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RESEARCH ARTICLE

CROP RESPONSE OF DIFFERENT FORMULATIONS OF PHOSPHATE SOLUBILIZING BACTERIA ON COW PEA

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A B S T R A C T

Biofertilizers are microbial inoculants which helps the plant growth and development through supply of plant nutrients. The commonly available biofertilizers are biological nitrogen fixers and phosphate solubilizers. Phosphate solubilizing biofertilizers (PSB) are carrier based preparation containing living cells of microorganisms like bacteria, fungi and actinomycetes which help in increasing crop productivity by way of helping in solubilization of insoluble phosphorus, stimulating plant growth by providing hormones, vitamins and other growth factors. The present study was aimed at to study the nursery performance of different formulations of phosphate solubilizing bacteria (PSB) in cow pea (*Vigna unguiculata* (L.) Walp.). The selected elite PSB strain was mass multiplied and prepared the bioformulation with different carrier materials such as composted coir pith, lignite, organic manure, vermicompost and vermiculite. The result indicated that the there was significant differences were observed in the growth as well as biochemical parameters of cow pea. The response was varied with carrier material. Among them, composted coirpith was the superior in both growth and biochemical responses of cow pea.

INTRODUCTION

Microorganisms are colonizing the rhizosphere can be classified according to their effects on plants and the way they interact with roots, some being pathogens whereas other trigger beneficial effects. Rhizobacteria inhabit plant roots and exert a positive effect ranging from direct influence mechanisms to an indirect effect. So, the bacteria inhabiting the rhizosphere and beneficial to plants are termed PGPR. There are several PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism; improved nutrient acquisition (Biofertilizers), phytohormone production (Biostimulants) and suppression of plant disease (termed Bioprotectants).

Biofertilizers are carrier based preparations containing beneficial microorganisms in a viable state. They improve the soil fertility and the promote plant growth. They are broadly classified into nitrogen fixers, phosphate solubilizers, phosphorus mobilizers and organic matter decomposers. They enhance certain biological processes by which the nutritionally important elements make available to the plants. Biofertilizers are environment friendly, low cost agricultural input playing a significant role in improving nutrient availability to the plants.

Phosphate Solubilizing Bacteria

There are various types of soil microbes which can solubilize the fixed form of P and make it available to plants such an organisms are called Phosphate solubilizing microorganisms (PSMs). PSMs include bacteria, fungi and actinomycetes. Several soil bacteria, particularly those belonging to the genera *Pseudomonas* and *Bacillus* possess the ability to convert the insoluble phosphate into soluble form by secreting organic acids resulting in improved phosphate availability to the plants are called Phosphate Solubilizing Bacteria (PSB).

Mass Multiplication

For large scale production and delivery to farm use, the carrier based cultures packed in polythene bags with proper sealing, is the common accepted practice in almost all producing countries. This method has several advantages over others except that the system involves bulk quantities to be delivered and shelf life is restricted from production to use up to 3-6 months. A number of carrier materials were used for mass multiplication of beneficial microorganisms such as lignite, charcoal, presumed agro industrial waste, compost (Mohammadi and Sohrabi, 2012).

A cost effective carrier materials which are non polluting, biodegradable, non-toxic, capable of maintaining high viable count and long shelf life amendable to nutrient supplement and high water holding capacity (Gomare *et al.,* 2013). Quality of the bioinoculants is one of the most important factors deciding their field performance (Baby, 2004).

Crop Response of PSB

The action of PSM was not only due to the release of plant available phosphorus but also the production of biologically active substances like indole acetic acid, gibberellins;

cytokinins production was also correlated with P solubilization. The favourable effect of the inoculants on plant growth and nutrient uptake was due to the production of growth promoting substances by PSM (Kundu *et al*., 2002). The available P_2O_5 content of the soil, nodulation, root and shoot biomass, straw and grain yield were increased due to PSM inoculation as compared to the inoculated controls. PSB could increase growth and yield in several crops including walnut (Xuan *et al*., 2011), apple (Aslantas *et al*., 2007), maize (Hameeda *et al*., 2008), soybean (Fernandez *et al*., 2007), chickpea (Akhtar and Siddiqui, 2009) and peanut (Taurian *et al*., 2010).

