# BIOFILM BIOFERTILIZER'S TECHNOLOGY, A REVITING METHOD FOR CROP PLANTS

*Phosphate solubilising biofilmed liquid biofertilizer produced using Bacillus sp. isolated from rhizospheric soil by batch fermentation method*

**[1]RAKHI ARORA, [2]KAJAL ARORA**

[1,2]Assistant Professor
Department of Microbiology
Swami Shri Swaroopanand Saraswati Mahavidyalaya, Hudco, Bhilai, C.G., India

***Abstract:*** **The over application of chemical fertilizers to fulfill the ever increasing demands on agriculture industry have now left us with decreased soil fertility, nutrient accumulation , directly or indirectly affecting environment and human health. The rhizospheric microbes, also known as PGPR help plants in various ways like nitrogen fixation, phosphorus solubilisation, etc also will surely help us with solving the hazards possessed by these agrochemicals. The present work documents 3 biofilm forming bacterial species named as P1, P2 &P3. As biofilmed liquid biofertilizer that will help in phosphate solubilsation thus improving soil fertility and ultimately crop yield. As this can become a boon for the agriculture industry and the only natural way to revitalize our soil with nutrients.**

***Keywords*: Biofertilizer, agrochemicals, biofilm, Phosphate Solubilisation, Bacillus, rhizoflora, rhizosphere.**

## I. INTRODUCTION

Soil nutrients are essential for plant growth. As, with the ever increasing population there has been tremendous increase in demand for food. After the amendment of Green Revolution the intensity of using chemical fertilizers to meet the demands by the agriculture industry has increased with was quite reliable for some time. But, at present due to the over application of these agrochemicals has completely destroyed soil fertility. The overuse of these agrochemicals have resulted in their accumulation in soil affecting soil rhizoflora, crop susceptibility to pathogens, alkalization/acidic fixation of soil making an irreparable damage to the environment.

The current hazard possessed by these agrochemicals is on human health and environment. Some of the recent studies showed that exposure of humans to these chemicals is a major reason to cause neuronal disorder, degenerative diseases, and the major reason of cancer in humans.

**Chemical fertilizers-** The organophosphatic fertilizers provide phosphorus to plants but only 25% of the phosphate is utilized and the left 75% of the chemical gets accumulated in the soil. Some of the recent studies show that the levels of biocidal contamination have adversely increased. This situation of environment detoriation due to excessive use of agrochemicals is endangering the situation of future. All these studies and findings is now lending us to the need for the provision of an environment friendly fertilizer known as biofertilizer.

**BIOFERTILIZER-** "Microorganisms used to enhance the availability of nutrients via nitrogen(by fixing atmospheric nitrogen) and phosphorus by (solubilisng phosphorus) and also promoting the production of growth hormones are known as biofertilizers also commonly called Plant Growth Promoting Rhizobacteria(PGPR)."
Biofertilizers are gaining popularity now a days as due to their ability to increase soil fertility also they can target pests acting as biocontrol agents, efficiently a sustainable choice over chemical and/or organic fertilizer. These PGPR inoculants promote growth of plants by suppressing plant diseases (as Bioprotectant) also by improving nutrient acquisition.

**BACILLUS AS BIOFERTILIZER-** Various sp. of bacillus play an important role in promoting plant growth by enhancing the biosynthesis of plant hormones like gibberllic acid and indole-3-acetic acid(IAA) having direct relation with the nutrient availability. The rhizospheric microorganisms enhance shoot development by improving or acting along to improve cell expression, division and differentiation.
Bacillus as phosphate solubilizer- Along with the above functions these microbes also act as phosphate solubilizer. They basically work by solubilisng the insoluble form of phosphate present in the soil and make them available to the plants. These rhizospheric microbes dissolve the phosphate from bound state to free state as they secrete organic acids which functions for lowering Ph of the soil.

Phosphate is the least mobile and easily available nutrient or mineral elements to the plants in almost all soil conditions. As phosphorus is abundantly available in both inorganic and organic forms. It becomes one of the major prime limiting factors for plant growth; the bioavailability of inorganic phosphorus in the rhizospheric region varies with the plant species, soil conditions and nutrient availability. To fulfill phosphorus deficiency, phosphate solubilisng microbes play a very important role.

**BIOFILMED LIQUID BIOFERTILIZERS-**The use of biofilms has been proposed as possible means to produce effective plant inoculums. A biofilm consists of microbial cells embedded into self produced polymeric substance (EPS) adherent to an inert or living surface, which provides structure and protection to the microbial community. The majority of plant associated bacteria found

on roots and soil colonize by producing this polymeric substance called biofilm. Hence, using PGPR strains that form biofilm could be a strategy to ease the formulation for production of inoculums/biofertilizer.
The microorganisms growing in a biofilm are comparatively more resistant to antimicrobial agents then the planktonic microbial cells.
Liquid Biofertlizers formulation is the new and promising updated technology which can retain itself for 3 months throughout the crop cycle. Liquid biofertilizers facilitates the long time survival of microorganisms by providing a suitable medium increasing the shelf life, cell protection and improved moisture retention capacity.
The present work has been done to study the biofilm forming microorganisms isolated from soil which can also help in solubilisng phosphorus to enhance crop yield and improving soil fertility.

