

Effect of bioformulations of Phosphate Solubilizing Bacteria (PSB) on the Growth and Biochemical Characters of the *Gossypium Hirsutum* and *Zea Mays*

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Abstract— Biofertilizers offer a new technology to Indian agriculture holding a promise to balance many of the shortcomings of the conventional chemical based technology. They are usually prepared as carrier based inoculants containing effective microorganisms. The present study was aimed at to study the nursery performance of different formulations of PSB in maize and cotton plants. The selected PSB was mass multiplied in the laboratory and incorporated into the nursery soil through different carrier material such as coirpith, vermicompost, organic manure, lignite and vermiculite. The effect of bioinoculants on the growth and biochemical characters were studied from the control and treated seedling of *Gossypium hirsutum* and *Zea mays*. The significant difference was observed in the growth and biochemical characters in both *Gossypium hirsutum* and *Zea mays*. The effect was differed with reference to the nature of carrier materials used for the preparation of bioformulations. The results indicated that the bioformulation prepared by composted coirpith had superior in plant growth and development.

Keywords— PSB, carrier, bioinoculants, crop response, cotton, maize.

I. INTRODUCTION

Biofertilizers are the bioinoculants of specific beneficial microorganisms that promote the growth and development of plant crops by converting the unavailable form of nutrients into available form. These biofertilizers also improve the soil fertility (Sivasakthivelan and Saranraj, 2013). They can be used as supplements of chemical fertilizers; they were relatively inexpensive and renewable sources of plant nutrient. Biofertilizers were selected strains of microorganisms which were beneficial to the growth of the plants. These microorganisms were cultured in laboratory, mixed with suitable carrier materials and then

applied to the fields. They maintain soil health, minimizes pollution of the environment by lowering the use of chemicals (Tripti and Anshumali, 2012).

Phosphate solubilizing bacteria play an important role in converting low grade insoluble inorganic phosphate sources like rock phosphate, bone meal, basic slag and the chemically fixed soil phosphorus into available form. Therefore, the use of phosphate solubilizing microbes in agricultural practice would not only offset the high cost of manufacturing phosphatic fertilizers but would also mobilize insoluble phosphorus in the fertilizers and soils to which they are applied. The mechanism of solubilization of insoluble phosphate is ability to secrete organic acids and phosphatase enzyme (Mahantesh *et al.*, 2015).

The carrier based inoculants produced in India generally have a short shelf life, poor quality, high contamination and unpredictable field performance. High quality biofertilizers would be expected to have higher population of desired microorganism, sufficient viability, and remain uncontaminated for longer period of storage. Today, advances in inoculants technology were concerned with improving quality, extending useful shelf life and developing new formulations for use under less favorable conditions. Suggested that liquid inoculants and alginate based granular formulations were two important new inoculants formulations which were an alternative to peat or lignite based ones (Brahmaprakash and Sahu, 2012). A cost effective carrier materials which were nonpolluting, 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).

Phosphate solubilizing bacteria of *Pseudomonas* and *Bacillus* species are producing phytohormones *i.e.* auxins, inhibition of deleterious pathogens or nutrient mobilization

and ammonification. The plant growth benefits due to the addition of PSB include increases in germination rate, root growth, yield, leaf area, chlorophyll content, tolerance to drought, shoot and root weight. Such groups of bacteria are termed as phosphate solubilizing bacteria and inoculation with PSB as biofertilizers enhances P accumulation and biomass production of plants (Abbasi *et al.*, 2015). Single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with maize straw and sugarcane husk increased plant height and fresh weight and protein, proline, sugar contents and antioxidant activities. Inoculation of PSB further increased plant growth, physiology and yield characters over single inoculation with different carriers. It is inferred that carrier based biofertilizer effectively increased growth, maintained osmotic balance and enhanced the activities of antioxidant enzymes and yield parameters (Hassan and Bano, 2016).

The cultivation of cotton and maize requires higher amount of phosphatic fertilizer for their growth as well as sustainable yield. But the added phosphatic fertilizers are rapidly transformed to fixed form or unavailable forms. This is mainly due the nature of soil. These fixed forms of phosphates are unavailable to plants. It is directly affected the plant growth, development and yield of cotton and maize. These problems can be easily solved by application Phosphate Solubilizing Bacteria (PSB). These beneficial microorganism can able to soluble the fixed forms of phosphate into soluble/available form and can make it available to the plants. The application/amendment of PSB with cotton and maize will increase the soil fertility and crop yield. Further, the sustainable yield can be achieved by application biofertilizers like PSB.