MATERIAL AND METHODS

Isolation and Mass Multiplication of Bioinoculants

Isolation and enumeration of PSB was carried out following dilution plate technique using hydroxy patite medium. PSB isolate was multiplied in the culture flask with nutrient broth. The broth culture was mixed with sterilized carrier materials. The viable count in the inoculum was checked before mixing the inoculums with carrier materials.

Carrier Materials

Various organic materials and agricultural wastes such as composted coir pith, lignite, organic manure, vermicompost and vermiculite were used for the mass multiplication of PSB. The carrier materials were sterilized, sieved and maintained proper water content in the carrier materials. The mass multiplied liquid culture was mixed with the carrier materials and used for nursery experiments.

Experimental Details

An experiment was conducted to study the nursery performance of PSB strain with reference to different carrier materials on cow pea (*Vigna unguiculata (*L.) Walp). The selected PSB strain was incorporated with different carriers. The biometric parameters such as shoot length, root length, plant fresh weight, plant dry weight and the biochemical parameters such as chlorophyll, protein, glucose, amino acid, nitrate reductase activity were analyzed in treated and untreated control plants. The experimental details were T1 – Control; T2 - Coirpith formulation; T3 - Vermicompost formulation – T4 - Organic manure formulation; T5 - Lignite formulation and T6 - Vermiculite formulation. The growth parameters such as shoot length, root length, plant fresh and dry weight were analyzed in both treated and untreated control plants. The biochemical parameters such as chlorophyll (Wellburn and Lichtenthaler, 1984), protein (Lowry *et al*., 1951), glucose (Jayaraman, 1981), free amino acid (Jayaraman, 1981) and nitrate reductase activity (Jaworski, 1971) were analyzed.

Statistical Analysis

The data obtained were subjected to analysis variance (ANOVA) and the significant means were segregated by critical difference (CD) at various levels of significance.

RESULTS

Phosphate solubilizing bacteria (PSB) were isolated based on the solubilization zone production in the hydroxy apatite medium from the rhizosphere soils of cluster bean (Plate 1). The CBP2 strain was isolated from rhizosphere soils of cluster bean. The elite PSB designates as CBP2 was selected and used for nursery experiments. This strain was superior in P solubilization zone formation, pH reduction, production of phosphatase enzyme and organic acids and liberation of available phosphorus.

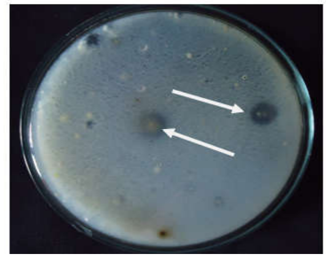

Plate 1 Solubilization zone produced by PSB

Mass Multiplication of Bioinoculant

The mass multiplication of biofertilizers involves careful selection of suitable carrier material for a long shelf life of selected bacterial strains. Quality of bioinoculants is one of the most important factors deciding their performance. A good carrier material is one which can keep up the viability of microbes for a longer period by providing organic food base to the organisms and retaining the moisture content.

Standardization of Age of the Culture

Mass multiplication of PSB was done in flask using nutrient broth. The pH of the media was kept around 7.0 and incubation temperature at 30±2ºC. For mass multiplication, three days old culture was used.

Preparation of Bioformulation

The carrier materials were sterilized and used for mass multiplication. A viable count ranged from 10^9 to 10^{10} ml^{-1} is preferred for the preparations of bioformulation and this population was attained within 3 - 5 days in the case of fast growing organisms such as *Pseudomonas* and *Bacillus*. Three day old culture was mixed with the sterilized carrier materials and air dried under shade condition and used for nursery experiments.

Quantity of Culture with Respect to Carrier Material

The quantity of culture filtrate which can be mixed with the carrier material varied with their water holding capacity. In the case of lignite 200ml/kg was found optimum, while in the case of vermicompost, organic manure 250 ml/kg and vermiculate and coirpith even with 500ml/kg, did not become pasty.

Effect of PSB Formulations on the Growth and biochemical Response of Nursery Plant

PSB strains were mass multiplied in the laboratory and treated with *Vigna unguiculata* (L.) Walp. Under nursery through different carriers such as coirpith, vermicompost, organic manure, lignite and vermiculite. Inoculation of PSB bioformulations increased the plant growth and also the dry matter content (Plate 2). Application of bioformulations also increased the physiological parameters like total chlorophyll, protein, amino acids, glucose and NR activity. The growth and physiological response were higher in all treatments compared to control. The response was varied based on the nature carrier material used for preparation of bioinoculants.

