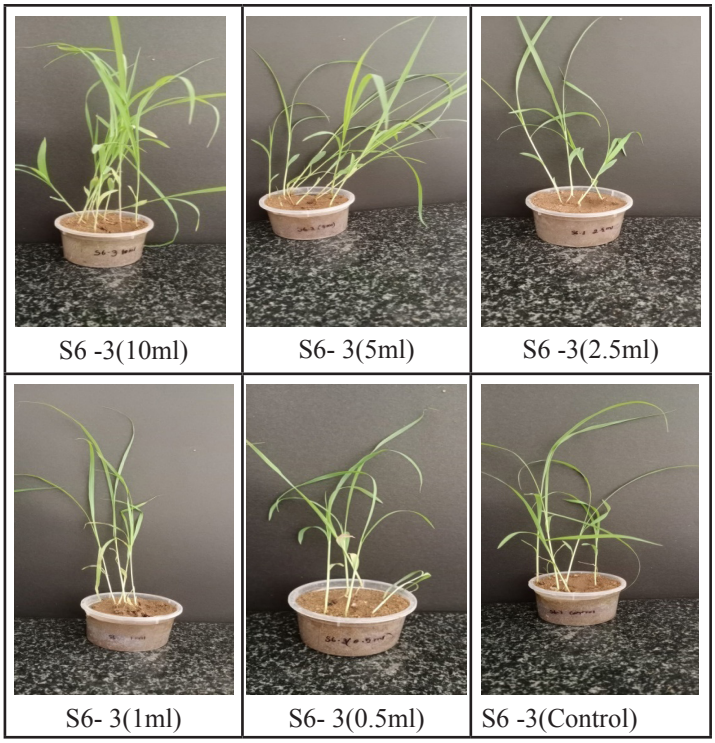

Figure 13 Microorganism's treatment for seeds

(S 6- 5*Enterobacter aerogenes, Enterobacter cloacae)*

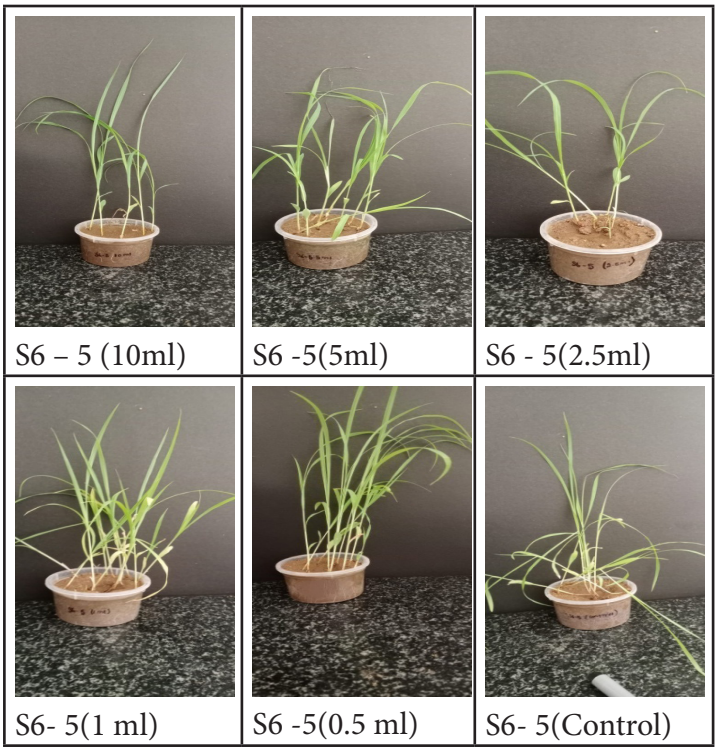

Table 2 Plant growth observations after the microbial treatment - Sample concentration vs Number of seeds

