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Production and yield attributes of biofertilizers on pulse crops

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Ravikumar, L. and Subramanian, N. (2018). Production and yield attributes of biofertilizers on pulse crops. International Journal of Agricultural Technology 14(7): 1777-1786.

Abstract Biofertilizers are becoming increasingly popular in many countries and for many crops. Biofertilizers are fertilizers containing living microorganisms, which increase microbial activity in the soil. Biofertilizers are low cost renewable source of nutrient that supplements the chemical fertilizer. Biofertilizers gained importance due to its low cost amongst small and marginal farmer**.** Inoculation of nitrogen fixing bacteria with bifertilizer increases the phosphorus level. The application of biofertilizer containing beneficial microbes showed a promoting effect on the growth of *Vigna radiata* (green gram) and *Vigna unguiculata* (cow pea) plants and improvement of soil properties through a 45 days greenhouse study. Among the various microbes, treated with *Pseudomonas* the plant height, fresh weight and dry weight were higher in *V. radiata* plant compared to *V. unguiculata*. The highest chlorophyll 'a' content (1.757 mg/g) was observed in combined microbes *Bacillus* + *Pseudomonas* + *Trichoderma* treated on *V. unguiculata* plant than *V. radiata.* Chlorophyll 'b' and carotenoid contents were also higher in the treatment of combined microbes *Bacillus* + *Pseudomonas* + *Trichoderma* treated on *V. unguiculata* plant compared to *V. radiata* and untreated control plants. In conclusion, efficient plant nutrition management should ensure both enhanced and sustainable agricultural production and safeguard the environment.

Keywords: Biofertilizer, Mass multiplication, Plant Growth Promoting Rhizobacteria (PGPR)

Introduction

Bio-fertilizers are one of the best modern tools for agriculture. It is a gift of our modern agricultural science. Bio fertilizers are applied in the agricultural field as a replacement to our conventional fertilizers. Conventional fertilizers contain compost; household wastes and green manure. Those are not as effective as chemical fertilizers. So, farmers often try to use chemical fertilizers in the field for crop development. But obviously the chemical fertilizers are not environment friendly (Mishra *et al*., 2013). Further, it presents recent developments in the area of field management that reveals the potential application of bio fertilizers and increased nutrient profiles, plant growth and productivity, and improved tolerance to environmental stress with a particular emphasis on mechanism of the feat of bio fertilizers. Bio fertilizers are usually

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prepared as carrier-based inoculants containing effective microorganisms. Incorporation of microorganisms in carrier material enables easy-handling, long-term storage and high effectiveness of bio fertilizers. Among various types of bio fertilizers, bacterial inoculant is one major group which includes rhizobia, nitrogen-fixing rhizobacteria, plant growth-promoting rhizobacteria, phosphate-solubilizing bacteria,. Basically, the carrier-based inoculant of these bacteria can be prepared by a common procedure. Several PGPR have been used worldwide as bio fertilizers, contributing to increasing crop yields and soil fertility and hence with the potential to contribute to more sustainable agriculture and forestry (Khalid *et al*., 2009). Some PGPR promote the growth by acting as bio fertilizer. Microorganisms mainly nitrogen fixer, phosphate solubilize and mycorrhizae are the main sources of bio fertilizer. The microorganisms used for the bio fertilizer are bacteria of *Bacillus*, *Pseudomonas*, *Lactobacillus*, photosynthetic bacteria, nitrogen fixing bacteria, fungi of *Trichoderma* and yeast (Bhattacharjee and Dey, 2014). *Rhizobium* is the most studied and important genera of nitrogen fixing bacteria (Pandit, 2015). Rhizobacteria group of *Pseudomonas* (*P. fluorescens*, *P. putida*, and *P. aeruginosa*) is known to be beneficial to plants. Some strains have long been known as a biological control agent. The bacteria are also known as plant growth promoting rhizobacteria (PGPR), either directly or as a result of its ability to control the disease. An explanation of some strains of *Pseudomonas* spp. associated with the plant, which can encourage the growth of plants or suppress plant diseases continues to grow, and knowledge of the mechanisms involved continue to increase (Widnyana and Javandira, 2016). *Trichoderma harzianum*, a filamentous fungus is used as a successful biological control agent to control different soil borne plant pathogens such as *Pythium* spp. *Rhizoctonia solani*, *Fusarium* spp., *Sclerotium rolfsii* etc. (Harman *et al*., 2004). *Trichoderma* spp. has also been exploited as a growth promoting agent. Thus it has the potential as a preferred input in Integrated Disease Management (IDM) systems (Kumar *et al*., 2014). *Vigna radiata* L. Wilczek (green gram), is economically one of the most important pulse crops of the *Vigna* group and is cultivated in many parts of Asia, Australia, West Indies, South and North America and tropical and subtropical Africa (Ali and Kumar, 2006). Being rich in quality protein, minerals and vitamins, it is an inseparable ingredient in the diets of vast majority of vegetation population in the Indian subcontinent. India is the largest producer of green gram in the world and accounts for 65% acreage and 54% production (Pratap *et al*., 2013). *Vigna unguiculata* L. Walp (cow pea), an annual crop, is one of the most important and widely cultivated legumes in the world, particularly in Africa, Latin America, and some parts of Asia and the United States .Cowpea cereal is a very important source of carbohydrates (63%)

