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Comparitive Study on Quality Parameters and Viability of Carrier and Liquid Biofertilizers

K. Bhavya*, R. Subhash Reddy and S. Triveni

Department of Agricultural Microbiology and Bioenergy, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderbad-30, India

*Corresponding Author E-mail: bhavyaraj55@gmail.com

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ABSTRACT

In the present study different carrier and liquid biofertilizers are collected from different production centers and research stations for Monitoring quality parameters which includes moisture percentage, pH, Consistency, Level of contaminants and Microbial population of beneficial bacteria in liquid and carrier based biofertilizers at monthly intervals. In carrier and liquid Biofertilizers gradual change in pH and consistency was observed. In Carrier based Biofertilizers quality was too low and decrease in count was more when compared to Liquid Biofertilizers.

Key words: *Carrier based Biofertilizers, Liquid based Biofertilizers, PSB, Rhizobium, Shelf life, Quality control.*

INTRODUCTION

In general, biofertilizers are living microorganisms, unlike chemical fertilizers; they themselves are not the source of nutrients but canhelp the plants in accessing the nutrient available in its surrounding environment. The microorganisms commonly used as biofertilizers may be nitrogen fixing soil bacteria nitrogen-fixing bacteria, phosphate solubilizing Bacteria. When applied to the field, the activities (nitrogen fixation, phosphate solubilization, production of phytohormones) of the plants are benefited resulting in improved growth and productivity. Therefore, viability of these organisms during production, formulation, storage, transportation/distribution and field application is directly related to plant growth promoting potential of a biofertilizer formulation. The complaint from farmers regarding the efficiency of biofertilizer is not uncommon and improper storage and longer duration between production and field application could be the best explanation for such incidents. This limits their use due to compatibility, stability and survival issues under different soil conditions. Hence, improved shelf life could be the key for further popularization of biofertilizer application.

Carrier based biofertilizers (CBF) are not so tolerant to the temperature which is mostly unpredictable and uncertain in the crop fields while temperature tolerance is the other advantage of the liquid biofertilizers.

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The range of possible contamination is very high as bulk sterilization does not provide the desirable results in the case of carrier based biofertilizers, where as the contamination can be controlled constructively by means of proper sterilization techniques and maintenance of intensive hygiene conditions by appropriate quality control measures in the case of liquid based biofertilizer. Moisture retaining capacity of the carrier based biofertilizers is very low, which does not allow the organism viable for longer period and the liquid based biofertilizer (LBF) facilitates the enhanced viability of the organism. The administration of liquid based biofertilizers in the fields is comparatively easier than carrier based biofertilizers[3,10]. LBF are believed to be the best alternative for the conventional carrier based biofertilizers in the modern agriculture which help in the enhanced crop yields, regaining soil health and sustainable global food production.

MATERIALS AND METHODS

The Present study was carried out at the Department of Agricultural Microbiology and Bioenergy, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad, Telangana. The materials used and methods followed are described below.

Equipment and apparatus used:

Hot air oven and autoclaves were used for sterilization of heat stable and media respectively. BOD incubators were used for incubating cultures at different temperatures. Cultures were stored and maintained in a refrigerator. The pH was measured by using digital pH meter. Cyclomixer was used for homogenization during serial dilution. Plate mixer was used for spread plate technique. Centrifuge was used for making cell-free cultures. Samples were weighed using a single pan electric balance. Compound electron microscope was used to observe the morphology of bacterial cultures. Quebech colony counter was used for counting the viable population of microorganisms. pH meter is used to estimate pH.

Table 1: Carrier and Liquid Biofertilizers

1	*Rhizobium*, PSB	K.N Biosciences (India). Pvt. Ltd
2	*Rhizobium*, PSB	Pratista Biofertilizers pvt ltd, hyd
3	*Rhizobium*, PSB	Agricultural Research Station, Amaravathi

Collection of biofertilizers

Different types of carrier and liquid based biofertilizers were collected from following different firms and stored at 4°C in refrigerator

Quality Control of Biofertilizers

Microbial Analysis of Biofertilizers

The Biofertilizers collected were analyzed for viable population of microorganisms i.e., *Rhizobium*, Phosphate solubilizing bacteria by the standard serial dilution plate count method[12] and plating on selective media as mentioned above. Plates were incubated at 28±2 ^{o}C in an incubator in triplicates. The microbial colonies appearing after the stipulated time period of incubation were counted as Colony forming units per gram (Cfu g^{-1}) fresh weight of the sample in the colony counter. For analysis of *Rhizobium* Biofertilizer, 0.1 ml from dilutions such as 10^{-3} to 10^{-6} were taken and plated on YEMA plates. Whereas for phosphate solubilizing bacterial biofertilizer, 0.1 ml from dilutions such as 10^{-3} to 10^{-6} were taken and spreaded on Pikovskaya's Agar medium.

