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Development of Effective Microbial Consortia for Better Crop Growth

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The soils present near coal mines have the properties of high concentration of metallic minerals, elevated pH and shortage of essential nutrients for organism growth. But being rich in minerals they could provide a good energy source for plants. Soil near Coal mines of Neyveli was taken and subject to treatment with effective microorganisms that have fertilizing, antibiotic, iron solubilising, sulphur solubilising and photosynthesis helping activities. The microbes helpful in plant photosynthesis were isolated after studying their effect on chlorophyll content and photosynthesis rate by increasing their load on leaves after Humic acid spray. Microorganisms, mainly bacteria, with the above activities were isolated, characterised and cultured. The mines soil and a normal garden soil were treated with these microbes and their effects on plant growth (*Abelmoscus esculentus* and *Trigonella foenum*) was analysed by measuring the root length and shoot length of the plants. Simultaneously the soil parameters like pH and EC and chemical factors like NPK content were measured after the treatment. The mining soil showed significant positive result on plant growth and also an improvement in the physical and chemical properties after the microbial treatment.

Key words: Photosynthesis, Microbial consortia, Humic acid, *Abelmoscus esculentus, Trigonella foenum,* and NPK.

Mine spoil heaps are composed of coarse rocks due to the deep coal mining operations and associated coal processing. These spoils are not suitable for both plant and microbial growth because of low organic matter content, unfavourable pH, and drought arising from coarse texture or oxygen deficiency due to compaction. The other limiting factors for re-vegetation of mine spoil may be salinity, actidity, poor water holding capacity, inadequate supply of plant nutrients and accelerated rate of erosion [1]. But these soils also contain high concentration of minerals and salts. Being rich in minerals and salts they might provide a very good energy source for plants.

Plants require nitrogen, phosphorous and potassium as essential elements for their growth and development. Nowadays chemical fertilizers are being used to increase the NPK content of the soil and hence to improve the plant growth and development. Fertilizers contain a large amount of inorganic salts which are acidic in nature. These salts when applied to the soil change the pH of the soil. Fertilizers also change the nature of the plant product. They reduce the flavour, taste or colour of the product. Some inorganic compounds which are not metabolized properly may get inserted into

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the plant product. The unused fertilizers that are left out in soil have an undesirable effect on it making it unusable for agriculture due to prolonged usage of fertilizers. This effect may lead to reduction in the percentage of cultivatable land by a large extent. Reduction in cultivatable lands will reduce the production of agricultural products.

All these ill effects are the aftermath of the usage of chemical fertilizers. In order to overcome the hazards caused by these fertilisers and to improve plant growth and production in an efficient and safe manner in the mines spoil measures have been taken through biological methods. Use of Eco-friendly biological method is an important criterion towards sustainable agriculture. One such method is the production of effective microorganisms by which the mines spoil can be treated through microorganisms and making it suitable for cultivation.

Effective microbes are the collection of microorganisms that have fertilizing and saprophytic activity which when applied to soil improves its quality and plant growth. It may also prevent the plant from insects and pathogens.

Development of effective microbial consortia for the treatment of mines spoil requires information about the microbes that can adapt themselves to the conditions of mines spoil and grow. Strains that have fertilizing activity can be used to develop the effective and efficient microbes. Most organics including animal manures and composts have populations of micro-organisms. Many of these are beneficial upon introduction to the soil; however they are soon overwhelmed by the existing soil microorganisms. Thus, the beneficial effects of micro-organisms introduced with the application of composts are often short lived. On application effective microbial cultures are also subject to the same fate when applied to the soil environment. But the advantage of effective microbial cultures is that beneficial microorganisms are in much greater numbers, and in optimally-balanced populations when introduced, so remain dominant in the soil for a much longer time.

The effectiveness of these cultures on soil can be increased by 3 to 4 applications of it at a regular interval of 8 – 10 days during the first 3- 4 weeks of plant growth. The organisms that can be included in this are

1. Nitrogen fixing bacteria
2. Phosphate solubilising bacteria
3. Bio fertilizer strains
4. Photosynthetic bacteria
5. VA mycorrhiza
6. Food grade bacteria
7. Iron oxidising bacteria
8. Sulphur oxidising bacteria
9. Other soil bacteria like *Streptomyces, Pseudomonas, Corynebacterium,* etc.,
10. Phylloplane living microorganisms
11. Rhizospere microorganisms.