II. MATERIAL AND METHODS

2.1. ISOLATION AND MASS MULTIPLICATION OF BIOINOCULANTS

Isolation and enumeration of PSB was carried out by dilution plate technique using hydroxy patite medium. The selected 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. Various organic materials such as composted coir pith, lignite, organic manure, vermicompost and vermiculite were used as carrier 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.

2.2. NURSERY EXPERIMENT

A nursery experiment was conducted to study the nursery performance of different formulations of PSB in *Gossypium hirsutum* and *Zea mays*. The seeds with uniform size, colour and weight were chosen for the experimental purpose and surface sterilized with 0.1% HgCl₂ for 1 minute and thoroughly washed with distilled water 3-5 times. Seeds were pre-soaked for 12 hours in distilled water and were sown in sterilized soil mixture. The soil mixture was prepared by mixing black soil, red soil and sand in the ratio of 1:1:1. The PSB formulations were applied 10g each at top soil of the pots. The treatment details were: 1.Control 2.Coir pith 3.Vermicompost 4.Organic manure 5.Lignite 6.Vermiculite. The growth characters such as seed germination percentage, germination index, seedling vigour index I and II, shoot length, root length, number of leaves, fresh weight and dry weight were analyzed in the treated and untreated plants. Biochemical characters such as total Chlorophyll (Wellburn and Lichtenthaler, 1984), Protein (Lowry *et al.*, 1951), glucose (Jayaraman 1981), amino acid (Jayaraman, 1981) and NR activity (Jaworski, 1971) were estimated.

2.3. STATISTICAL ANALYSIS

The data were reported as mean \pm SE and in the figure parentheses represent the per cent activity. Values are expressed as means \pm standard deviation of three independent data.

III. RESULTS

3.1 ISOLATION AND MASS MULTIPLICATION OF BIOINOCULANTS

Phosphate solubilizing bacteria (PSB) were isolated based on the solubilization zone production in the hydroxy apatite medium from the rhizosphere soils of Brinjal. Totally 10 PSB strains were isolated. Based on preliminary screening, PSB strain BP1 was selected as elite strain and used for further studies. The selected BP1 strain was mass multiplied in the laboratory with different carrier materials and used for nursery experiments. The carrier materials such as composted coirpith, vermicompost, organic manure, lignite and vermiculite were used for mass multiplication. A viable count ranged from 10⁹ to 10¹⁰ ml⁻¹ preferred for the preparations of bioformulation. Three day old culture was mixed with the sterilized carrier materials and used for the nursery experiment.

3.2 EFFECT OF PSB FORMULATIONS ON THE GROWTH CHARACTERS

Effect of bioformulations on the growth characters were studied from the control and treated seedlings of

Gossypium hirsutum and *Zea mays*. The germination rate was higher in the plants treatment with coirpith formulation over the control plants. Among the bioformulations, the low germination rate was organic manure in *G. hirsutum* and vermiculite formulation in *Z. mays*. The germination index was varied according to the bioformulation treatment. Little difference was shown in germination index among different bioinoculant treatments but the effect was higher to the control. The values of the seedling vigour index of the *Gossypium hirsutum* and *Zea mays* varied according to the bioformulation treatment. The highest value of seedling vigour index was observed in the plants treated with coirpith formulation. In both *G. hirsutum* and *Z. mays*, seedling vigour index II was significantly increased in the treatment of coirpith formulation followed by vermicompost formulation. The result revealed that the shoot length was higher in the plants of *G. hirsutum* and *Z. mays* treated with PSB with coirpith as carrier followed by vermicompost formulation. The effect was least with vermiculite bioformulation and control. Results also indicated that the root length was higher in all treated plants over control. It was observed that the plants grown with coirpith formulation produced taller roots than other treatments in both *G. hirsutum* and *Z. mays*. The number of leaves per plant was significantly influenced by bioformulation treated plants than untreated control plants. The highest number of leaves was found in plants treated with coirpith formulation in both *G. hirsutum* and *Z. mays*. Among different formulation tested, coirpith formulation significantly increased the plant fresh weight followed by vermicompost and least in vermiculite formulation. Like fresh weight, same trend was observed in plant dry weight also (Table 1 and table 2).