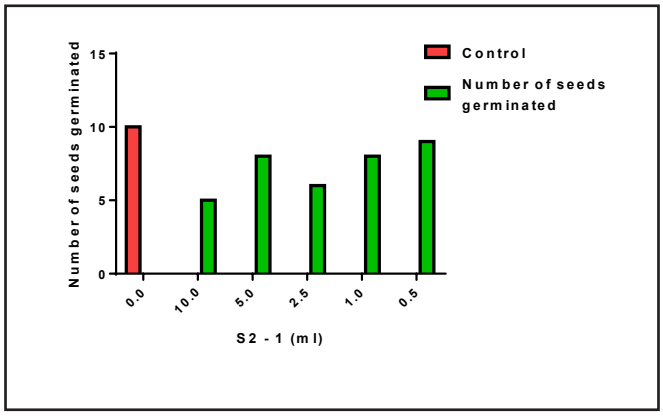

Figure 14 Number of seeds germinated after the treatment of S2-1

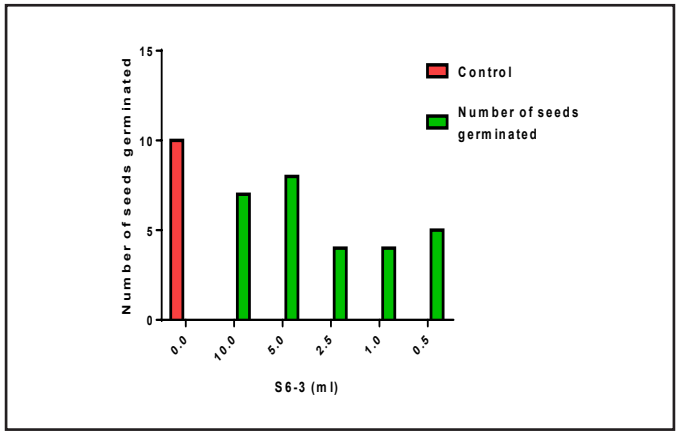

Figure 15 Number of seeds germinated after

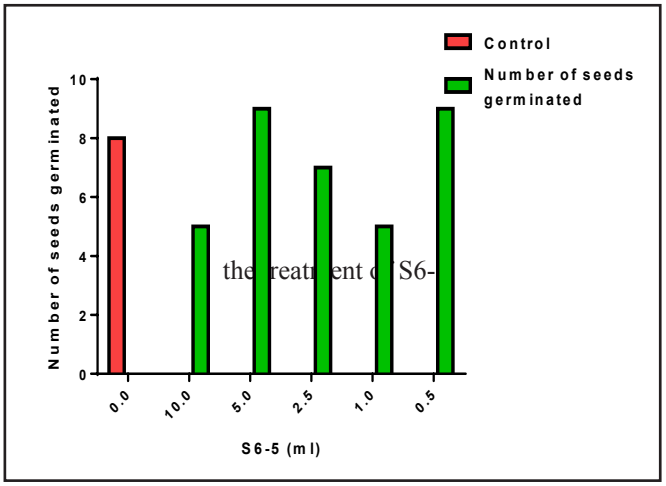

Figure 16 Number of seeds germinated after the treatment of S6-5

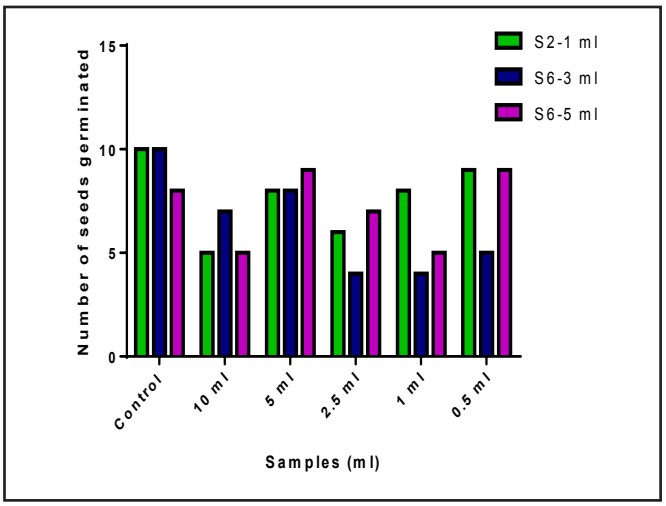

Figure 17 Comparison of number of seeds germinated after the treatment of S2-1, S6-3 and S6