MATERIAL AND METHODS

Study area

The mines spoil from coal (Lignite) mines of Neyveli, India was taken for the study. The pH of the soil was found to be 6.45 and it had a high content of mineral salts which were inhibitory for the growth of microorganisms as well as plants. Microorganisms which have fertilising activity and saprophytic activity were isolated and characterised in the following manner using selective and formulated media.

1. *Streptomyces* sp., from soil sample grown in Yeast Mannitol Glucose Agar at 26°C for 12 hours . Filamentous colonies were isolated and subcultured.
2. *Corynebacterium* sp., from soil sample grown in Corynebacterium Agar at 28°C for 12 hours. Methylene blue test was used to confirm the growth of *Corynebacterium* sp.
3. *Azotobacter* sp., from Maize root soil in Azotobacter Agar at 30°C for 4 days.
4. *Rhizobium leguminosarum* from root nodules of *Arachis hypogeal* in YEMA medium at 28°C for 3-7 days.
5. Phosphobacteria from various soil samples using Pikovskaya's medium at 28°C for 4-5 days.
6. *Azospirullum* sp., from Maize root in Azospirillum Agar at 28±2°C for 2-3 days.
7. Iron oxidising bacteria from Bottom slag of coal combustion and soil in Iron oxidising medium at 32±2°C for 7 days.
8. Sulphur oxidising bacteria from soil in Sulphur medium at 34±2°C for 10 days.
9. *Acetobacter diazotrophicus* from sugarcane in Acetobacter Agar at 28°C for

24 hours.

10. *Lactobacillus* sp., from curd in King's B medium at 28°C for 24 hours.
11. Plant growth promoting beneficial bacteria from soil in Nutrient agar at 28°C for 24 hours.

Analysis of the effect of phyllosphere microbes on plant growth

Six test plants were selected from the fields of CARD, Neyveli namely Mango, Cashew, Jatropha, Jack, Cluster bean and Coconut. Chlorophyll content in their leaves using Chlorophyll meter and photosynthesis rate using TPS-2 portable system were measured before and after 0.1% Humic acid spray. Humic acid was used to increase the phyllosphere microbial load which was evident from previous experimentations.

Based on the results of the effect of Phyllosphere microbes on plant growth beneficial ones from them were isolated using Leaf imprinting technique. They were grown on Nutrient agar at 28°C for 24-48 hours. These microbes were named based on their colony morphology and plant from which it was isolated.

After the growth of all microbes on solid media, mass culturing was done on liquid media (Nutrient broth) and the studies were carried out.

Preparation of Effective microbial consortia

Six different microbial mixtures were made using the isolated microbes.

Batch – 1

Azospirillum lipoferum, Azotobacter chroococcum, Rhizobium leguminosarum and *Pseudomonas sp.*

Batch – 2

Iron oxidising bacteria, *Thiobacillus thiooxidans* and two other beneficial soil bacteria cultured separately.

Batch – 3

Three Phyllosphere bacteria cultured separately.

Batch – 4

Phosphobacteria isolated from five different soil samples and cultured separately.

Batch – 5

Acetobacter diazotrophicus, Corynebacterium sp., Streptomyces griseus, Bacillus megaterium and *Lactobacillus acidophilus*.

Batch – 6 (Effective Microbial Consortia)

Mix 10 ml of all the bacterial culture

Incubation of all the six batches of cultures was done at room temperature for 1 hour under aseptic conditions. Co – existence of the microbes were found positive after the isolation test carried out after the treatment on mine spoil and garden soil.

Treatments of mine spoil and plant growth on it

2 parts of Mine spoil was mixed with 1 part of sand (carrier) and 1 part of compost and watered. Similarly normal garden soil was also prepared and used as control. These were packed into polythene bags (2 Kg per bag). After one day well dried seeds of *Trigonella foenum* (Fenugreek) and *Abelmoscus esculentus* (lady's finger) were sown to the soils and watered. After an hour the effective microbial cultures were poured to the soils (separate bag for each Batch). After 10 days the same microbial treatment was done to the plants and soil. Then the plant parameters like root and shoot length and soil parameters like pH, NPK content and electrical conductivity were measured.

Estimation of Available Phosphorous in Soil

Weigh 5g of soil and transfer it to 250ml conical flask. Add 50 ml Bray's No: 1 extractant and shake in a reciprocatory mechanical shaker at 136 rpm for 30 minutes. Filter through Whatmann No: 42 dry filter paper. Collect the filtrate in clean dry conical flask. 5ml of filtrate was pipetted out into test tubes and the volume was diluted using distilled water and then 4ml of reagent B was added and the volume was made to 25ml. then incubated for 10 minutes for colour development and the blue colour developed was read at 660nm calorimetrically and adjusted the meter to 100% transmittance with the blank. From the standard curve for P, find out the concentration of P at ppm in the solution against percent transmittance in above steps.