## II. MATERIAL AND METHODS

**Isolation of Bacillus sp. from soil sample**, Rhizospheric microbes were isolated from soil that were composed from different areas in BHOPAL (Madhya Pradesh) and inoculated in Bacillus Selective Agar medium and was incubated for 24 hrs. Colony traits of isolated colonies were recorded. Gram staining was performed.
**Biochemical characterization**- Characterization of the chosen microorganisms by various biochemical tests was done.
**Detection of biofilm forming ability of microorganisms-** All the isolated cultures was subjected to assay methods for detection of biofilm formation. A total 7 assay methods were done including coverslip assay, dry weight assay, cell surface hydrophobicity assay exopolysaccharide assay, etc.
**Determination of phosphate solubilising capacity by biofilm forming and non biofilm forming microorganisms-** Prepared pikosvsky medium and inoculated the isolated strains in sterilized petriplates and incubated at 37 C for 3-4 days in an inverted position.
**Fermentation-** (preparation of inoculums as biofilmed biofertilizer) fermentation of the selected bacterial isolates using batch fermentation method in pikosvsky medium to produce biofilmed liquid biofertilizer.
**Bioassay-** of *Cicer arientinum* in presence of produced inoculums.

## III. RESULTS

The presented work documented 3 bacterial species isolated from soil composed in different areas of Bhopal (Madhya Pradesh). These bacterial species were identified (using morphological, biochemical parameter), screened for biofilm formation, phosphate solubilisation and their effect on growth of the plant (*Cicer arientinum*).

Table 1 Results of gram staining of isolated bacterial species.

| Microorganism | Result of Gram staining | Bacterial shape |
|---|---|---|
| P1 | Gram-ve | Rod shaped |
| P2 | Gram -ve | Small rods |
| P3 | Gram-ve | Rod shaped |

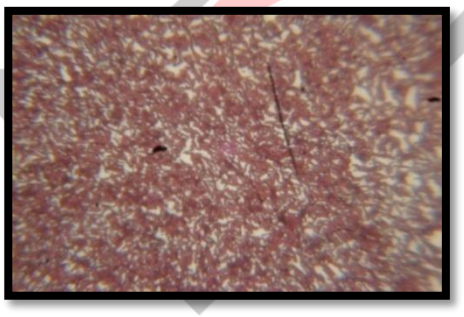

Fig 1- gram staining of isolated bacterial sp.

Table 2 Results of biochemical tests

| S.No | Biochemical test | P1 | P2 | P3 |
|---|---|---|---|---|
| 1 | Cellulase production test | -ve | -ve | -ve |
| 2. | Amylase production test | +ve | -ve | +ve |
| 3. | Production of pectolytic enzymes | -ve | -ve | -ve |
| 4. | Casein hydrolysis | +ve | +ve | +ve |
| 5. | Urease test | -ve | -ve | -ve |
| 6. | Gelatin hydrolysis | -ve | +ve | -ve |
| 7. | Hydrogen sulfide production test | -ve | -ve | -ve |
| 8. | Carbohydrates catabolism | +ve | +ve | +ve |
| 9. | Indole production test | -ve | -ve | -ve |
| 10. | Catalase test | +ve | +ve | +ve |
| 11. | Citrate utilization test | -ve | +ve | +ve |
| 12. | Methyl red and Voges-Proskauer | | | |

| | | | | |
|---|---|---|---|---|
| | MR test | +ve | -ve | -ve |
| | VP test | -ve | +ve | +ve |
| 13. | Fermentation of carbohydrates | | | |
| | Lactose | -ve | +ve | +ve |
| | Sucrose | -ve | +ve | +ve |
| | Mannitol | -ve | +ve | +ve |
| | Dextrose | -ve | +ve | +ve |
| | Starch | +ve | +ve | +ve |
| 14. | Microbial reaction in litmus milk | | | |
| | Acidic ph | -ve | +ve | -ve |
| | Alkaline ph | -ve | -ve | -ve |
| | Reduction | +ve | +ve | +ve |
| | Acid curd | +ve | -ve | +ve |
| | Rennet curd | -ve | +ve | -ve |
| | Gas formation | +ve | +ve | +ve |
| | No change | - | - | - |
| 15. | Nitrate reduction test | +ve | +ve | +ve |
| 16. | Arginine dihydrolase test | -ve | +ve | +ve |

Table 3 Bacterial identification using PIBWIN software

| S.No | BACTERIAL ID | IDENTIFIED BACTERIA |
|---|---|---|
| 1. | P1 | *Bacillus species* |
| 2. | P2 | *Bacillus species* |
| 3. | P3 | *Bacillus species* |

Table 4 effect of carbon source on biofilm formation

| CARBON SOURCE | Dextrose | Sucrose | Maltose | Mannitol | Lactose |
|---|---|---|---|---|---|
| P1 | 0.596 | 1.766 | 0.156 | 0.276 | 1.061 |
| P2 | 0.593 | 0.575 | 1.063 | 0.760 | 0.646 |
| P3 | 0.786 | 0.847 | 0.556 | 0.842 | 1.051 |