Table 2 Plant growth observations after the microbial treatment - Sample concentration vs Plant height-

S.NO.	SAMPLE CONCENTRATION (ml)	PLANT HEIGHT (cm)		
		S2-1	S6-3	S6-5
1.	Control	29	28	25.5
2.	10	18.5	28	18
3.	5	20	29	24
4.	2.5	28	25	20
5.	1	28.5	28	20
6.	0.5	28.5	18	20

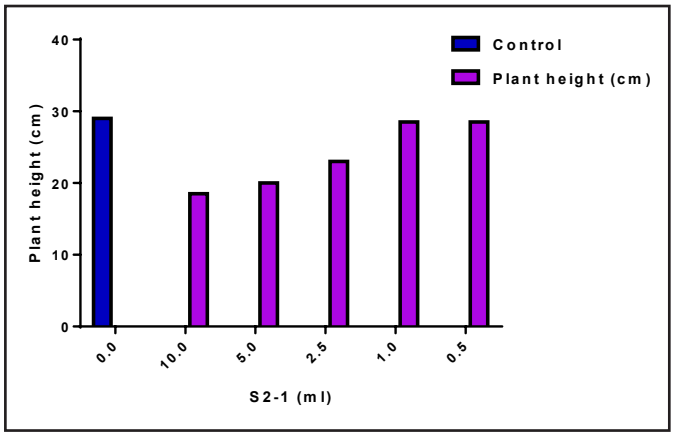

Figure 18 Plant height after the treatment of S2-1

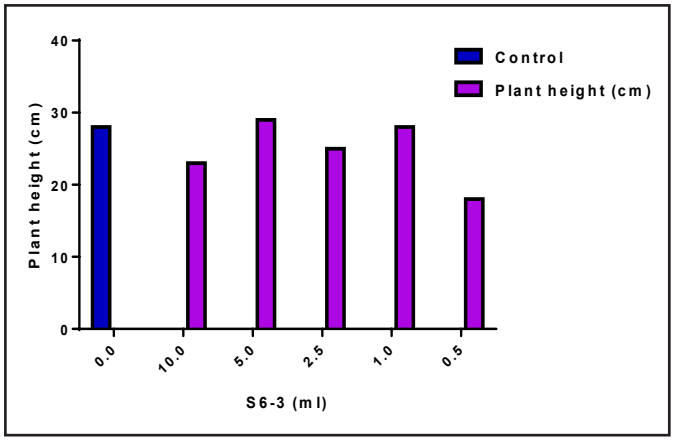

Figure 19 Plant height after the treatment of S6-**3**

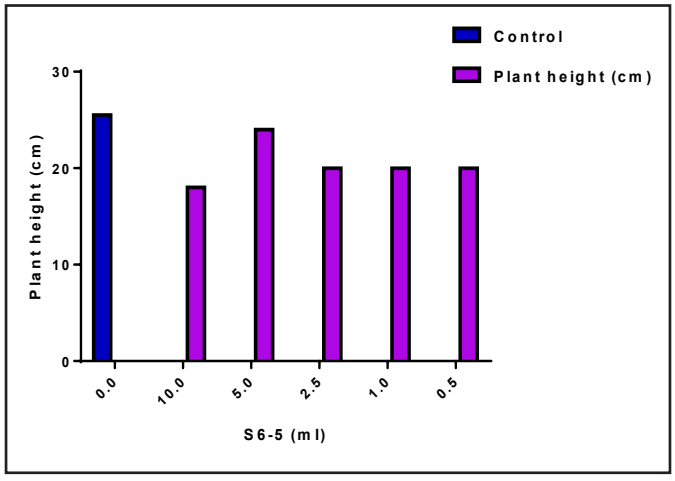

Figure 20 Plant height after the treatment of S6-5

References

1. Alexandre G (2010) Coupling metabolism and chemotaxis-dependent behaviours by energy taxis receptors. Microbiology 156:2283–2293
2. Anchal Sharma, V.D. Diwevidi, Smita Singh, Khsitiz Kumar Pawar, Mahesh Jerman, L.B. Singh, Satish Singh and Deepak Srivastawa (2013). Biological Control and its Important in Agriculture, International Journal of Biotechnology and Bioengineering Research,Volume 4, Number 3, pp. 175-180.
3. Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Smith, D. L. (2018). Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. Frontiers in Plant Science, 9.
4. Bender, S. F., Plantenga, F., Neftel, A., Jocher, M., Oberholzer, H.-R., Köhl, L., van der Heijden, M. G. (2013). Symbiotic relationships between soil fungi and plants reduce N2O emissions from soil. The ISME Journal, 8(6), 1336–1345.
5. Bhardwaj, D., Ansari, M., Sahoo, R., and Tuteja, N. (2014). Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microbial Cell Factories, 13(1), 66.
6. Blagodatskaya, E., Blagodatsky, S., Anderson, T.-H., & Kuzyakov, Y. (2014). Microbial Growth and Carbon Use Efficiency in the Rhizosphere and Root-Free Soil. PLoS ONE, 9(4), e93282.
7. Cameron Wagga, S. Franz Bendera, Franco Widmerc, and Marcel G. A. vander Heijden (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality, vol. 111, no. 14.
8. Choudhary DK, Johri BN (2009) Interactions of Bacillus spp. And plants-with special reference to induced systemic resistance (ISR). Microbiol Res 164:493–513
9. David Chandler, Alastair S. Bailey, G. Mark Tatchell, Gill Davidson, Justin Greaves and Wyn P. Grant (2011). The development, regulation and use of biopesticides for integrated pest management, The Royal Society 366.