Determination of Available Nitrogen in Soil

20g of soil was placed in distillation flask. Then 20 ml of water was added and 100 ml of 0.32% potassium permanganate solution was added. 25 ml of N/50 H_2SO_4 was pipette out in a conical flask. Then 2 to 3 drops of methyl red indicator was added and the end of the delivery tube was dipped into it. 100 ml of 2.5% NaOH solution was poured into the flask and corked immediately. Ammonia gas was

distilled from the distillation flask and collected in H_2SO_4 solution. The distillation was continued till the evolution of ammonia ceases completely (test by bringing a moist red litmus paper near the outlet of the condenser, which turns blue as long as ammonia is being evolved). Excess of H_2SO_4 was titrated against N/50 NaOH and the volume of NaOH used was noted. The end point is reached when the colour changes from pink to yellow.

Determination of Available Potassium in Soil

5g of soil was weighed from the given samples and named as C_1, C_2, C_3, T_1, T_2 andT_3 and taken in 250ml conical flask. 25ml of ammonium acetate was added to the soil sample and kept in shaker for 30 minutes at 136 rpm. Then the samples are filtered using Whatmann filter paper. The filtrate contents are fed into the automyzer of flame photometer. The readings are located on the standard curve which gives potassium concentration in the extract.

RESULT AND DISCUSSION

The biofertilizer bacterial strains, plant growth promoting beneficial strains, Sulphur and Iron oxidising bacteria were isolated from the respective sources and characterised by Gram staining, Motility determination and by various biochemical tests like MR-VP test, Catalase test, Casein hydrolysis, Starch hydrolysis, Citrate utility test, Urease test and TSI test. Humic acid being a very good plant growth promoter increased total bacterial load on the phyllosphere of the six plants (Fig.1). This increased load of microorganisms had

Table 1. Chlorophyll content in different plant species before and after Humic acid treatment.

Plant	CCI - before humic acid spray	CCI - after humic acid spray	% Increase in CCI
Mango	46.63	52.96	16.5
Cashew	18.07	22.0	26.3
Jatropha	15.5	17.53	18.0
Cluster Bean	35.4	62.0	75.1
Coconut	12.9	15.2	17.8
Jack	62.7	91.67	57.9

CCI – Chlorophyll Content Index

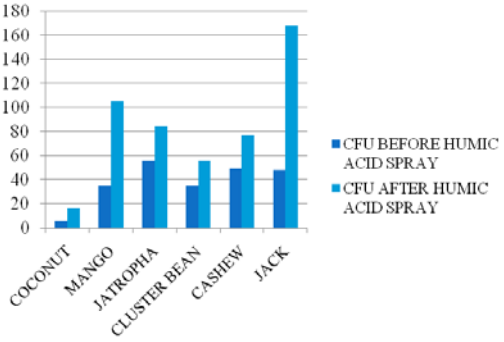

Fig.1. Comparison of Phyllosphere bacterial load before and after Humic acid spray

Table 2. Photosynthesis in different plant species before Humic acid treatment

Plant	CO_2 REF	EVA	GS	Leaf Temp	C int.
Mango	362.4	0.40	32	29.0	438
Cashew	359.0	0.63	49	29.8	395
Jatropha	360.2	0.30	34	28.4	316
Cluster Bean	361.1	1.19	156	28.7	377
Coconut	359.2	0.83	101	28.3	384
Jack	359.2	0.59	43	29.3	408

Where,

			Unit
CO_2 REF	-	Carbon-di-oxide reference	ppm
EVA	-	Evapotranspiration	mol /m^2s
GS	-	Stomatal Conductance	mmol/m^2/s
LEAF TEMP	-	Leaf Temperature	℃
C Int.	-	Internal CO_2 Concentratio0n	ppm

considerable effect on chlorophyll content of the plants and photosynthesis of some plants (Table 1, 2 & 3). It was also seen that there was no pathogenicity caused by the microbes on the phyllosphere, thus proving that they might help the plant in their respiration and photosynthesis and also by inhibiting the growth of pathogens[5,].

Table 3. Photosynthesis in different plant species after Humic acid treatment.