Estimation of moisture content

1gm of moisture sample was weighed and recorded as "wet weight of sample". Dried the wet sample to a constant weight, at a temperature not exceeding 239º F (115º C) using the suitable drying equipment, Allowed the sample to cool. Weighed the cooled sample again, and recorded as the "dry weight of sample". The moisture content of the sample was calculated using the following equation:

$$\%W = \frac{A-B}{A}$$

Where: %W = Percentage of moisture in the sample,
A = Weight of wet sample (grams), and
B = Weight of dry sample (grams)

Level of contaminants

Level of contaminants in biofertilizers was evaluated immediately after collection by serial dilution method at 10^{-5} dilution point. Level of contaminants such as other culturally varied bacteria on selective media measured in (Cfu g^{-1}).

pH

Weigh 20 g of biofertilizer into 100 ml beaker and add 50 ml of distilled water. Stir the contents with a glass rod and allow it for 30 min measure the pH with pH meter.

RESULT AND DISCUSSION

pH of the *Rhizobium* - carrier based biofertilizer collected from different production centers ranged from 7.0 to 7.5, pH of PSB biofertilizer ranged from 6.5 to 7.5. pH of the *Rhizobium* – liquid based biofertilizer collected from different production

centres ranged from 7.0 to 7.5, pH of PSB - liquid based biofertilizer ranges from 6.5 to 7.5. There was more fluctuation of pH in case of carrier based biofertilizer than liquid based biofertilizers.

Moisture content (MC)

Moisture content of carrier based biofertilizers must be 20 – 35 per cent. Biofertilizers brought from ARS(Agriculture research station) Amaravathi had MC of 40 – 60 %.Biofertilizers of KN Biosciences and Pratista had moisture content of about 35 – 45 %. The moisture content of biofertilizers gradually decreased with the time period. Shelf life of liquid based biofertilizers was more when compared to carrier based biofertilizers these results are in conformity with the findings of Brar *et al*.(2012) reported that liquid biofertilizers has more shelf life because of sufficient amount of nutrients, cell protectants and inducers and Liu *et al*. (2009) reported that liquid biofertilizers have several advantages including high cell count, zero contamination, longer shelf life, greater protection against environmental stress

Consistency

Consistency of carrier based biofertilizers must be powdery and flowable for easy application in the fields Biofertilizers brought from ARS Amaravathi and KN Biosciences are powdery in nature. Consistency of liquid based biofertilizers brought from different production centers were turbid in nature.

Rhizobium - carrier based biofertilizer was collected on July 29, 2015 from K.N Biosciences (Mfg date - July 20 2015). The initial population of *Rhizobium* was 1.5×10^6 CFU g^{-1} on YEMA with Congo Red medium. The viability of microorganisms was evaluated on monthly intervals upto January month. The microbial analysis revealed that there was a decline in the population of *Rhizobium* from August (1.5×10^6 CFU g^{-1}) to January (4.5×10^3 CFU g^{-1}). Level of contaminants observed were 1.7×10^5, 1.8×10^5 during last two months i.e December and january. The quality was not good as prescribed population was not found even within one month

Rhizobium - carrier based biofertilizer was collected on July 29, 2015 from Pratista Biofertilizers (Mfg date - July 15,2015). The initial population of *Rhizobium* on YEMA with Congo Red medium was 1.59×10^8 CFU g^{-1} .The viability of microorganisms was evaluated on monthly intervals upto January month. The microbial analysis revealed that there was a decline in the population of *Rhizobium* from August (1.59×10^8 CFU g^{-1}) to January (4.0×10^5 CFU g^{-1}). Level of contaminants observed were 1.8×10^5, 2.0×10^5 during last two months i.e December and january. The biofertilizer retained desired population till four months, but there was considerable contamination in two (september). Hence biofertilizer quality was retained upto two months.