3.3. EFFECT OF PSB FORMULATIONS ON THE BIOCHEMICAL CHARACTERS

In the nursery experiment, the inoculation of PSB with *Gossypium hirsutum* and *Zea mays* increased the biochemical characters such total chlorophyll, protein content, aminoacids content, glucose content and NR activity. The total chlorophyll content was higher in plants treated with coirpith formulation followed by vermicompost formulation and least in organic manure formulation. In both *G. hirsutum* and *Z. mays*, the protein content was significantly higher in plants treated with PSB as coirpith formulation and least in organic manure. The result revealed that there was marked difference in the glucose content among the treatments. In both *G. hirsutum* and *Z. mays* glucose content was higher in plant treated with coirpith formulation. Application of biofertilizer increased the

aminoacid content in the leaves of *G. hirsutum* and *Z. mays* in all treated plants. Among them, the effect was higher in coirpith formulation over other formulations and control. The NR activity was maximum in plants treated with PSB as coirpith formulation over other treated plants in both *G. hirsutum* and *Z. mays* (Table 3 and table 4).

IV. DISCUSSION

4.1. MASS MULTIPLICATION OF PSB STRAIN

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 were used such as lignite, vermiculite, charcoal, agro industrial waste, compost *etc.* 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 was important one in the biofertilizer production as well as in the crop response (Uma Maheswari and Kalaiyarasi, 2015).

The nature of carrier material, shelf life and inoculums potential were important in the quality of bioinoculants. Quality of bioinoculants was one of the most important factors deciding their performance. A good carrier material was 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). Presently lignite powder was being used as carrier material by most of the bioinoculant producing units in India. Availability of quality lignite powder was also in doubt because of adulteration by agents and improper mesh size in the pulverizing unit. Several scientists have suggested compost as carrier material for biofertilizers. But the role of good compost is maintaining microbial population. The role of vermicompost as carrier in maintaining shelf life of inoculated bacterial culture has been studied for selecting as alternative carrier material to lignite (Gandhi and Sivakumar, 2010; Murray *et al.*, 2003). The quantity of culture required for mass multiplication was differed based on the physical properties of carrier materials. It was 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 was varied. 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 was 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).

4.2 CROP RESPONSE OF PSB

Ahemad and Kibret (2014) stated that mobilization of mineral nutrients like P in soil by bacteria could be the main mechanism for increased growth and development of plants which makes these nutrients in more readily plant available forms. Improvement in shoot length, root length, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight, yield per plant and P contents of root and shoot by inoculation of PSB strains in combination with organic amendment was more prominent as compared to sole inoculation of PSB strains PSB containing phosphatase enzymes liberate P through mineralization of organic matter which promote the growth and yield of plants (Walpola and Yoon, 2013).

A significant difference in the root, shoot length and shoot dry weight of tomato plants was observed at 60 days of plant growth due to various inoculation treatments. The treatment receiving inoculation of PSB5 recorded maximum root length and was significantly superior over all other inoculation treatments followed by PSB7. All treatments showed significant increase in root growth over absolute control (Assefa and Fenta, 2017). Phosphate solubilizing bacteria improved the plant growth, yield and phosphorus content of several crops and used as bioinoculant to enhance the sustainable production. All strains showed a positive effect on plant growth. A significant increment in plant height, shoot dry weight was determined in plants treated with *Pseudomonas tolaasii*, while *Pseudomonas koreensis* has remarkably increased P content compared to the uninoculated control. *Pseudomonas* strain was selected and evaluated under field conditions in combination with triple superphosphate as P fertilizer. The presence of *Pseudomonas* strain stimulated seedling emergence, shoot length, grain yield, grain weight, total dry biomass and P content of maize plants. In general, *P. tolaasii*, inoculation was more efficient as bioinoculant without P fertilizer (Viruel *et al.*, 2014).

The application of PSB either individually or combined, effected on the growth and biomass production of several crops (Hariprasad and Niranjana, 2009). Biofertilizer produced the plant growth regulating substances, which promotes root growth found the significant effect on number of primary flowers number of secondary flowers, total number of flowers, number of primary fruit, number of

secondary fruit and total number of fruit by the application of PSB in strawberry (Zargar *et al.*, 2008). Biochemical parameters of chlorophyll, total carbohydrate, total protein and total fat contents found higher in biofertilizer enriched vermicompost treatments. Increased amount of chlorophyll contents seems to correlate the increased photosynthetic properties (Khomami and Moharam, 2013). 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 was 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 PSB (Yadav and Chandra, 2014).