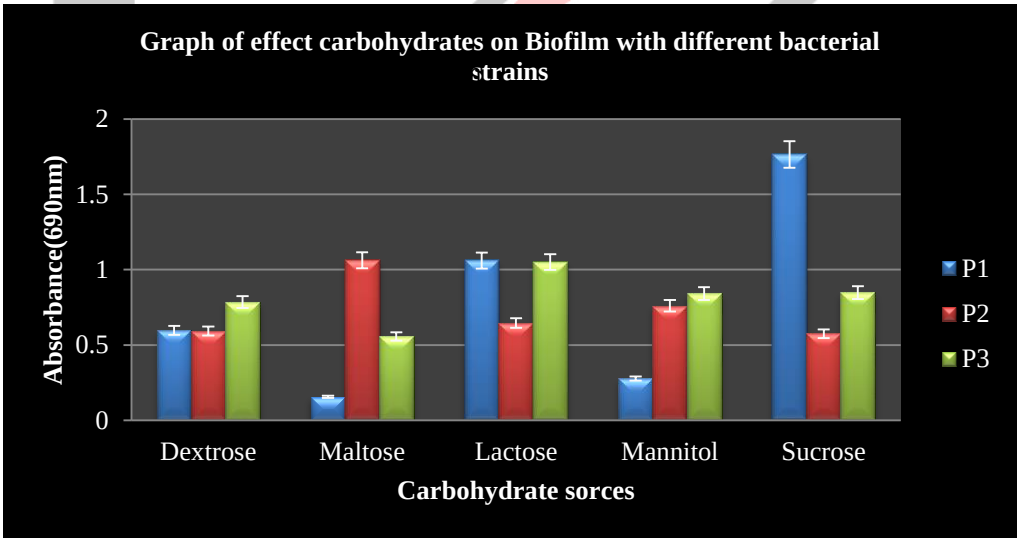


Fig 2- effect of carbohydrates on biofilm formation

TABLE 5: exopolysaccharide produced by bacterial isolates

| Carbon sources | Dextrose | Lactose | Sucrose | Mannitol | Maltose |
|---|---|---|---|---|---|
| P1 | 0646 | 0.646 | 0.760 | 0.491 | 0.456 |
| P2 | 0.699 | 0.617 | 0.569 | 0.576 | 0.703 |
| P3 | 0.821 | 0.595 | 0.638 | 0.764 | 0.735 |

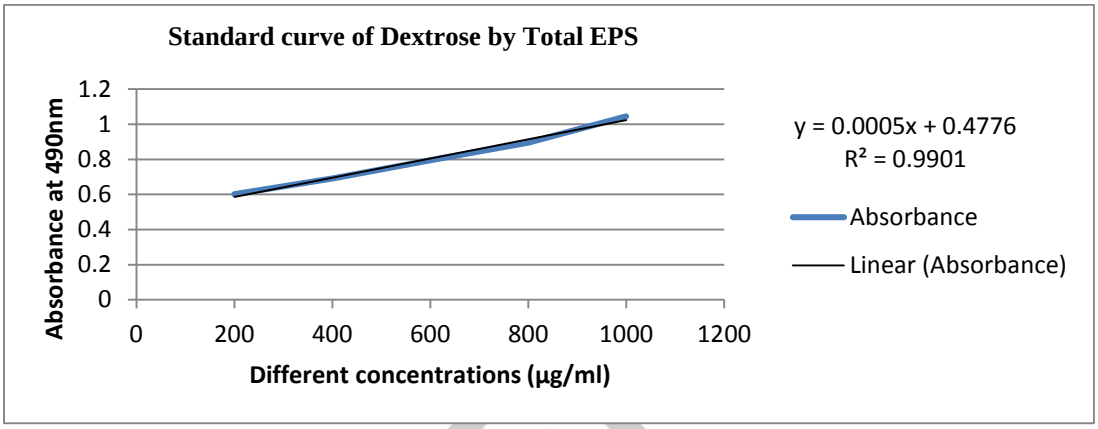


Fig 3- showing standard curve of dextrose by total exopolysaccharide substance

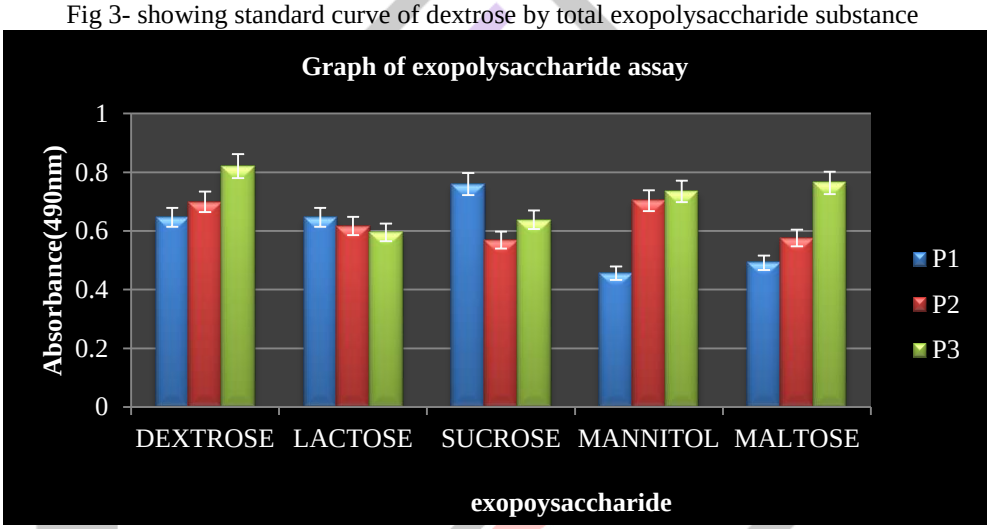


Fig .4- showing the different amounts of exopolysaccharide produced by different bacterial strains