Rhizobium - carrier based biofertilizer was collected on August 15, 2015 from ARS Amaravathi, ANGRAU (Mfg date - July 29,2015). The initial population of *Rhizobium*

found on YEMA with Congo Red medium was 5.4 x 10^8 CFU g^{-1}. The viability of microorganisms was evaluated on monthly intervals upto January month. The microbial analysis revealed that there was a decline in the population of *Rhizobium* from August (5.4 x 10^8 CFU g^{-1}) to January (7.4 x 10^5 CFU g^{-1}). After five months i.e in January month microbial count was reduced to 7.4 x 10^5 CFU g^{-1}.The biofertilizer retained desired population till four months and there was no contamination. Hence shelf life of biofertilizer was retained upto four months.

PSB - carrier based biofertilizer was collected on July 29, 2015 from K.N Biosciences (Mfg date – July 20, 2015). The initial population of PSB found on Pikovskaya's agar was 3.8 x 10^7 CFU g^{-1} .The viability of microorganisms was evaluated on monthly intervals upto January month. The microbial analysis revealed that there was a decline in the population of PSB from August (3.8 x 10^7 CFU g^{-1})to January(5.0 x 10^4 CFU g^{-1}). After five months microbial count was reduced to 5.0 x 10^4 CFU g^{-1} and no contamination was observed. The quality was not good as prescribed population was not found even within one month

PSB - carrier based biofertilizer was collected on July 29, 2015 from pratista Biofertilizers (Mfg date – July 15, 2015).The initial population of PSB on Pikovskaya's agar was 3.2 x 10^8 CFU g^{-1}.The viability of microorganisms was evaluated on monthly intervals upto January month. The microbial analysis revealed that there was a decline in the population of PSB from August (3.2 x 10^8 CFU g^{-1}) to January (3.0 x 10^4 CFU g^{-1}). After five months microbial count was reduced to 3.0 x 10^4 CFU g^{-1}.The biofertilizer retained desired population till two months and there was no contamination. Hence biofertilizer quality was retained upto two months.

PSB – carrier based biofertilizer was collected on August 15, 2015 from ARS Amaravathi ANGRAU (Mfg date – July 29, 2015).The initial population of PSB found on Pikovskaya's agar was 7.4 x 10^8 CFU g^{-1} .The viability of microorganisms was evaluated on monthly intervals upto January month. The microbial analysis reveals that there was a decline in the population of PSB from August (7.4 x 10^8 CFU g^{-1}) to January (7.8 x 10^5 CFU g^{-1}). After five months microbial count has reduced to 7.8 x 10^5 CFU g^{-1} and no contamination was observed.The biofertilizer retained desired population till four months and there was no contamination. Hence shelf life of biofertilizer was retained upto four months.

Shelf life of Liquid based biofertilizers

PSB - liquid based biofertilizer was collected on July 29, 2015 from K.N Biosciences (Mfg date – july 20, 2015). The initial population of PSB was found on Pikovskaya's agar was 2.6 x 10^7 CFU g^{-1} .The viability of microorganisms was evaluated on monthly intervals upto January month. The microbial analysis revealed that there was a decline in the population of PSB from August (2.6 x 10^7 CFU g^{-1}) to January (2.5 x 10^5 CFU g^{-1}). After five months microbial count has reduced to 2.5 x 10^5 CFU g^{-1}. The biofertilizer retained desired population till three months and there was no contamination. Hence quality of biofertilizer was retained upto three months.

PSB - liquid based biofertilizer was collected July 29, 2015 from pratista Biofertilizers (Mfg date – july 15, 2015). The initial population of PSB was taken on Pikovskaya's agar was 2.6 x 10^7 CFU g^{-1} .The viability of microorganisms was evaluated on monthly intervals upto January month. The microbial analysis revealed that there was a decline in the population of PSB from August (2.6 x 10^7 CFU g^{-1}) to January (2.5 x 10^5 CFU g^{-1}). After five months microbial count was reduced to 2.5 x 10^5 CFU g^{-1} . The biofertilizer retained desired population till three months and there was no contamination. Hence quality of biofertilizer was retained upto three months.