10. El Moujahid, L., Le Roux, X., Michalet, S., Bellvert, F., Weigelt, A., & Poly, F. (2017). Effect of plant diversity on the diversity of soil organic compounds. PLOS ONE, 12(2),e0170494.
11. Elizabeth A. Worrall, Aflaq Hamid, Karishma T. Mody, Neena Mitter and Hanu R. Pappu (2020). Nanotechnology for Plant Disease Management, Agronomy, 8, 285.
12. Elizabeth M. Bach, Kelly S. Ramirez, Tandra D. Fraser and Diana H. Wall (2018). Soil Biodiversity Integrates Solutions for a Sustainable Future, Sustainability, 12, 2662;
13. Foster S, Tyler VE. Tyler's Honest Herbal: A Sensible Guide to the Use of Herbs and Related Remedies. 4th ed. New York: The Haworth Press; 1999. p. 97-9.
14. Garcia-Gonzalez, J., and Sommerfeld, M. (2015). Biofertilizer and biostimulant properties of the microalga *Acutodesmus dimorphus*. Journal of Applied Phycology, 28(2), 1051–1061.
15. Glick, B. R. (1995). The enhancement of plant growth by freeliving bacteria. Can. J. Microbiol. 4, 1109–1114.
16. Glick, B.R. (1995).The enhancement of plant growth by free living bacteria. Can. J. Microbiol. 4, 1109–1114.
17. Guasch-Ferre, M., Liu, X., Malik, V. S., Sun, Q., Willett, W. C., Manson, J. E., et al. (2017). Nut consumption and risk of cardiovascular disease. J. Am. Coll. Cardiol. 70 (20), 2519–2532.
18. Guerra, C. A., Heintz-Buschart, A., Sikorski, J., Chatzinotas, A., Guerrero-Ramírez, N., Cesarz, S., Eisenhauer, N. (2020). Blind spots in global soil biodiversity and ecosystem function research. Nature Communications, 11(1).

19. Hartmann, M., Frey, B., Mayer, J., Mäder, P., and Widmer, F. (2014). Distinct soil microbial diversity under long-term organic and conventional farming. The ISME Journal, 9(5), 1177–1194.

20. Hassani, M. A., Duran, P., and Hacquard, S. (2018). Microbial interactions within the plantholobiont. Microbiome, 6(1).

21. Jia, T., Cao, M., & Wang, R. (2018). Effects of Restoration Time on Microbial Diversity in Rhizosphere and Non-Rhizosphere Soil of Bothriochloa ischaemum. International Journal of Environmental Research and Public Health, 15(10), 2155.