PSB application significantly increased the biochemical parameters like the Chlorophyll a, and b, carotenoid, protein and ascorbic acid (Singh *et al.*, 2014). Ferreira *et al.* (1987) reported that wheat plants inoculated with PSB showed greater activity of the nitrate reductase enzyme. Inoculation with nitrogen fixing bacteria always increased leaf NRA suggesting a greater supply of NO₃ to the plants over uninoculated control. The increased NO₃ uptake may relate to increased root development in response to production of hormones. The biochemical characters of protein contents of leaves of pot grown plants was 33-35% higher over control when plants were inoculated with *Bacillus cereus* and *Pseudomonas moraviensis*. Co-inoculation of both *Bacillus cereus* and *Pseudomonas moraviensis* significantly increased protein content. Further, sugar contents of leaves were also significantly increased (Galal, 2003).

V. CONCLUSION

The isolated PSB strains were screened *in vitro* for the selection of elite strain. The selected strain of PSB was mass multiplied and used for nursery experiment. PSB strain was incorporated into the nursery soil through different carriers. The bioinoculation of PSB was increased the growth and biochemical characters of *Gossypium hirsutum* and *Zea mays*. The response was varied with carrier materials used in bioinoculation of PSB.

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Table.1: Effect of PSB formulations on the growth characters of *Gossypium hirsutum*

Treatment	Seed Germination (%)	Germination Index	Seedling Vigour Index I	Seedling Vigour Index II	Shoot Length (cm)	Root Length (cm)	Number of leaves (per plant)	Fresh Weight (g)	Dry Weight (g)
Control	76	0.92	18.2 ±0.49 (100)	1.12 ±0.02 (100)	24 ±0.30 (100)	9.5 ±0.32 (100)	15 ±0.15 (100)	4.25 ±0.02 (100)	1.48 ±0.13 (100)
Coirpith formulation	96	2.04	36.5 ±0.37 (200)	5.21 ±0.06 (465)	38 ±0.32 (158)	17.3 ±0.41 (182)	33 ±0.30 (220)	12.83 ±0.75 (302)	5.43 ±0.47 (367)
Vermicompost formulation	94	1.85	34.8 ±0.35 (191)	3.49 ±0.60 (312)	36 ±0.15 (150)	16.8 ±0.72 (177)	32 ±0.40 (213)	11.40 ±0.47 (268)	3.80 ±0.65 (257)
Organic manure formulation	90	1.53	30.2 ±0.49 (166)	1.98 ±0.54 (172)	28 ±0.35 (117)	13.2 ±0.40 (139)	22 ±0.47 (147)	6.67 ±0.12 (157)	2.65 ±0.18 (179)
Lignite formulation	96	2.04	32.6 ±0.15 (179)	3.42 ±0.01 (305)	34 ±0.25 (142)	15.6 ±0.35 (164)	25 ±0.37 (166)	9.98 ±0.10 (235)	3.57 ±0.13 (241)
Vermiculite formulation	91	1.59	30.4 ±0.47 (167)	2.22 ±0.02 (198)	33 ±0.49 (137)	12.8 ±0.30 (135)	27 ±0.58 (180)	8.14 ±0.05 (192)	2.42 ±0.27 (164)

Table.2: Effect of PSB formulations on the growth characters of *Zea mays*

Treatment	Seed Germination (%)	Germination Index	Seedling Vigour Index I	Seedling Vigour Index II	Shoot Length (cm)	Root Length (cm)	Number of leaves (per plant)	Fresh Weight (g)	Dry Weight (g)
Control	75	0.78	13.4 ±0.15 (100)	0.70 ±0.03 (100)	20 ±0.25 (100)	9.0 ±0.09 (100)	13 ±0.28 (100)	3.55 ±0.11 (100)	1.05 ±0.01 (100)
Coirpith formulation	93	1.97	33.5 ±0.40 (250)	2.14 ±0.01 (305)	36 ±0.15 (180)	15.7 ±0.20 (174)	25 ±0.35 (192)	8.41 ±0.34 (237)	2.31 ±0.07 (220)
Vermicompost formulation	90	1.82	26.1 ±0.25	1.68 ±0.07	30 ±0.40	14.0 ±0.15	22 ±0.40	6.92 ±0.71	1.93 ±0.03