Plant	CO_2 REF	EVA	GS	Leaf Temp	C int.	% INC. IN CO_2 intake
Mango	363.4	0.40	45	29.1	407	1.16
Cashew	368.2	0.39	46	28.6	427	1.60
Jatropha	366.8	0.41	10	28.6	210	1.83
Cluster Bean	364.4	0.39	29	30.4	424	1.44
Coconut	358.3	0.49	54	28.8	403	NIL
Jack	362.9	0.42	31	31.1	471	0.50

Where,			Unit
CO_2 REF	-	Carbon-di-oxide reference	ppm
EVA	-	Evapotranspiration	mol /m^2s
GS	-	Stomatal Conductance	mmol/m^2/s
LEAF TEMP	-	Leaf Temperature	°C
C Int.	-	Internal CO_2 Concentratio0n	ppm

Table 4. Root and Shoot length of plants in Garden Soil

Plant	Fenugreek		Lady's finger	
Treatment	Root length in cm	Shoot length in cm	Root length in cm	Shoot length in cm
Control	8.4	7.0	12.5	10.5
B_1	15.2	9.5	23.5	12.0
B_2	13.0	7.6	8.5	8.5
B_3	17.7	9.4	17.5	13.0
B_4	14.5	11.5	15.5	16.5
B_5	19.0	10.1	17.0	15.5
B_6	20.0	12.5	27.5	15.5

Table 5. Root and Shoot length of plants in Mine Spoil

Plant	Fenugreek		Lady's finger	
Treatment	Root length in cm	Shoot length in cm	Root length in cm	Shoot length in cm
Control	7.9	6.5	12.0	9.0
B_1	14.8	11.3	18.5	12.8
B_2	11.7	7.1	12.1	11.0
B_3	16.5	8.8	17.5	14.5
B_4	15.7	12.4	16.5	14.8
B_5	20.8	12.5	23.2	11.6
B_6	21.2	14.7	28.6	15.2

The effective microbial culture prepared from the isolated microbes showed a significant change on the plants' (*Abelmoscus esculentus* and *Trigonella foenum*) growth in mines spoil. This was proved by the increase in the root and shoot length of the plants (Table 4 & 5). This result coincides with the results of experimentation with the strains of *Azotobacter* and *Azospirillum* on the growth of *Echinochloa frumentacea*[5]. Also the number of leaves grown on the treated soils was more than that of the non-treated ones. No diseased condition was seen on any of the plants which add up to the fact that all the strains isolated were not pathogenic or in a way they became suppressed to produce toxins.

The germination of seeds was faster in mine spoil than garden soil from which shows that reclamation of mine spoil by biological way will improve its quality higher than that of Agricultural soil. Comparing the plants growth parameters like root and shoot length it was found that the maximum root and shoot length of *Abelmoscus esculentus* and *Trigonella foenum* were shown by Batch 6 which contains all the microorganisms. Next to it the maximum root length in both the plants was shown by Batch 5 and maximum shoot length was shown by Batch 4. Batch 2 which had the Iron oxidising and Sulphur oxidising Bacteria along with Rhizosphere bacteria showed the least growth in terms of root and shoot length. Comparing the two plants *A.esculentus* showed better results on treatment.

The p^H of the control in garden soil was found to be 7.1 i.e. the untreated soil, whereas the same was observed to be 7.83 in effective microorganisms treated Batch 6 soil followed by Batch 5 and Batch 4 (Table 6). The electrical conductivity, nitrogen, phosphorous and potassium content in Batch 6 has an remarkable increase when compared to the untreated control soil.

It was seen that the acidic pH of the mine spoil was stabilised by the treatment with Effective Microbes. The pH got increased up to 7.72 with the treatment by Batch 6 culture followed by Batch 1 and Batch 5 (Table 7). There was a considerable increase in all the measured soil parameters in microbes treated Batch 6. Thus effective microorganisms prove to be a better solution for stabilizing the adverse conditions of mine spoil.

Table 6. Garden soil Parameters after the treatment.