22. Jitendra Kulkarni, Nitin Kapse and D.K Kulkarni; (2009). Plant-based pesticides for control of *Helicoverpa amigera* on Cucumis; by Asian Agric. History, 13 (4), 327-332.

23. Katoh, M., Risky, E., and Sato, T. (2017). Immobilization of Lead Migrating from Contaminated Soil in Rhizosphere Soil of Barley (Hordeum vulgare L.) and Hairy Vetch (Vicia villosa) Using Hydroxyapatite. International Journal of Environmental Research and Public Health, 14(10), 1273.

24. Kris-Etherton, P. M., Hu, F. B., Ros, E., and Sabate, J. (2008). The role of tree nuts and groundnuts in the prevention of coronary heart disease: multiple potential mechanisms. J. Nutr. 138 (9), 1746s–1751s.

25. Kumar, A., Maurya, B. R., and Raghuwanshi, R. (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). Biocatal. Agric. Biotechnol. 3, 121–128.

26. Kumar, A.,Maurya,B.R.,and Raghuwanshi, R. (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticumae stivum*L.). Biocatal. Agric. Biotechnol. 3, 121–128.

27. Kurucheve, V.; Ezhilan, J.G. and Jayaraj, J. (1997). Screening of higher plants for fungi toxicity against *Rhizoctonia solaniIn vitro*. Indian Phtopath. 50 (2): 235-241.

28. Lijbert Brussaard, Valerie M. Behan-Pelletier, David Bignell, Patricia Folgarait (1997). Biodiversity and ecosystem functioning in soil, Royal Swedish Academy of Sciences, Vol. 26 No. 8.

29. Lim, J.-H., and Kim, S.-D. (2013). Induction of Drought Stress Resistance by Multi-Functional PGPR Bacillus licheniformis K11 in Pepper. The Plant Pathology Journal, 29(2), 201–208.

30. Lori, M., Piton, G., Symanczik, S., Legay, N., Brussaard, L., Jaenicke, S., Foulquier, A. (2020). Compared to conventional, ecological intensive management promotes beneficial proteolytic soil microbial communities for agro-ecosystem functioning under climate change-induced rain regimes. Scientific Reports, 10(1).

31. Luca Ruiu (2018). Microbial Biopesticides in Agroecosystems, Agronomy 2, 8, 235.

32. Lucy M, Reed E, Glick BR (2004) Applications of free living plant growth-promoting rhizobacteria. Antonie Leeuwenhoek Int J G 86:1–25

33. Lugtenberg, B., and Kamilova, F. (2009). Plant-Growth-Promoting Rhizobacteria. Annual Review of Microbiology, 63(1), 541–556.

34. Maarastawi, S. A., Frindte, K., Linnartz, M., and Knief, C. (2018). Crop Rotation and Straw Application Impact Microbial Communities in Italian and Philippine Soils and the Rhizosphere of Zea mays. Frontiers in Microbiology, 9.

35. Majeed, A., Abbasi, M. K., Hameed, S., Imran, A., & Rahim, N. (2015). Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Frontiers inMicrobiology, 6. doi:10.3389/fmicb.2015.00198

36. Majeed, A., Abbasi, M. K., Hameed, S., Imran, A., and Rahim, N. (2015). Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Frontiers in Microbiology, 6.

37. Mandela Fernandez-Grandon. G, Steven J. Harte, Jaspher Ewany, Daniel Bray and Philip C. Stevenson (2020). Additive Effect of Botanical Insecticide and Entomopathogenic Fungi on Pest Mortality and the Behavioral Response of Its Natural Enemy, Plants, 9, 173.