Plate 2 Growth response of PSB with different carrier materials in cow pea

Growth Response

The result revealed that the shoot length was higher in the plants treated with PSB with coirpith as carrier followed lignite formulation. The effect was least with organic manure bioformulation and control. Results also indicated that the root length was higher in all treated plants over control. The effect was varied with different formulations. It was observed that the plants grown with coirpith formulation produced taller roots. PSB inoculation increased the plant fresh weight. Among different formulations tested, coirpith formulation significantly increased the plant fresh weight followed by lignite formulation and least in organic manure formulation. Like fresh weight, same trend was observed in plant dry weight also (Table 1).

Table 1 Growth response of different formulations of PSB in cow pea

S. No	Treatment	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
1	Control	26.2^{d}	8.0^{d}	1.5^{d}	0.75^{d}
2	Coirpith formulation	40.5^{a}	15.0^{a}	4.1^{a}	2.50^{a}
3	Vermicompost formulation	36.2^{b}	10.2^{c}	2.7^{c}	1.35^{c}
4	Organic manure formulation	31.1^{c}	12.4^{b}	1.9^{d}	0.90^{b}
5	Lignite formulation	38.3^{b}	13.2^{b}	3.5^{c}	1.75^{c}
6	Vermiculite formulation	36.2^{b}	10.0^{c}	3.0^{b}	1.50^{b}
CD P=0.01%		2.3	1.3	0.48	0.05

Biochemical Response

In the nursery experiment, PSB inoculation with *Vigna unguiculata* increased the biochemical parameters such total chlorophyll, protein, amino acids, glucose and NR activity. The effect was varied with types of formulations. The result indicated that the total chlorophyll content was higher in plants treated with coirpith formulation followed by vermiculite formulation. But there was not much variation in total chlorophyll content with formulations with organic manure, lignite and vermicompost. Estimation of protein content in the leaves of *Vigna unguiculata* indicated that protein content was significantly higher in coirpith formulation and least in organic manure. The result also revealed that there was marked difference in the glucose content among treatments. Among them, glucose content was higher in plants treated with coirpith formulation. The least glucose content was noticed in vermicompost formulation. Application of biofertilizer increased the free amino acid in the leaves of *Vigna unguiculata* in all treated plants. The effect was higher in coirpith formulations over other formulations and control. NR activity was estimated in leaves of treated and control plants. The results indicated that NR activity was higher in plants treated with PSB as coirpith formulation over other treated plants (Table 2).

Table 2 Biochemical response of PSB strain with different carrier materials in cow pea

S. No	Treatment	Total Chlorophyll (mg/g LFW)	Protein (mg/g LFW)	Glucose (mg/g LFW)	Free amino Acid (mg/g LFW)	RNA(µ mole /g LFW)
1	Control	0.94^{e}	2.51^{d}	9.6^{d}	2.7^{d}	4.45^{f}
2	Coirpith formulation	2.92^{a}	4.39^{a}	29.6^{a}	5.7^{a}	6.55^{a}
3	Vermicompost formulation	1.97^{c}	3.48^{b}	12.0^{c}	4.6^{b}	5.93^{b}
4	Organic manure formulation	1.67^{d}	3.15^{c}	20.0^{b}	3.1^{c}	5.75^{c}
5	Lignite formulation	1.66^{d}	4.27^{a}	12.8^{c}	4.7^{b}	$4.81.4^{e}$
6	Vermiculite formulation	2.19^{b}	4.31^{a}	20.8^{b}	3.6^{c}	5.57^{d}
CD P=0.01%		0.37	0.23	0.34	0.004	0.059

DISCUSSION

Mass Multiplication of Bioinoculants

Biofertilizers are usually prepared as carrier-based inoculants containing effective microorganisms. Incorporation of microorganisms in carrier material enables easy-handling, long-term storage and high effectiveness of biofertilizers. Basically, the carrier-based inoculant of these bacteria can be prepared by a common procedure. In the bioinoculants preparation, various carrier materials are used such as lignite, vermiculite, charcoal, agro industrial waste, compost *etc.* In the present study, PSB strains were mass multiplied with different carriers such as coir pith, vermicompost, organic manure, lignite and vermiculite. The efficiency of different types of bioinoculants was varied based on the nature of carrier material used for preparation of bioinoculants. Therefore, selection of carrier material is important one in the biofertilizer production as well as in the crop response (Uma Maheswari and Kalaiyarasi, 2015). A number of carriers materials were used for mass multiplication of beneficial microorganisms such as lignite, charcoal, presumed agro industrial waste, compost (Muthukumarasamy *et al*., 1996).