and proteins (25%), with low fat content (1.5%), and are rich in vitamins, minerals (Ca, P, Fe), folate, thiamin, and riboflavin. Cowpea is chiefly used as a grain crop; however, it also finds use as animal fodder or as a vegetable (Muchero *et al*., 2009; Behura *et al*., 2015).

Materials and methods

Microorganisms

Bacillus subtilis, Pseudomonas fluorescens, *Rhizobium* and *Trichoderma* cultures were isolated from soil. All the cultures were maintained on King's B agar, Nutrient agar and Potato dextrose agar plate and incubated at 37℃ for 18–24 h.

Preparation of mother culture

The inoculums prepared by growing single colony for overnight culture streaked on to King's B, Nutrient and PDA agar slants and incubated at 37°C for 2–3 days.

Collection of carrier materials

Talcum powder purchased from The Precision Scientific Company (PSC), Coimbatore, Tamil Nadu, India. Lignite powder collected from Thirumurugan Mines, Palaiyur, Salem, Tamil Nadu, India.

Carrier preparation

Carrier materials were first powdered and passed through a 100 mesh sieve before physicochemical characterization. The main criteria used to select carrier materials were the ability to adjust the pH to neutral (pH 7.0), high water holding capacity, low cost, and wide availability. The pH of all of the materials was adjusted to pH 7.0 with $CaCO_3$ before use. Two carriers: (1) Talcum powder and (2) Lignite.

Carrier sterilization

For each of the two carriers, 500 g of carrier material was placed into each of 4 cotton bags (50 cm × 16 cm). The cotton bags were approximately 1.0 mm thick. These packages were sterilized by autoclaving. For irradiation, the

packages were placed in 0.08 mm thick polypropylene plastic bags. For the autoclaving treatment, the samples were autoclaved for 40 min at 121 ℃. The sterilized bags were placed into another large sterile cotton bag after cooling overnight in the autoclave. After sterilization, the packages were dried for 12 h at 60 ℃ in a blow-type oven.

Preparation of seed culture

A loop full of the mother culture is aseptically transferred to 5 ml of King's B, Nutrient and PDA broth taken in test tubes. The tube incubated in the shaker for 36–48 h till massive growth of culture occurs.

Mass multiplication of microorganisms

The organisms were revealed in suggested broth medium and sub-cultured in King's B, Nutrient and Potato dextrose broth media respectively. A loop full of *Bacillus*, *Pseudomonas*, *Rhizobium* and *Trichoderma* respectively was transferred to 10 ml of respective selective medium and incubated. After incubation, 1 ml of the inoculum was transferred to 500 ml of respective broth and kept in shaking incubator for mass multiplication.

Analysis of photosynthetic pigment contents

The photosynthetic pigments namely chlorophyll 'a', 'b' and carotenoid contents were determined according to the method of (Arnon, 1949).

$$\text{Chlorophyll 'a'} = \frac{(22.9 \times A663) - (2.69 \times A645) \times V}{W \times 1000}$$

$$\text{Chlorophyll 'b'} = \frac{(12.9 \times A663) - (4.68 \times A645) \times V}{W \times 1000}$$

$$\text{Carotenoid} = A480 - (0.114 \times A663) - (0.638 \times A645)$$

$$V = \text{Volume of the extract}$$

$$W = \text{Weight of the fresh leaves}$$

Results

Mass multiplication of microbes was used with talcum powder and lignite in the combinations (1:2) as carrier substrate for biofertilizers preparation (*Bacillus subtilis, Pseudomonas fluorescens, Rhizobium* and *Trichoderma*) (Fig.