PSB - liquid basedbiofertilizer was collected on August 15, 2015 from ARS Amaravathi ANGRAU (Mfg date – july 29, 2015). The initial population of PSB was found on Pikovskaya's agar was 5.4 x 10^8 CFU g^{-1} .The viability of microorganisms was evaluated on monthly intervals upto January month. The

microbial analysis revealed that there was a decline in the population of PSB from August (5.4 x 10^8 CFU g^{-1}) to January (7.1 x 10^5 CFU g^{-1}). After five months microbial count was reduced to 7.1 x 10^5 CFU g^{-1}. The biofertilizer retained desired population till five months and there was no contamination. Hence quality of biofertilizer was retained upto five months.

Table 2: Quality of carrier based *Rhizobium* biofertilizers from different production centres

		August	September	October	November	December	January
K.N BIOSCIENCES Mfg date July 21 2015	Population	1.5 x 10^6 CFU g^{-1}	3.2 x 10^5 CFU g^{-1}	2.5 x 10^5 CFU g^{-1}	2.0 x 10^5 CFU g^{-1}	2.0 x 10^4 CFU g^{-1}	4.5 x10^3 CFU g^{-1}
	Consistency	fine powder	fine powder	fine powder	fine powder	fine powder	fine powder
	pH	7.5	7.5	7.5	7.5	7.0	7.0
	Moisture percentage	40.5	40	40	35.3	32.5	32.5
	Level of contaminants	–	–	–	–	1.7 x 10^5	1.8 x 10^5
PRATISTA BIOFERTILIZERS Mfg date July 15 2015	Population	1.59 x 10^8 CFU g^{-1}	1.5 x 10^8 CFU g^{-1}	8.2 x 10^7 CFU g^{-1}	7.2 x 10^7 CFU g^{-1}	3.6x 10^6 CFU g^{-1}	4.0 x10^5 CFU g^{-1}
	Consistency	Clumps	Clumps	Clumps	sticky	sticky	Sticky
	pH	7.5	7.5	7.5	7.2	7.2	7.0
	Moisture percentage	42.2	41.98	41.98	40.2	39.8	39.8
	Level of contaminants	–	–	–	–	1.8 x 10^5	2.0 x 10^5
ARS AMARAVATHI (ANGRAU) Mfg date July 29 2015	Population	5.4 x 10^8 CFU g^{-1}	5.0 x 10^8 CFU g^{-1}	6.4 x 10^7 CFU g^{-1}	6.2 x 10^7 CFU g^{-1}	4.5x 10^6 CFU g^{-1}	7.4x10^5 CFU g^{-1}
	Consistency	Clumps	Clumps	Clumps	clumps	clumps	Clumps
	pH	7.5	7.5	7.5	7.2	7.2	7.0
	Moisture percentage	35.0	33.3	30.9	28.0	27	27
	Level of contaminants	–	–	–	–	–	–

Table 3: Quality of carrier based PSB biofertilizers from different production centres

K.N BIOSCIENCES Mfg date July 20 2015	Population	3.8 x10^7 CFU g^{-1}	2.0 x10^7 CFU g^{-1}	3.2 x10^6 CFU g^{-1}	9.0 x10^5 CFU g^{-1}	8.2 x 10^5 CFU g^{-1}	5.0 x 10^4 CFU g^{-1}
	Consistency	Powdery	powdery	Powdery	Powdery	Powdery	Powdery
	pH	7.5	7.5	7.5	7.2	7.0	7.0
	Moisture percentage	48.9	45.1	41.5	40.5	40	39.9
	Level of contaminants	–	–	–	–	–	–
PRATISTA BIOFERTILIZERS Mfg date July 15 2015	Population	3.2 x10^8 CFU g^{-1}	2.8 x10^7 CFU g^{-1}	1.1 x10^7CFUg^{-1}	4.9 x 10^6 CFU g^{-1}	3.4 x 10^5 CFU g^{-1}	3.0 x 10^4 CFU g^{-1}
	Consistency	Powdery	Powdery	Powdery	Sticky	sticky	Sticky
	pH	7.2	7.2	7.0	7.0	7.5	7.5
	Moisture percentage	40.28	40.18	39.54	37.85	37.85	34.9
	Level of contaminants	–	–	–	–	–	–
ARS AMARAVATHI (ANGRAU) Mfg date July 20 2015	Population	7.4x10^8	5.4x10^8	6.8x10^7	5.4x10^7	5.5x10^6	7.8x10^5
	Consistency	Powdery	Powdery	Powdery	Powdery	Powdery	Powdery
	pH	6.5	6.5	6.5	7.0	7.0	7.0
	Moisture percentage	59.0	55.6	45.0	42.0	40.3	40.1
	Level of contaminants	–	–	–	–	–	–