Treatment	Seed Germination (%)	Germination Index	Seedling Vigour Index I	Seedling Vigour Index II	Shoot Length (cm)	Root Length (cm)	Number of leaves (per plant)	Fresh Weight (g)	Dry Weight (g)
			(195)	(240)	(150)	(156)	(169)	(195)	(182)
Organic manure formulation	82	1.30	28.0 ±0.30 (209)	1.12 ±0.02 (160)	35 ±0.20 (175)	14.3 ±0.45 (159)	23 ±0.41 (177)	5.18 ±0.60 (146)	1.41 ±0.13 (134)
Lignite formulation	87	1.55	27.8 ±0.73 (207)	1.42 ±0.03 (203)	32 ±0.55 (160)	13.7 ±0.40 (152)	20 ±0.81 (154)	5.77 ±0.49 (163)	1.64 ±0.05 (156)
Vermiculite formulation	81	1.28	18.2 ±0.20 (136)	0.92 ±0.02 (131)	25 ±0.65 (125)	12.2 ±0.55 (136)	19 ±0.40 (146)	4.39 ±0.11 (123)	1.26 ±0.24 (120)

Table.3: Effect of PSB formulations on the biochemical characters of *Gossypium hirsutum*

Treatment	Total Chlorophyll (mg/g LFW)	Protein (mg/g LFW)	Glucose (mg/g LFW)	Aminoacid Content (mg/g LFW)	NRA (μmol NO ₂ formed/g LFW/h)
Control	1.07 ±0.01 (100)	2.91 ±0.03 (100)	11.13 ±0.16 (100)	1.64 ±0.10 (100)	1.68 ±0.04 (100)
Coirpith formulation	2.83 ±0.02 (264)	6.80 ±0.19 (234)	22.35 ±0.42 (201)	3.90 ±0.35 (238)	3.13 ±0.17 (186)
Vermicompost formulation	2.32 ±0.07 (217)	5.66 ±0.23 (195)	19.92 ±0.21 (179)	2.94 ±0.32 (179)	2.95 ±0.09 (175)
Organic manure formulation	1.35 ±0.04 (126)	3.85 ±0.04 (132)	14.70 ±0.32 (132)	2.35 ±0.11 (143)	1.95 ±0.14 (116)
Lignite formulation	1.94 ±0.05 (181)	4.36 ±0.07 (150)	17.43 ±0.17 (157)	2.21 ±0.17 (134)	2.32 ±0.05 (138)
Vermiculite formulation	1.45 ±0.06 (135)	4.14 ±0.09 (142)	19.86 ±0.25 (178)	3.12 ±0.11 (190)	2.72 ±0.16 (162)

Table.4: Effect of PSB formulations on the biochemical characters of *Zea mays*

Treatment	Total Chlorophyll (mg/g LFW)	Protein (mg/g LFW)	Glucose (mg/g LFW)	Aminoacid Content (mg/g LFW)	NRA (μmol NO ₂ formed/g LFW/h)
Control	0.92 ±0.03 (100)	2.65 ±0.05 (100)	9.81 ±0.03 (100)	1.12 ±0.02 (100)	1.18 ±0.02 (100)
Coirpith formulation	2.69 ±0.05 (292)	5.43 ±0.26 (204)	21.40 ±0.72 (218)	3.14 ±0.03 (280)	2.55 ±0.17 (216)

Treatment	Total Chlorophyll (mg/g LFW)	Protein (mg/g LFW)	Glucose (mg/g LFW)	Aminoacid Content (mg/g LFW)	NRA ($\mu\text{mol NO}_2$ formed/g LFW/h)
Vermicompost formulation	2.24 ± 0.11 (243)	4.71 ± 0.07 (178)	17.09 ± 0.53 (174)	2.21 ± 0.11 (197)	2.04 ± 0.07 (173)
Organic manure formulation	1.12 ± 0.24 (121)	3.15 ± 0.09 (119)	13.62 ± 0.75 (139)	1.47 ± 0.04 (131)	1.48 ± 0.06 (125)
Lignite formulation	1.63 ± 0.05 (177)	4.32 ± 0.57 (163)	15.18 ± 0.15 (155)	1.85 ± 0.05 (165)	2.18 ± 0.09 (184)
Vermiculite formulation	1.38 ± 0.06 (150)	3.22 ± 0.13 (122)	16.33 ± 0.42 (166)	2.51 ± 0.14 (224)	1.96 ± 0.05 (166)