1). The viability count of biofertilizer organisms in stored carrier material was individually carried out once in 15 days for a total period of ten months. The colony forming unit (CFU) more than 1.7×10^6 g^{-1} viable cell of *B. subtilis*, 8.6×10^6 g^{-1} viable cell of *P. fluorescens*, 4.7×10^6 g^{-1} viable cell of *Rhizobium* and 2.4×10^8 g^{-1} propagates of *Trichoderma* were observed in 1:2 combinations of culture and carrier materials (Fig. 2). The effects of carrier material and storage temperature on the viable cell number, pH and moisture content of the biofertilizers are important because the functioning and reliability of the biofertilizer to increase crop yield may be affected by it. Thus, it is important to emphasis on proper method of storage and temperature in which will prolong the shelf-life of the biofertilizer. Selection of the proper type of carrier materials is also very important. It should be able to sustain a high amount of bacterial inoculants for as long as possible. A high number of bacteria present and the capability to sustain for a long period of time ensure that the biofertilizer is in good condition and is readily applied to the soil.

Figure 1. Mass production of microorganisms

Figure 2. Preparation of microbial biofertilizer

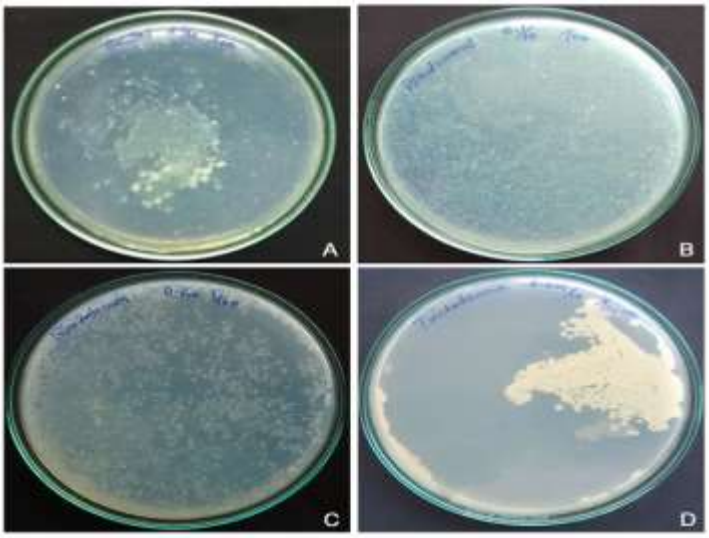

Figure 3. Viable cell count of inoculants in carrier materials by CFU **A**) *Bacillus*, **B**) *Pseudomonas*, **C**) *Rhizobium* and **D**) *Trichoderma*

Effect of various microbial biofertilizers on growth of Vigna radiata (green gram) and Vigna unguiculata (cow pea) plants after 45 days treatment

In the present study, green gram and cow pea plants were grown in pots supplemented with various microbial fertilizers to evaluate the effect on plant growth, shoot length, root length, fresh and dry weight. In general, green gram and cow pea plants treated with biofertilizers showed significant improvement in the growth like length of plant, shoot length and root length, fresh ad dry weight (Tables 1,2,3,). The photosynthetic pigment contents level of inoculated plants were significantly higher than compared to untreated control plants.