Table 4: Quality of liquid based *Rhizobium* biofertilizers from different production centres

K.N BIOSCIENCES Mfg date July 20 2015	Population	1.2 x 10^7 CFU g^{-1}	5.0 x10^6 CFU g^{-1}	4.2 x10^6 CFU g^{-1}	1.2 x10^6 CFU g^{-1}	3.5 x10^5 CFU g^{-1}	1.2 x 10^4 CFU g^{-1}
	Consistency	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	pH	7.0	7.0	7.0	7.0	7.0	7.0
	Level of contaminants	–	–	–	–	–	–
PRATISTA BIOFERTILIZERS Mfg date July 15 2015	Population	2.6x10^8 CFU g^{-1}	2.4x10^8 CFU g^{-1}	4.4x10^7 CFU g^{-1}	2.4x10^7 CFU g^{-1}	6.5x10^6 CFU g^{-1}	7.4x10^5 CFU g^{-1}
	consistency	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	pH	7.0	7.0	7.0	7.0	7.0	7.0
	Level of contaminants	–	–	–	–	–	–
ARS AMARAVATHI (ANGRAU) Mfg date July 29 2015	population	8.4x10^8 CFU g^{-1}	6.4x10^8 CFU g^{-1}	5.4x10^8 CFU g^{-1}	4.4x10^8 CFU g^{-1}	6.5x10^6 CFU g^{-1}	8.4x10^5 CFU g^{-1}
	consistency	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	pH	7.0	7.0	7.0	7.0	7.0	6.5
	Level of contaminants	–	–	–	–	–	–

Table 5: Quality of liquid based PSB biofertilizers from different production centres

Samples		**August**	**September**	**October**	**November**	**December**	**January**
K.N BIOSCIENCES Mfg date July 20 2015	Population	2.6 x10^7 CFU g^{-1}	2.0 x10^7 CFU g^{-1}	1.6 x10^7 CFU g^{-1}	3.1 x10^6 CFU g^{-1}	7.5 x10^5 CFU g^{-1}	2.5 x 10^5 CFU g^{-1}
	Consistency	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	pH	7.0	7.0	7.0	7.0	7.0	7.0
	Level of contaminants						
PRATISTA BIOFERTILIZERS Mfg date July 15 2015	Population	3.4x10^8 CFU g^{-1}	3.1x10^8 CFU g^{-1}	8.4x10^7 CFU g^{-1}	6.9x10^6 CFU g^{-1}	6.8x10^6 CFU g^{-1}	7.1x10^5 CFU g^{-1}
	Consistency	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	pH	7.0	7.0	7.0	7.0	7.0	7.0
	Level of contaminants						
ARS AMARAVATHI (ANGRAU) Mfg date July 29 2015	Population	5.4x10^8 CFU g^{-1}	3.3x10^8 CFU g^{-1}	8.4x10^7 CFU g^{-1}	6.2x10^7 CFU g^{-1}	6.8x10^6 CFU g^{-1}	7.1x10^5 CFU g^{-1}
	Consistency	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid	Thick liquid
	pH	7.0	7.0	7.0	6.5	6.5	6.5
	Level of contaminants						

According to Biofertilizer Control Order specifications,the viable count of carrier biofertilizers must be 5 x 10^7 cell g^{-1}. In this present study quality of carrier based biofertilizers from different production centers results revealed that, biofertilizers from K.N Biosciences produced carrier based biofertilizers prescribed viable count was not seen in first month, whereas Pratista produced carrier based biofertilizers supported and maintained optimum viable count upto four months and ARS(Amaravathi) produced carrier based biofertilizers supported and maintained optimum viable count upto four months.

According to Biofertilizer Control Order specifications,the viable count of liquid based biofertilizers must be 1x10^8 cell ml^{-1}. In this present study results reavealed that only ARS(Amaravathi), produced liquid based biofertilizers supported and maintained viable count up to five months and there is no contamination.