38. Manuel Delgado-Baquerizo, Peter B. Reich, Chanda Trivedi, David J. Eldridge, Sebastián Abades, Fernando D. Alfaro, Feli Lijbert Brussaard pe Bastida, Asmeret A. Berhe, Nick A. Cutler, Antonio Gallardo, Laura Garcia-Velazquez, Stephen C. Hart, Patrick E. Hayes, Ji-Zheng He, Zeng-Yei Hseu, Hang-Wei Hu, Martin Kirchmair, Sigrid Neuhauser, Cecilia A. Perez, Sasha C. Reed, Fernanda Santos, Benjamin W. Sullivan, Pankaj Trivedi, Jun-Tao Wang, Luis Weber-Grullon, Mark A. Williams and Brajesh K. Singh (2020). Nature Ecology and Evolution, vol 4, page 210–220.

39. Mapelli, F., Marasco, R., Fusi, M., Scaglia, B., Tsiamis, G., Rolli, E., Daffonchio, D. (2018). The stage of soil development modulates rhizosphere effect along a High Arctic desert chronosequence. The ISME Journal, 12(5), 1188–1198.

40. Marques, A. P. G. C., Pires, C., Moreira, H., Rangel, A. O. S. S., and Castro, P. M. L. (2010). Assessment of the plant growth promotion abilities of six bacterial isolates using Zea mays as indicator plant. Soil Biol. Biochem. 42, 1229–1235.

41. Megha M. Akashe, Uday V. Pawade, Ashwin V. Nikam (2018). Classification of pesticides: a review, Res. Ayurveda Pharm. 9 (4).

42. Mehnaz, S., Kowalik, T., Reynolds, B., and Lazarovits, G. (2010). Growth promoting effects of corn (Zea mays) bacterial isolates under greenhouse and field conditions. Soil Biol. Biochem. 42, 1848–1856. doi: 10.1016/j.soilbio.2010.07.003

43. Moisan, K., Cordovez, V., van de Zande, E. M., Raaijmakers, J. M., Dicke, M., and Lucas-Barbosa, D. (2019). Volatiles of pathogenic and non-pathogenic soil-borne fungi affect plant development and resistance to insects. Oecologia 190:589–604.

44. Ojiewo, C. O., Janila, P., Bhatnagar-Mathur, P., Pandey, M. K., Desmae, H., Okori, P., Varshney, R. K. (2020). Advances in Crop Improvement and Delivery Research for Nutritional Quality and Health Benefits of Groundnut (Arachis hypogaea L.). Frontiers in Plant

Science, 11.