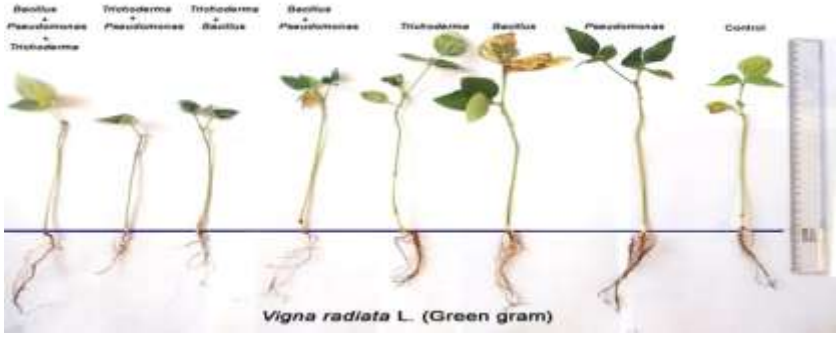

Figure 4. Effect of various microbial biofertilizers on treated and untreated (Control) green gram plants (45 days treatment)

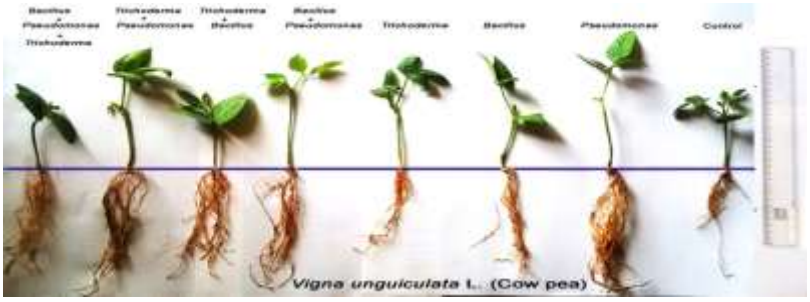

Figure 5. Effect of various microbial biofertilizers on treated and untreated (Control) cow pea plants (45 days treatment)

Table 1. Effect of various microbial fertilizers on plant height of *Vigna radiata* (green gram) and *Vigna unguiculata* (cow pea) 45 DAI

Treatment	Plant length (cm) (Mean ±SD)	
	V. radiata	***V. unguiculata***
Control	30.0 ±3.46	18.4 ±1.13
Bacillus	35.4 ±1.55	24.4 ±0.84
Pseudomonas	37.5 ±1.83	28.1 ±1.20
Trichoderma	34.5 ±2.12	24.6 ±3.04
Bacillus + Pseudomonas	29.0 ±2.34	27.3 ±1.55
Bacillus + Trichoderma	23.7 ±2.61	23.9 ±1.97
Pseudomonas + Trichoderma	23.0 ±1.76	26.7 ±1.83
Bacillus + Pseudomonas +Trichoderma	32.5 ±2.12	22.0 ±2.12

Table 2. Effect of various microbial fertilizers on shoot and root length of *V. radiata* (green gram) and *V. unguiculata* (cow pea) (45 DAI)

Treatment	***V. radiate***		***V. unguiculata***	
	Shoot length (cm) (Mean ±SD)	**Root length (cm) (Mean ±SD)**	**Shoot length (cm) (Mean ±SD)**	**Root length (cm) (Mean ±SD)**
Control	21.5 ±1.27	8.5 ±2.01	12.5 ±0.70	5.9 ±1.83
Bacillus	25.6 ±1.34	9.4 ±1.34	14.5 ±0.91	9.9 ±1.76
Pseudomonas	28.3 ±1.24	9.2 ±1.62	17.0 ±1.41	11.5 ±2.61
Trichoderma	27.0 ±2.16	7.0 ±0.95	13.7 ±1.76	10.9 ±1.27
Bacillus+ Pseudomonas	22.0 ±2.82	7.0 ±1.76	14.7 ±1.41	12.6 ±0.14
Bacillus+ Trichoderma	17.5 ±1.76	6.2 ±1.23	11.7 ±1.76	12.5 ±0.21
Pseudomonas+ Trichoderma	16.2 ±1.55	6.8 ±1.87	16.1 ±1.55	10.6 ±3.39
Bacillus+ Pseudomonas+ Trichoderma	21.5 ±2.89	11.0 ±1.41	12.0 ±2.82	10.0 ±0.70

Table 3. Effect of various microbial fertilizers on chlorophyll a, chlorophyll b and carotenoid contents of *V. radiata* (green gram) and *V. unguiculata* (cow pea) (45 DAI)