Quantity of Culture with Respect to Carrier Material

The quantity of culture required for mass multiplication is differed based on the physical properties of carrier materials. It is also determine the quality of bioinoculants. Based on the water holding capacity and particle size of the carrier materials, the quantity of requirement of culture is varied. In the present study, the quantity of culture filtrate varied with the carrier material with their water holding capacity. Coirpith required higher concentration of culture than others; because the water holding capacity is more with coirpith than other carrier materials. High level of organic matter content increased the water holding capacity and neutral pH for better survival of the microorganisms (Roychowdhury *et al.,* 2015).

Standardization of Age of the Culture

The selected PSB strains were cultured in the flask and mixed with various carrier materials. A viable count ranged from 10^{9}

to 10^{10} ml^{-1} is preferred for the preparations of bioformulation and this population was attained within 3 – 5 days in the case of fast growing organisms such as *Azospirillum, Pseudomonas* and *Bacillus* sp. In the case of slow growing organisms, it took about 6-7 days to reach such counts. Most of the laboratories the practice is in using logarithmic or late logarithmic phase culture with fermentation period of 30 - 72 h or 10 - 15 day old culture. The nature of carrier material, shelf life and inoculums potential are important in the quality of bioinoculants. Quality of bioinoculants is one of the most important factors deciding their performance. A good carrier material is one which can keep up the viability of microbes for a longer period by providing organic food base to the organisms and retaining the moisture content (Yadav and Chandra, 2012; Sivasakthivelan and Saranraj, 2013).

Crop Response of Bioformulation

Application of PSB increased the plant growth and also the dry matter content. It also increased the physiological parameters like total chlorophyll, protein, amino acids, glucose and NR activity. The response was varied based on the nature of carrier material used for preparation of bioinoculants. From these, it is clear that PSB not only solubilize the P but also increased plant growth and development. PSB was not only due to the release of plant available phosphorus but also produce the biologically active substances like indole acetic acid, gibberellins, cytokinin. The favourable effect of the inoculants on plant growth and nutrient uptake was due to the improved phosphate nutrition and production of growth promoting substances by PSM (Yadav and Chandra, 2014).

Further, the inoculation of *Pseudomonas* strains on winter wheat increased the plant height, root and shoot mass and number of tillers under growth chamber conditions. PSB improved the nodulation, root and shoot biomass, straw and grain yield compared to the uninoculated controls. Field study with *Bacillus megaterium* and *B. circulans* in rice and wheat significantly increased the yield which was equivalent to the yield obtained which up to 50 kg P_2O_5/ha and also significant increased in the yield of rice, chickpea, pigeonpea, soyabean, groundnut *etc.* (Hedge and Dwivedi, 1994). The positive benefits from inoculation were attributed to several mechanisms such as phytohomones production, phosphate solubilization, enhanced nutrient uptake (Fernandez *et al.*, 2007). In a pot experiment, the total dry matter yield, phosphorus and calcium content in shoot of soybean were increased with the use of low grade rock phosphate inoculated with phosphate solubilizers and farmyard manure (Packialakshmi and Aliya Riswana, 2014).

CONCLUSION

Soil P precipitated as orthophosphate and adsorbed by Fe and Al oxides is likely to become bio-available by bacteria through their organic acid production and acid phosphatase secretion. Although, high buffering capacity of soil reduces the effectiveness of PSB in releasing P from bound phosphates; however, enhancing microbial activity through P solubilizing inoculants may contribute considerably in plant P uptake. Phosphorus solubilizing bacteria are very effective for increasing the plant available P in soil as well as the growth and yield of crops. So, exploitation of phosphate solubilizing bacteria through biofertilization has enormous potential for making use of ever increasing the growth and development of crop plants.

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References

Akhtar, M.S and Siddiqui, Z.A. 2009. Effects of phosphate solubilizing microorganisms and *Rhizobium* sp. on the growth, nodulation, yield and root-rot disease complex of chickpea under field condition. *Afr. J. Biotechnol*. 8:3489-3496.