TABLE 6: cell surface hydrophobicity assay

| Bacterial ID | Percentage of cell hydrophobicity |
|---|---|
| P1 | 0.584 |
| P2 | 0.209 |
| P3 | 0.041 |

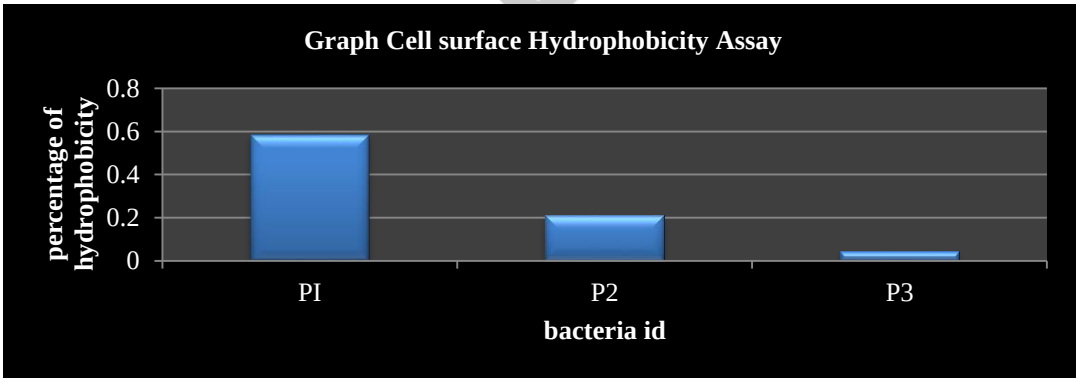


Fig 5- hydrophobicity in percentage

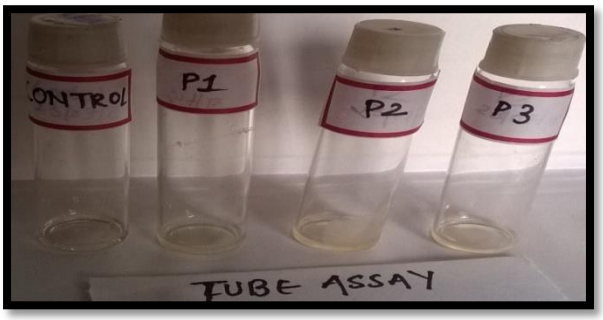


Fig 6 Tube Assay (formation of ring on tube surface showing adherence)

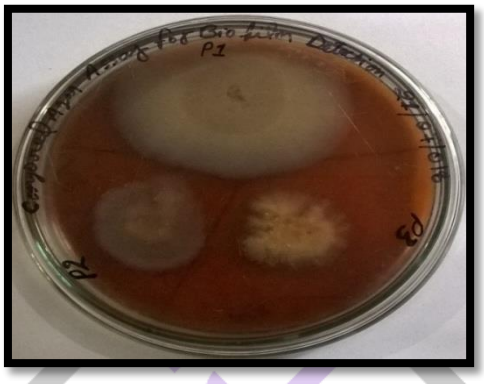


Fig 7- Congo red assay (morphology and growth on brain heart infusion showing increased chances of forming biofilm)

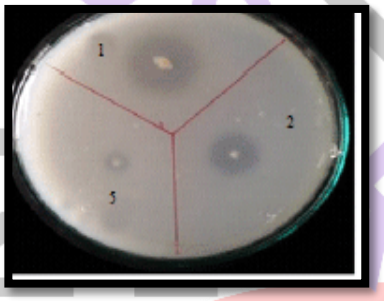

Fig 8- showing the zone of clearance/ phosphate solubilisation by isolated strains.

Fig 9- bioassay of Cicer arientinum in presence of produced inoculums

Table 7–showing bioassay of fermented bacillus sps-

| Bacterial ID | Shoot length | Root length | No, of roots | No. of leaves | No. of branches | Leaf size |
|---|---|---|---|---|---|---|
| Control | 22cm | 25cm | 19 | 9 | 7 | 1.1cm |
| Standard PSB | 27cm | 25.5cm | 20 | 8 | 9 | 1.4cm |
| P1 | 28cm | 18cm | 25 | 13 | 17 | 1.3cm |
| P2 | 15.5cm | 9.5cm | 12 | 10 | 13 | 0.5cm |
| P3 | 15.5cm | 8cm | 7 | 10 | 10 | 0.5cm |