Treatment	*V. radiate*			*V. unguiculata*		
	Chlorophyll 'a' (mg/g FW)	**Chlorophyll 'b' (mg/g FW)**	**Carotenoid (mg/g FW)**	**Chlorophyll 'a' (mg/g FW)**	**Chlorophyll 'b' (mg/g FW)**	**Carotenoid (mg/g FW)**
Control	1.030±0.002	0.023±0.0007	3.068±0.021	1.079±0.0353	0.007±0.0095	0.947±0.042
Bacillus	0.820±0.001	0.027±0.0021	2.474±0.021	1.572±0.007	0.099±0.0021	1.505±0.007
Pseudomonas	1.171±0.066	0.029±0.0042	3.267±0.114	1.266±0.012	0.026±0.0070	1.022±0.098
Trichoderma	1.269±0.004	0.082±0.0007	3.391±0.067	1.258±0.005	0.024±0.0035	0.879±0.134
Bacillus+ Pseudomonas	1.248±0.004	0.049±0.0021	3.306±0.028	1.375±0.006	0.057±0.0014	1.135±0.176
Bacillus+ Trichoderma	0.883±0.108	0.012±0.1090	2.347±0.007	1.534±0.007	0.095±0.0035	1.457±0.183
Pseudomonas + *Trichoderma*	1.053±0.002	0.033±0.0021	3.051±0.012	1.019±0.019	0.065±0.0021	1.186±0.155
Bacillus + *Pseudomonas* + *Trichoderma*	1.431±0.003	0.044±0.0070	3.366±0.049	1.757±0.004	0.188±0.0028	2.008±0.176

Discussion

Phosphate solubilizing bacteria isolated from rhizosphere soil samples of maize and tomato plants. They were identified as *Bacillus megaterium* and *Pseudomonas aeruginosa*. Mass production was carried out at the optimized condition in submerged batch fermentation. These mass cultures can be used further as potential biofertilizer by packing in suitable carrier materials and added to rhizosphere soils directly or through application with the seeds (Prasad, 2014).

PGPBs increased growth and yield parameters of dry bean. In addition, some of the PGPBs suppressed the diseases of bean caused by natural bacterial and/or fungal infections (Tozlu *et al*., 2012). Molla *et al*. (2012) studied the *Trichoderma* composted kitchen wastes can serve as prospective biofertilizer for improvement in yield and quality of tomato cultivation.

The comparative effect of bacterial biofertilizers such as *Rhizobium*, Phosphobacteria and *Azospirillum* on growth and yield of green gram (*Phaseolus radiata* L.) and cowpea (*Vigna siensis* Edhl.) was studied. The bacteria were isolated from the soil samples and identified by staining and biochemical tests. The seeds were inoculated with bacterial biofertilizers with various treatments and showed in sterile polythene bag containing sterilized soil. After 65 days of plant growth, the morphological and bio-chemical parameters of cowpea were increased in combined inoculation of *Rhizobium*, Phosphobacteria and *Azospirillum* than green gram plants (Senthilkumar and Sivagurunathan, 2012).

Summary

Biofertilizers are becoming increasingly popular in many countries and for many crops. Biofertilizers are fertilizers containing living microorganisms, which increase microbial activity in the soil. The role of biofertilizer in agricultural production is of great importance. Inoculation of nitrogen fixing bacteria with bifertilizer increases the phosphorus level. The application of biofertilizer containing beneficial microbes showed a promoting effect on the growth of *Vigna radiata* (green gram) and *Vigna unguiculata* (cow pea) plants and improvement of soil properties through a 45 days greenhouse study. Among the various microbes, treated with *Pseudomonas* the plant height, fresh weight and dry weight were higher in *V. radiata* plant compared to *V. unguiculata*. The highest chlorophyll 'a' content (1.757 mg/g FW) was observed in combined microbes *Bacillus* + *Pseudomonas* + *Trichoderma* treated on *V. unguiculata* plant than *V. radiata.* Chlorophyll 'b' and carotenoid contents were also higher in the treatment of combined microbes *Bacillus* + *Pseudomonas* + *Trichoderma* treated on *V. unguiculata* plant compared to *V. radiata* and untreated control plants.

In conclusion, efficient plant nutrition management should ensure both enhanced and sustainable agricultural production and safeguard the environment. Microbial fertilizer has its advantages and disadvantages in terms of nutrient supply, soil quality and crop growth. Developing a suitable nutrient management system that integrates use of these kinds of microbial biofertilizers

may be a challenge to reach the goal of sustainable agriculture; however much research is still needed.

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