45. Opender Koul (2011). Microbial bio pesticides opportunities and challenges, Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 6, No. 056.
46. Ozturk, A., Caglar, O., and Sahin, F. (2003). Yield response of wheat and barley to inoculation of plant growth promoting rhizobacteria at various levels of nitrogen fertilization. J. Plant Nutr. Soil Sci. 166, 262–266. doi: 10.1002/jpln.200390038
47. Pacifici E, Polverari L, Sabatini S (2015) Plant hormone cross-talk: the pivot of root growth. J Exp Bot 66:1113–1121
48. Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM (2012) Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 28:489–521
49. Pio A. Javier and Marilyn B. Brown2 (2018). Bio-fertilizers and bio-pesticides research and development at uplb, University of the Philippines Los Banos (UPLB).
50. Pitumpe Arachchige, P.S., Rosso, L.H.M., Hansel, F.D., Ramundo, B., Torres, A.R., Ciampitti, I.A., Jagadish, S.V.K (2020). Temporal biological nitrogen fixation pattern in soybean inoculated with Bradyrhizobium. Agrosyst. Geosci. Environ, 3, e20079.
51. Raguchander. D. Saravana kumar and P. Balasurramanian (2011). Molecular approaches to improvement of biocontrol agents of plant diseases, journal of Biological control, 25 (2)71-84.
52. Raj Kumar Mishra, Abhishek Bohra, Naimuddin Kamaal, Krishna Kumar, Kiran Gandhi, Sujayanand GK, P. R. Saabale, Satheesh Naik SJ, Birinchi Kumar Sarma, Dharmendra Kumar, Monika Mishra, Dhirendra Kumar Srivastava and Narendra Pratap Singh (2018). Utilization of biopesticides as sustainable solutions for management of pests in legume crops: achievements and prospects, Egyptian Journal of biological Pest Control, 28:3.
53. Rajveer Kaur, Gurjot Kaur Mavi and Shweta Raghav (2019). Pesticides Classification and its Impact on Environment, Int. J. Curr. Microbiol. App.Sci*8*(3): 1889-1897.
54. Saad, M. M., Eida, A. A., & Hirt, H. (2020). Tailoring Plant-Associated Microbial Inoculants in Agriculture - A Roadmap for Successful Application. Journal of Experimental Botany.
55. Sabate, J., Oda, K., and Ros, E. (2010). Nut consumption and blood lipid levels: a pooled analysis of 25 intervention trials. Arch. Intern. Med. 170 (9), 821–827.
56. Schutz, L., Gattinger, A., Meier, M., Müller, A., Boller, T., Mäder, P., and Mathimaran, N. (2018). Improving Crop Yield and Nutrient Use Efficiency via Biofertilization A Global Meta-analysis. Frontiers in Plant Science, 8.
57. Sessitsch A, Howieson JG, Perret X, Antoun H, Martı´nez-Romero E (2002). Advances in Rhizobium research. Crit Rev Plant Sci 21:323–378.
58. Simonsen, A. K., Dinnage, R., Barrett, L. G., Prober, S. M., and Thrall, P. H. (2017). Symbiosis limits establishment of legumes outside their native range at a global scale. Nature Communications, 8, 14790.
59. Someshwar Bhagat, Ajanta Birah , Rakesh Kumar , M. S. Yadav , and C. Chattopadhyay (2016). Plant Disease Management: Prospects of Pesticides of PlantOrigin, Advances in Plant Biopesticides, DOI 10.1007/978-81-322-2006-0-7.
60. Thakur, M. P., Phillips, H. R. P., Brose, U., De Vries, F. T., Lavelle, P., Loreau, M., Cameron, E. K. (2019). Towards an integrative understanding of soil biodiversity. Biological Reviews.
61. Thomas, L., and D. L. Crawford. (1998). Cloning of clustered *S. viridosporus*T7A lignocellulose catabolism genes encoding peroxidase and endoglucanaseand their extracellular expression in Pichia pastoris. Can. J. Microbiol. 44:364–372.
62. Tresch, S., Frey, D., Bayon, R.-C. L., Mäder, P., Stehle, B., Fliessbach, A., and Moretti, M. (2019). Direct and indirect effects of urban gardening on aboveground and belowground diversity influencing soil multifunctionality. Scientific Reports, 9(1).
63. Uheda E, Daimon H, Yoshizako F. (2001). Colonization and invasion of peanut (*Arachis hypogaea* L.) roots by gusA-marked *Bradyrhizobium* sp. Canadian Journal of Botany 79: 733–738.
64. Vaishali Kandpal (2014). Biopesticides, International Journal of Environmental Research and Development, Volume 4, Number 2, pp. 191-196.
65. Wagg, C., Bender, S. F., Widmer, F., and van der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences, 111(14), 5266–5270.
66. Wessells, K. R., and Brown, K. H. (2012). Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. PloS One 7 (11), e50568.
67. Willett, W., Rockstrom, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., et al. (2019). Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems. Lancet 393 (10170), 447–492.
68. Wintermans, P. C. A., Bakker, P. A. H. M., and Pieterse, C. M. J. (2016). Natural genetic variation in Arabidopsis for responsiveness to plant growth-promoting rhizobacteria. Plant Molecular Biology, 90(6), 623–634.
69. Zhang, J., Liu, J., Meng, L., Ma, Z., Tang, X., Cao, Y.,et al. (2012). Isolation and characterization of plant growth-promoting rhizobacteria from wheat roots by wheat germ agglutinin labeled with fluorescein isothiocyanate. J. Microbiol. 50, 191–198.
70. Strickland, M.S.; Rousk, J. Considering fungal: Bacterial dominance in soils–methods, controls, and ecosystem implications. Soil Biol. Biochem. 2010, 42, 1385–1395.
71. Tiedje, J.M.; Asuming-Brempong, S.; Nüsslein, K.; Marsh, T.L.; Flynn, S.J. Opening the black box of soil-bacterial diversity. Appl. Soil Ecol. 1999, 13, 109–122.