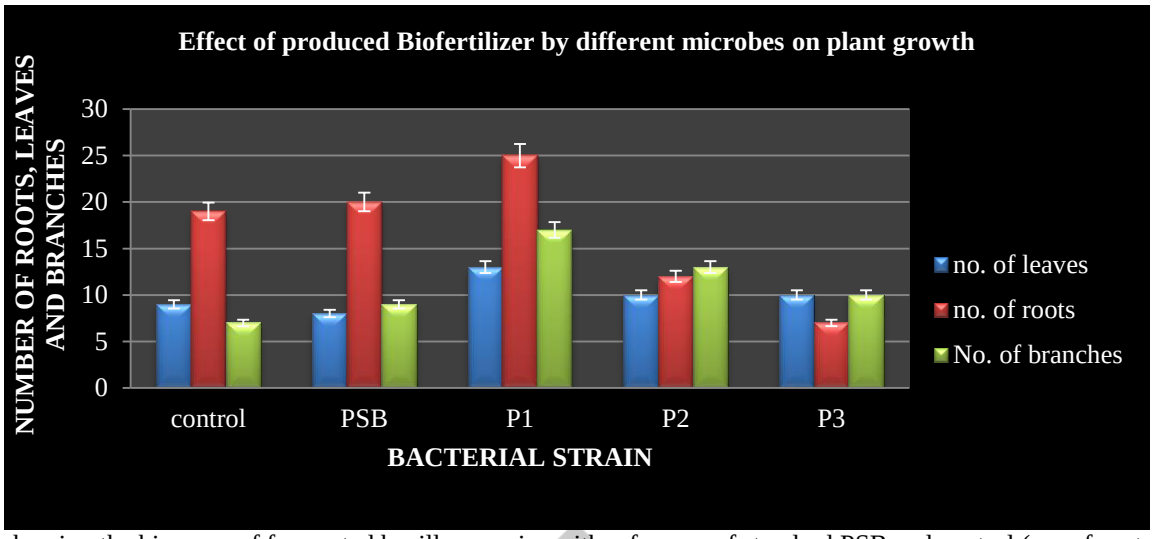


Fig 10-showing the bioassay of fermented bacillus species with reference of standard PSB and control (no. of roots, no. of branches, and no. of leaves).

## IV. DISCUSSION

Table 1/Fig 1- showing the results of gram staining of bacterial isolates P1 P2 and P3.. Table 2- showing the results of various biochemical tests performed to study the effects of various factors on growth of bacterial isolates. Table 3- showing the results obtained using PIBWIN software. For detecting the biofilm forming capacity of bacterial isolates they were grown in LB broth medium and were analyzed using spectrophotometric methods. Bacterial strains were then grown in medium containing different carbon sources that may differ from the sources available in the plant for examining the effect on biofilm formation Table 4/ fig 2- showing the effect of different carbon sources on biofilm formation/ growth of bacterial isolates. P1 stain has higher growth in presence of sucrose, P2 and P3 with maltose and lactose respectively. Table 5/ fig 4- exopolysaccharide assay performed for determining the amount of exopolysaccharide produced in presence of different carbon and nitrogen sources. Showing P1 produces highest exopolysaccharide with sucrose, P1 with Mannitol and dextrose whereas P3 with dextrose on comparing with the standard dextrose curve (fig3). Table 6/fig 5- cell surface hydrophobicity assay the values of hydrophobicity are higher when grown in liquid medium results show that P1 is more hydrophobic than P2 and P3 i.e. will form more denser biofilm. Fig 6 - P2 forms a ring onto tube after decanting liquid medium. Fig 8- showing phosphate solubilisation by 3 bacterial isolates in pikosvsky medium indicating P1 has the maximum phosphate solubilisation on comparing with P2 and P3. TABLE 7/ Fig 9 & 8- Bioassay of *Cicer arientinum* clearly show that P1 had the high phosphate solubilisation and can promote plant growth as all 5 sown seeds in the pot grew well with almost equal root and shoot length whereas in all other including market PSB and control pot only 2 of 5 seeds were showing growth.

From the above work it can be concluded that P1 bacterial isolate can be used as biofertilizer and also for further study.

## V. CONCLUSION

The present work or the whole study concluded that through the tremendous use of various agrochemicals basically chemical fertilizers have destroyed the soil fertility and has become a major threat to animal and human health as it is been known to affect the beneficial soil rhizoflora. The whole study was done to take out this trend of using inorganic fertilizers and bring out a new revolution towards sustainable agriculture. The whole experimentation was based on the production of liquid biofertilizers (PSB) by using phosphate solubilising bacteria which is more effective for increasing fertility of soil and for growth of the plant. By using phosphate solubilising biofertilizers would be a new technique to solubilize the insoluble phosphate aggregated in the soil through the use of various organophosphatic fertilizers

As we studied that the microbes present in the soil like bacillus, paniebacillus, pseudomonas can solubilize phosphate which is good for the growth of plants and so we need to enhance the microbial population of these bacteria into the soil to make the soil more fertile to make this possible we studied the biofilm formation capacity of various microbes isolated from the soil rhizoflora. So that the crop yield can be increased

Three samples Rhizosphere of different soil were studied. Three different microbes were isolated P1, P2 and P3 were analyzed by using various physiochemical parameters as well as their microbial analysis has also been done. These microbes had different effects on the plant growth.