CONCLUSION

Different carrier and liquid based biofertilizers are brought from different firms for evaluation of their quality. In case of carrier based biofertilizers, population of beneficial bacteria, pH, consistency, moisture content, level of contaminants were estimated. In case of liquid based biofertilizers, population of beneficial bacteria, pH, consistency and level of contaminants were estimated. The shelf life of biofertilzers were estimated using suitable media for viable count. Microbial population of beneficial bacteria was monitored in liquid and carrier based biofertilizers at monthly intervals.

In Liquid based biofertilizers viable count was constant for four months but a gradual decrease was observed in fifth and sixth months. Quality parameters of Liquid based biofertilizers are good and constant for six months. In carrier based biofertilizers, the quality was too low, moisture content was high and and decrease in count was more. The shelf life of biofertlizers from ARS(Amaravathi) was good followed by Pratista biofertilizers. Use of biofertilizers within four months from manufactured date is beneficial as viable count was observed more and no contamination was seen.

REFERENCES

1. Banerjee, M., Yesmin, R.L. and Vessey J.K., Plantgrowth promoting rhizobacteria as biofertilizer and biopesticides. pp. 137-181. In: Handbook of microbial bio-fertilizers. Rai, M.K. (Ed.). Food Production Press,U.S.A ((2006).
2. Brar, S.K., Sarma, S.J. and Chaabouni, E., Shelf life of biofertilizersss. An accord between formulation andgenetics, Journal of Biofertilizer and Biopesticides. **3 (5)**: 1-2 (2012)
3. Deaker, R., Roughley, R.J and Kennedy, I.R., Legume seed inoculation technology a review, Soil Biology and Biochemistry. **36** : 1275-1288 (2004).
4. Janagard, M. S., Raei, Y., Gasemi,G. K. and Aliasgarzad, N., Soybean response to biological and chemical fertilizers, *International Journal of Agriculture and Crop Sciences*. **5 (3)** : 261-266 (2013).
5. Liu, J., Tian, S., Li, B. and Qin, G., Enhancing viability of two biocontrol yeasts in liquid formulation by applying sugar protectant combined with antioxidant.,Biocontrol. **54:** 817-824 (2009).
6. Makoi, J.H.J.R. and Ndakidemi, P.A., Selected soil enzymes: Examples of their potential roles in the ecosystem, African Journal of Biotechnology. **7(3):** 181-191 (2008).
7. Ngampimol, H and Kunathigan, V. 2008. The study of shelf life for liquid biofertilizer from vegetable waste. *Australian Journal of Technology*. 11**:** 204-208.
8. Nita, P., Pallavi, G., Shubhangi, S., Hemlata, S., Neha, P. and Balasaheb, K., Liquid formulations of *Acetobacter diazotrophicus* L1 and *Herbaspirillum seropedicae* J24 and their field trials on wheat, *International Journal of Environmental Sciences*. **3:** 3(2012).

9. Rahmansyah, M., Antonius, S. and Sulistinah, N., Phosphatase and urease instability caused by pesticides present in soil improved by grounded rice straw, ARPN Journal of Agricultural and Biological Science. **4(2):** 56-62 (2009).
10. Singleton, P.W., Keyser, H.H and Sande, E.S., Development and evaluation of liquid inoculants and nitrogen fixation of legumes in Vietnam D. Herridge, AICAR Proceedings. **109:** 52-66 (2002).
11. Turan, M., Ataoglu, N. and Sahin, F., Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture, Sustainable Agricultural. **28:** 99-108 (2006).
12. Vlassak, K.L., Van, H and Duchateau, L., Isolation and characterization of fluorescent Pseudomonas associated with the roots of rice and banana grown in Srilanka, Plant and Soil. **145:** 51-63 (1992).
13. Wu, S.C., Caob, Z.H., Lib, Z.G., Cheunga, K.C. and Wong, M.H., Effects of bio-fertilizer containing N-fixer, Pand K solubilizers and AM fungi on maize growth: a greenhouse trial, Geoderma. 125**:** 155-166 (2005).
14. Zahir, A.Z., Arshad, M. and Frankenberger, W.F., Plant growth promoting rhizobacteria: applications and perspectives in agriculture, Advances in Agronomy. **81:** 97-168 (2004).