Aslantas, R, Cakmakci, R and Sahin, F. 2007. Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. *Sci. Hortic*. 111:371-377.

Baby, U.I 2004. Suustainable productivity. *Contemporary Tea Time* 10:51-52.

Fernandez, L.A, Zalba, P, Gomez, M.A and Sagardoy, M.A. 2007. Phosphate solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. *Biol. Fert. Soil.* 43: 805-809.

Gomare, K.S, Mese, M, Shetkar Y. 2013. Isolation of *Azotobacter* and cost effective production of biofertilizer. *Indian J. Appl. Res*., 3(5): 54 56.

Hameeda, B., Harini, G., Rupela, O.P, Wani, S.P and Reddy, G. 2008. Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macro fauna. *Microbiol. Res*. 163: 234-242.

Hedge, D.M and Dwivedi, B.S. 1994. Crop response to biofertilizers in irrigated areas. *Fert. News* 39 (4):19-26.

Jaworski, E.G. 1971. Nitrate reductase assay intact plant tissues. *Biochem. Biophy. Res. Commun*. 43: 1274-1279.

Jayaraman, J. 1981. Laboratory manual in Biochemistry, Willey-eastern company limited. Madras. pp. 1-65.

Kundu, B.S, Geva, R, Sharma, N, Bhatia, A and Sharma, R. 2002. Host specificity of phosphate solubilizing bacteria. *Ind. J. Microbiol.* 42:19-21.

Lowry, O.H, Rosebrough, M. J, Farr, A. L and Randall, R.J. 1951. Protein measurement with Folin-phenol reagent. *J. Biol. Che*, 193: 262-275.

Mohammadi, K and Sohrabi, Y. 2012. Bacterial biofertilizers for sustainable crop production: A Review. *ARPN J Agric. Bio. Sci.* 7 (5): 307-316.

Muthukumarasamy, R., Revathi, G, Murthy, V.G Mala, S.R., Vadivelu, M and Soloyappan, A.R. 1996. Alternate carrier material for biofertilizers. Paper presented in *National Seminar on Biofertilizer Production - Problems and Constraints.* Tamil Nadu Agricultural University, Coimbatore. pp. 24-25.

Packialakshmi, N and Aliya Riswana, T. 2014. Comparative study of vermicast and charcoal used as a carrier inoculums to the biofertilizer preparation. BMR Biotechnology. 1(1): 1-6.

Roychowdhury, D, Paul, M and Kumar Banerjee, S. 2015. Isolation, identification and characterization of phosphate solubilising bacteria from soil and the production of biofertilizer. *Int.J.Curr.Microbiol.App.Sci* 4(11): 808-815.

Sivasakthivelan, P and Saranraj, P. 2013. *Azospirillum* and its Formulations: A Review. *International Journal of Microbiological Research* 4 (3): 275-287.

Taurian, T, Anzuay, M.S, Angelini, J.G, Tonelli, M.L., Luduena, L, Pena, D, Inanez, F and Fabra, A. 2010. Phosphate-solubilizing peanut associated bacteria, screening for plant growth-promoting activities. *Plant Soil*. 329: 421-431.

Uma Maheswari, N and Kalaiyarasi, M. 2015. Comparative Study of Liquid Biofertilizer and Carrier Based Biofertilizer on Green Leafy Vegetables. *Int. J. Pharm. Sci*. Rev. Res., 33(1): 229-232.

Wellburn, A.R. and Lichtenthaler, H. 1984. In: Advances in photosynthesis research (ed. Sybesma) Martinus Nijhoff, Co. *The Hague*. pp. 9-12.

Xuan Yu, A, Xu Liu, D, TianHui Zhu, Z, GuangHai Liu, R.T and Cui Mao G. 2011. Isolation and characterization of phosphate solubilizing bacteria from walnut and their effect on growth and phosphorus mobilization. *Biol .Fert. Soil* 47:437-446.

Yadav, A.K and Chandra, K. 2012. National Seminar on Organic and Biological Inputs - New Innovations and Quality Control, Published by National Centre of Organic Farming, DAC, Ghaziabad, pp 19-24.

Yadav, A.K and Chandra, K. 2014. Mass production and quality control of microbial inoculants. Proc. Indian Natn. Sci. Acad. 80 (2): 483-489.

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