Treatment	p^H	EC in milli simonds	N_2 in Kg/Hectare	P in Kg/Hectare	K in Kg/Hectare
Control	7.1	0.252	380.56	68.40	347.2
B_1	7.3	0.358	502.13	69.64	454.6
B_2	6.8	0.396	412.51	68.57	356.12
B_3	7.4	0.314	443.98	69.91	389.3
B_4	7.46	0.328	456.32	71.12	412.6
B_5	7.71	0.373	478.52	70.39	439.7
B_6	7.83	0.417	512.74	71.25	465.92

Table 7. Mine Spoil Parameters after the treatment

Treatment	p^H	EC in milli simonds	N_2 in Kg/Hectare	P in Kg/Hectare	K in Kg/Hectare
Control	6.45	0.286	376.34	68.97	421.12
B_1	7.4	0.324	578.13	74.34	584.34
B_2	6.7	0.415	426.74	70.46	445.6
B_3	7.2	0.348	549.17	71.90	472.9
B_4	7.3	0.319	553.12	78.87	532.14
B_5	7.4	0.427	567.46	76.51	568.74
B_6	7.72	0.524	590.12	79.23	622.72

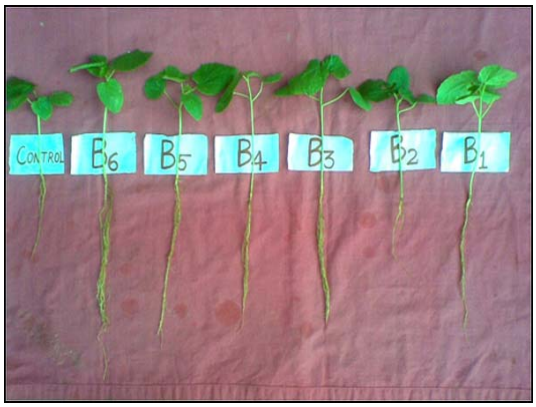

Fig. 2. Plate showing the Root and Shoot growth of Lady's Finger in Garden Soil.

Fig. 3. Plate showing the Root and Shoot growth of Lady's Finger in Mine Spoil.

Fig. 4. Plate showing the Root and Shoot growth of Fenugreek in Garden Soil

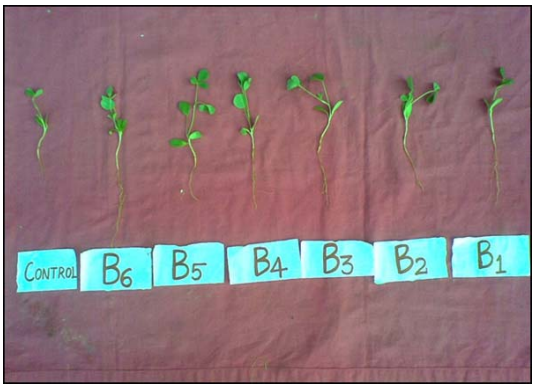

Fig. 5. Plate showing the Root and Shoot growth of Fenugreek in Mine Spoil

To be mentioned specifically the effect of these cultures on the mines spoil was more when compared to the garden soil. The germination of seeds in mines spoil was faster than that in the garden soil. Also the growth of plants in these two soils was differing in a way that the mines spoil showed better results. Even the soil properties like pH and NPK content were improved very much so that it became favourable for plant growth. The significant change between the plant growths in mines spoil and garden soil could be contributed to the fact that the mines spoil had become more fertile than the garden soil when treated with the effective microbial culture. Thus the cultivation of plants on treated mines spoil could be an effective solution for the increasing need for agricultural products and faster production.

CONCLUSION

i. The application of humic acid to the plants showed a significant increase in the number of phyllospere microbes. Also it increased the chlorophyll content of the leaves and thereby the photosynthesis.

ii. Treatment with effective microbial cultures

enhanced the plant growth (*Abelmoscus esculentus* and *Trigonella foenum*) both on mine spoil and garden soil.

iii. The root length and shoot length of the effective microbes treated plants were higher than that of the non-treated ones. Also the germination time was lesser for the seeds sowed on treated mines and garden soil than that sowed on the control soils.

iv. The cultures did not have any pathogenic effect on the plants.

v. Among the two soils that were treated microbial cultures mine spoil showed better results compared to that of garden soil.

vi. The soil properties like pH and NPK content were improved very much so that it became favourable for plant growth. Thus mines spoil proves to be a fertile soil for plant growth when treated with the beneficial microbial cultures.