## REFERENCES


- ALY H. EL-NAGGAR * "EFFECT OF BIOFERsTILIZER, ORGANIC COMPOST AND MINERAL FERTILIZERS ON THE GROWTH, FLOWERING AND BULBS PRODUCTION OF NARCISSUS TAZETTA" L. FLORICULTURE, ORNAMENTAL HORTICULTURE AND LANDSCAPE GARDENING DEPT., FACULTY OF AGRIC., (ELSHATBY), ALEXANDRIA UNIV. EGYPT.
- Arguelles-Arias A[1], Ongena M, Halimi B, Lara Y, Brans A, Joris B, Fickers P. **Bacillus amyloliquefaciens GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens.** Microb Cell Fact. 2009 Nov 26;8:63. doi: 10.1186/1475-2859-8-63.
- Baron L. "**Beyond the Green Revolution: singin' the population blues."** 1992 Sep;24(4):1, 4. ZPG Report.

- Basheer J[1], Ravi A[1], Mathew J[1], Krishnankutty RE[2] "**Assessment of Plant-Probiotic Performance of Novel Endophytic Bacillus sp. in Talc-Based Formulation."** 2018 Jan 25. doi: 10.1007/s12602-018-9386-y Probiotics Antimicrob Proteins.
- Bhardwaj† , Mohammad Wahid Ansari†, Ranjan Kumar Sahoo and Narendra Tuteja* "**Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity"** Microbial Cell Factories 2014, 13:66 http://www.microbialcellfactories.com/content/13/1/66
- E. R. Tarakhovskaya, Yu. I. Maslov, M. F. Shishova, **"Phytohormones in algae"** April 2007, Volume 54, Issue 2, pp 163–170| Russian Journal of Plant Physiology.
- Firoz Ahmad Ansari*, Iqbal Ahmad* "**Biofilm Development, Plant Growth Promoting Traits and Rhizosphere Colonization by *Pseudomonas entomophila* FAP1: A Promising PGPR" DOI:** 10.4236/aim.2018.83016 Biofilm Research Lab., Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India.
- Gupta R.R., Singal R., Shanker A., Kuhad R.C., Saxena R.K. (1994) **A modified plate assay for secreening phosphate solubilizing microorganisms,** J. Gen. Appl. Microbiol. 40, 255–260.
- Hafsa Cherif-Silini*, Allaoua Silini, Mostefa Ghoul, Bilal Yahiaoui and Fouzia Arif "**Solubilization of phosphate by the Bacillus under salt stress and in the presence of osmoprotectant compounds"** Vol. 7(37), pp. 4562-4571, 13 September, 2013 DOI: 10.5897/AJMR2013.5696 ISSN 1996-0808 ©2013 Academic Journals http://www.academicjournals.org/AJMR African Journal of Microbiology Research
- He MX[1], Gao Y, Hu ZX, Xu Y, Long R, Sun QY. "Screening, identification, and phosphate-solubilizing capability of phosphate-solubilizingbacterial strain B25." 2012 Jan;23(1):235-9. Ying Yong Sheng Tai Xue Bao.
- HERRMANN L[1], LESUEUR D. "CHALLENGES OF FORMULATION AND QUALITY OF BIOFERTILIZERS FOR SUCCESSFUL INOCULATION." 2013 OCT;97(20):8859-73. DOI: 10.1007/S00253-013-5228-8 EPUB 2013 SEP 15.
- Hilda Rodríguez *, Reynaldo Fraga "**Phosphate solubilizing bacteria and their role in plant growth promotion**" Department of Microbiology, Cuban Research Institute on Sugarcane By-Products (ICIDCA), P.O. Box 4026, CP 11 000, Havana, Cuba https://www.google.co.in/url?sa=i&source=images&cd=&cad=rja&uact=8&ved=2ahUKEwiwz5G9wd3cAhUlTI8KHVBtAGoQjRx6BAgBEAU&url=https%3A%2F%2Fsocratic.org%2Fquestions%2Fwhere-is-biofilm-found&psig=AOvVaw206rZSSfK1nZHj-VvjfQd7&ust=1533819493763012 (biofilm development)