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REFERENCES

1. Raman Kumar Dutta and Agarwal, M. Effect of tree plantations on the soil characteristics and microbial activity of coal mine spoil land. *Tropical Ecology,* 2002; **43**(2): 315-324.
2. Murcia, F.J., Gomez, M.J, Palop, A, and Garcia, G. Assesment of Mining Soil improvement after construction and demolition wastes amendment additions evaluated by Microbiological analysis. *Global NEST Journal*, 2007; **9**: 224-228.
3. http://en.wikipedia.org/wiki/efficient_microbes
4. Okuda, A and Higa, T. Purification of Waste Water with Effective Microorganisms and its Utilization in Agriculture.
5. Chandrasekar, B.R., Ambrose, G. and Jayabalan, N. Influence of biofertilizers and nitrogen source level on the growth and yield of *Echinochloa frumentacea* (Roxb.) Link. *Journal of Agricultural Technology*, 2005; **1**(2): 223-234.
6. Gadgil, P.D. and Bhide, V.P. nitrogen fixation by *Azotobacter* in association with some associated soil microorganisms. 1959; 60-63.
7. Dong, Z., Canny, M.J., McCully, M.E., Roboredo, M.R., Cabadilla, C.F., Ortega, E. and Rodes, R. A Nitrogen-Fixing Endophyte of Sugarcane stems' – A new role for the Apoplast. *Plant physiol*, 1994; **105**: 1139-1147.
8. Dong, Z., Heydrich, M., Bernard, K. and McCully, M.E. Further Evidence that the N2-Fixing Endophytic Bacterium from the Intercellular Spaces of Sugarcane Stems Is *Acetobacter diazotrophicus*. *Applied and Environmental Microbiology*, 1995; **61**(5): 1843-1846.
9. Naher, U.A., Othman, R, Shamshuddin, Z.H.J, Saud, H.M and Ismail, M.R. Growth Enhancement colonization of rice seedlings by *Rhizobium* and *Corynebacterium* spp. *International Journal of Agriculture & Biology*, 2009; **11**: 586-590.
10. Primavesi, A.M. Effect of *Lactobacillus* inoculants, Organic amendments and Mineral elements on yield of Onion and Field bean.
11. Sharma, K., Dak,G., Agarwal, A., Bhatnagar, M. and Sharma, R.Effect of Phosphate solubilising bacteria on the germination of *Cicer arietinum* seeds and seedling growth. *Journal of Herbal Medicine and Toxicology*, 2007; **1**(1): 61-63.
12. Yazdani, M., Bahmanyar, M.A., Pirdashti, H. and Esmail, M.A. Effect of Phosphate solubilising Microorganisms (PSM) and Plant Growth Promoting Rhizobacteria (PGPR) on yield and Yield components of Corn (*Zea mays L.*). *World Academy of Science, Engineering and Technology*, 2009; 49.
13. Son, T.T.N., Diep, C.N. and Giang, T.T.M. Effect of *Bradyrhizobia* and Phosphate solubilising Bacteria application on Soyabean in Rotational system in the Mekong Delta. *Omirice*, 2006; **14**: 48-57.
14. Anitha, A and Rebeeth, M. *In vitro* Antifungal Activity of *Streptomyces griseus* Against Phytopathogenic Fungi of Tomato Field. *Academic Journal of Plant Sciences*, 2009; **2**(2): 119-123.
15. Akhtar, M.J., Asghar, H.N., Shahzad, K and Arshad, M. Role of Plant Growth Promoting Rhizobacteria Applied in Combination with compost and mineral fertilizers to improve growth and yield of Wheat (*Triticum aestivum L.*). *Pakistan Journal of Botany*, 2009; **41**(1): 381-390.
16. Kloepper, J.W., and Schroth, M.N. Plant

Growth Promoting Rhizobacteria and plant growth under gnotobiotic conditions. *Phytopathology,* 1981; **71**: 642-644.

17. Gholami, A Shahsavani, S and Nezarat, S. The Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Germination, Seedling Growth and Yield of Maize. *World Academy of Science, Engineering and Technology,* 2009; 49.
18. Johnson, D.B., Ghauri, M.A. and Said, M.F. Isolation and Characterisation of an Acidophilic, Heterotrophic Bacterium capable of Oxidizing Ferrous Iron. *Applied and Environmental Microbiology,* 1992; **58**(5); 1423-1428.
19. Mishra, A.K., and Roy, P. A note on the Growth of *Thiobacillus ferrooxidans* on Solid medium. *Journal of Applied Bacteriology,* 1919; **47**: 289-292.
20. Biswas, J. C, Ladha, J. K and Dazzo, F. B. Rhizobia Inoculation Improves Nutrient Uptake and Growth of Lowland Rice. *Soil Sci. Soc. Am. J,* 2000; **64**: 1644-1650.
21. Antoun, H., Beauchamp, C. J., Goussard, N., Chabot, R. and Lalande, R. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting Rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.) *plant and soil,* 1998; **204**: 57-67.
22. http://en.wikipedia.org/wiki/humic_acid
23. www.agrimont.de/gfx/pdfen/7 Effects_of_humic_acids.pdf