- Ismail AR[1], El-Henawy SB[1], Younis SA[1], Betiha MA[1], El-Gendy NS[1], Azab MS[2], Sedky NM[3]. **"Statistical enhancement of lipase extracellular production by Bacillus stratosphericus PSP a batch submerged fermentation process."** J Appl Microbiol. 2018 Jun 16. doi: 10.1111/jam.14023. [Epub ahead of print].
- Itelima JU1, PhD, Bang WJ1, PhD, Sila MD1, Onyimba IA2, OJ3 PhD, Egbere, PhD(psb) **Biofertilizer - A key player in enhancing soil fertility and crop productivity.**
- JAMIL SHAFI , HUI TIAN AND MINGSHAN JI "BACILLUS SPECIES AS VERSATILE WEAPONS FOR PLANT PATHOGENS" ARTICLE HISTORY RECEIVED 29 APRIL 2016 ACCEPTED 23 JANUARY 2017.
- Khosro M, Yousef S. **Bacterial bio-fertilizers for sustainable crop production**: A review APRN Journal of Agricultural and Biological Science. 2012;7(5):237-308.
- LICHTENBERG E[1], ZIMMERMAN R. "ADVERSE HEALTH EXPERIENCES, ENVIRONMENTAL ATTITUDES, AND PESTICIDE USAGE BEHAVIOR OF FARM OPERATORS." 1999 APR; 19(2):283-94. RISK ANAL.
- Lin W[1], Ding JJ[1], Li YZ[1,2], Xu CY[1], Li QZ[1], Zheng Q[1], Zhuang S[1]. **"[Effects of organic and inorganic fertilizers on emission and sources of $N_2O$ in vegetable soils.]"** Ying Yong Sheng Tai Xue Bao. 2018 May;29(5):1470-1478. doi: 10.13287/j.1001-9332.201805.029.
- Liu P[1,2], Chen Z[1,2], Yang L[1,2], Li Q[1,2], He N[3,4] . **"Increasing the bioflocculant production and identifying the effect of over expressing epsB on the synthesis of polysaccharide and γ-PGA in Bacillus licheniformis."** 2017 Sep 26;16(1):163. doi: 10.1186/s12934-017-0775-9. Microb Cell Fact.
- MAHANTY T[1], BHATTACHARJEE S[1], GOSWAMI M[2], BHATTACHARYYA P[2], DAS B[3], GHOSH A[4], TRIBEDI P[5]. "BIOFERTILIZERS: A POTENTIAL APPROACH FOR SUSTAINABLE AGRICULTURE DEVELOPMENT." ENVIRON SCI POLLUT RES INT. 2017 FEB;24(4):3315-3335. DOI: 10.1007/S11356-016-8104-0. EPUB 2016 NOV 26.
- MAHROUS, N. M.1;A. A. RAGAB(2);H. H. ABOTALEB(3);M. H. TAHA(1)AND MARIAM.S.EL-METWALLY(2) "EFFECT OF INORGANIC, ORGANIC AND BIO FERTILIZERS ON YIELD AND YIELD COMPONENTS OF SUNFLOWER" 1-DEPARTMENT OF AGRONOMY, FACULTY OF AGRICULTURE, CAIRO UNIVERSITY, GIZA, EGYPT 2-CENTRAL LAB. OF ORGANIC AGRICULTURE, AGRICULTURAL RESEARCH CENTER, GIZA, EGYPT 3-SOIL ,WATER AND ENVIRONMENT INSTITUTE , AGRICULTURAL RESEARCH CENTER , GIZA, EGYPT
- Manjunath M[1], Kumar U[2], Yadava RB[3], Rai AB[3], Singh B **"Influence of organic and inorganic sources of nutrients on the functional diversity of microbial communities in the vegetable cropping system of the Indo-Gangetic plains".** C R Biol. 2018 Jul - Aug;341(6):349-357. doi: 10.1016/j.crvi.2018.05.002. Epub 2018 May 31.
- Mohammad Saghir KHAN*, Almas ZAIDI, Parvaze A. WANI **"Role of phosphate-solubilizing microorganisms in sustainable agriculture – A review"** (Accepted 18 May 2006) Agronomy for Sustainable Development, Springer Verlag/EDP Sciences/INRA, 2007, 27 (1), pp.29-43. <hal-00886352
- Mukhtar S[1], Shahid I[1], Mehnaz S[1], Malik KA[2]. **"Assessment of two carrier materials for phosphate solubilizing biofertilizers and their effect on growth of wheat (Triticum aestivum L.)."** Microbiol Res. 2017 Dec;205:107-117. doi: 10.1016/j.micres.2017.08.011 Epub 2017 Aug 30.

- N.UMA MAHESWARI*, T.ELAKKIYA "EFFECT OF LIQUID BIOFERTILIZERS ON GROWTH AND YIELD OF VIGNA MUNGO L." ACCEPTED ON: 17-09-2014; FINALIZED ON: 30-11-2014. INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES REVIEW AND RESEARCH AVAILABLE ONLINE AT WWW.GLOBALRESEARCHONLINE.NET ©
- Narula N., Kumar V., Behl R.K., Duebel A.A., Gransee A., Merbach W. (2000) **Effect of P solubilizing Azotobacter chroococcum on N, P, K uptake in P responsive wheat genotypes grown under green house conditions,** J. Plant Nutr. Soil Sci. 163, 393–398.
- Nautiyal C.S. (1999) **An efficient microbiological growth medium for screening of phosphate solubilizing microorganisms**, FEMS Microbiol. Lett. 170, 265–270.
- NAYANA.SHARMA "EFFECTS OF CHEMICAL FERTILIZERS AND PESTICIDES ON HUMAN HEALTH AND ENVIROMENT" (ORCID ID: 0000-0003-0298-7470) PAPER NO.: 628 RECEIVED: 07-04-2017 ACCEPTED: 10-11-2017 INTERNATIONAL JOURNAL OF AGRICULTURE ENVIROMENT AND BIOTECHNOLOGY.
- Pomorska K[1]. **"General evaluation of risk associated with the use of pesticides and other chemical substances on animal breeding and plant production farms."** 1999;5(3):449-57Int J Occup Saf Ergon. .
- Raja N. **Bipesticides and biofertilizers: ecofriendly sources for sustainable agriculture.** Journal of Biofertilizer Biopesticide. 2013;(3):112-5.
- Rakesh Kumar1, Narendra Kumawat2*and Yogesh Kumar Sahu3 1Scientist (Agronomy**), "Role of Biofertilizers in Agriculture"** ICAR Research Complex for NEH Region, Nagaland Centre Jharnapani -797 106 2AICRP Maize, Zonal Agricultural Research Station, Jhabua 457 661, MP 3Krishi Vigyan Kendra, Jhabua 457 661, MP, India *Email of corresponding author: kumawatandy@gmail.com
- SHAHZAD R[1], WAQAS M[2], KHAN AL[3], ASAF S[1], KHAN MA[1], KANG SM[1], YUN BW[4], LEE IJ "SEED-BORNE ENDOPHYTIC BACILLUS AMYLOLIQUEFACIENS RWL-1 PRODUCES GIBBERELLINS AND REGULATES ENDOGENOUS PHYTOHORMONES OF ORYZA SATIVA." PLANT PHYSIOL BIOCHEM. 2016 SEP;106:236-43. DOI: 10.1016/J.PLAPHY.2016.05.006 EPUB 2016 MAY 6.
- SI HO HANA, JI YOUNG ANB, JAEHONG HWANGC, SE BIN KIMA AND BYUNG BAE PARKA* "THE EFFECTS OF ORGANIC MANURE AND CHEMICAL FERTILIZER ON THE GROWTH AND NUTRIENT CONCENTRATIONS OF YELLOW POPLAR (LIRIODENDRON TULIPIFERA LIN.) IN A NURSERY SYSTEM" 14 DECEMBER 2015. J.AGRIC.&ENV.SCI.ALEX.UNIV.,EGYPT VOL.9 (1)2010
- SIMON S[1], PETRÁŠEK J. "WHY PLANTS NEED MORE THAN ONE TYPE OF AUXIN." PLANT SCI. 2011 MAR;180(3):454-60. DOI: 10.1016/J.PLANTSCI.2010.12.007. EPUB 2010 DEC 22.
- Tahir M[1], Khalid U[2], Ijaz M[3], Shah GM[1], Naeem MA[1], Shahid M[4], Mahmood K[5], Ahmad N[1], Kareem F[2]. **"Combined application of bio-organic phosphate and phosphorus solubilizing bacteria (Bacillus strain MWT 14) improve the performance of bread wheat with low fertilizer input under an arid climate."** Braz J Microbiol. 2018 Apr 24. pii: S1517-8382(17)30400-8. doi: 10.1016/j.bjm.2017.11.005. [Epub ahead of print]
- Triveni S[1], Prasanna R, Saxena AK**. "Optimization of conditions for in vitro development of Trichoderma viride-based biofilms as potential inoculants."** Folia Microbiol (Praha). 2012 Sep;57(5):431-7. doi: 10.1007/s12223-012-0154-1. Epub 2012 May 8.
- Triveni S[1], Prasanna R, Saxena AK. **"Optimization of conditions for in vitro development of Trichoderma viride-based biofilms as potential inoculants."** Folia Microbiol (Praha). 2012 Sep;57(5):431-7. doi: 10.1007/s12223-012-0154- Epub 2012 May 8.
- Vashkulat NP. **"[The effect of the territorial loads of pesticides and mineral and organic fertilizers on morbidity in children up to 14 years old in Ukraine]."** 1998 Oct-Nov;(7):167-70 Lik Sprava. .
- Vessey JK**. Plant growth promoting Rhizobacteria as bio-fertilizers.** Journal of Plant and Soil. 2003;225(43):571-86.
- Weisenburger DD[1]. **"Human health effects of agrichemical use."** 1993 Jun;24(6):571-6. Hum Pathol.
- YANG RONG1, SU YONG-ZHONG1, WANG TAO2, YANG QIN3 "EFFECT OF CHEMICAL AND ORGANIC FERTILIZATION ON SOIL CARBON AND NITROGEN ACCUMULATION IN A NEWLY CULTIVATED FARMLAND"
- Zaidi A[1], Khan MS, Ahemad M, Oves M. **"Plant growth promotion by phosphate solubilizing bacteria"** doi: 10.1556/AMicr.56.2009.3.6. Acta Microbiol Immunol Hung. 2009 Sep; 56(3):263-84.
- Zhang T[1], Xu H[1], Ru SH[2], Su DC[1]**. "[Distribution of Phosphorus in Soil Profiles after Continuous Application of Different Fertilizers]."** 2017 Dec 8;38(12):5247-5255. doi: 10.13227/j.hjkx.201705027Huan Jing Ke Xue. .
- ZHENG BX[1,2,3,4], IBRAHIM M[1,2], ZHANG DP[5], BI QF[1,6], LI HZ[1,2], ZHOU GW[7], DING K[1], PEÑUELAS J[3,4], ZHU YG[1,7], YANG XR[8]. "IDENTIFICATION AND CHARACTERIZATION OF INORGANIC-PHOSPHATE-SOLUBILIZING BACTERIA FROM AGRICULTURAL FIELDS WITH A RAPID ISOLATION METHOD." 2018 MAR 27;8(1):47 AMB EXPRESS