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Research Article

Usage of Encapsulated Plant Growth Promoting- Microbial Consortia and Testing its Efficacy on Cajanus Cajan

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ABSTRACT

From the past few decades, usage of plant growth promoting bacteria in the agricultural fields as individual inoculants is well known. But the usage of these microbial inoculants was limited due to the several disadvantages like inconsistency, survivability in different climatic conditions, host specificity etc. In the current research work, encapsulated PGP microbial consortium was designed, and its efficacy was tested in *Cajanus cajan*. The microbial consortium contains the plant growth promoting microbe viz. Bacillus megaterium (Microbial Type culture collection–2412), *Azotobacter chroococcum* (Microbial Type culture collection–3853) and *Pseudomonas flourescens* (Microbial Type culture collection–103) and *Trichoderma viride* (Microbial Type culture collection–793) and the total microbial load in the consortium was assessed to be $2.9x10^9$. Pot trial experiment was carried out to assess the efficacy of the Encapsulated Microbial Consortium (EMC) by comparing with the untreated control. When different parameters were compared *Cajanus cajan* plants treated with EMC showed good results *i.e* shoot length (125.5 cm), shoot weight (264.2 g), root length (42.4 cm), root weight (89.4 g), no. of branches (18 no's) and no. of pods (148 no's). Based on these research results, the encapsulated microbial consortium showed better efficacy and can be effectively used as seed inoculants.

Keywords: *Cajanus cajan*; Encapsulation; Microbial consortia

INTRODUCTION

Rhizosphere is the soil area in which microbes influence the root system by adhering to the plant roots up to a few millimetres [1]. The plants release several exudates as signalling molecules which will be attracted by the beneficial microbial communities [2]. Plant growth promoting bacteria are defined as the microorganisms which are involved in the crop production and the microbes which are isolated from the root zone of the plants are termed as the plant growth promoting rhizobacteria [3]. There are wide ranges of plant growth promoting rhizobacteria and each microbe has their own capability to enhance the growth of the plant [1].

Though the inoculants of *Azotobacter spp.*, *Azospirillum spp.*, *Rhizobium spp.*, *Pseudomonas flourescens*, *Bacillus subtilis* and *Thiobacillus novellus* are available in the market as individual inoculants, there inconsistency and lack of performance in different agro-climatic conditions have bought the consortia-based formulations into the light [4]. The microbial consortia-based formulations will improve the natural way of nitrogen, phosphorous and potassium uptake and protects the plant against plant pathogens providing the systemic acquired resistance [5].

Encapsulation of microbial cells in the polymeric gel matrix will enhance the survivability of the cells under harsh environmental conditions. Research studies revealed that alginate beads have the capability to entrap more microbial cells and enhanced mechanical strength [6]**.** In the current research work, the encapsulated microbial consortium was tested for its bio-efficacy by taking *Cajanus cajan* as host plant which is commonly called as Pigeon pea having rich protein content [7].

Present research work was aimed to encapsulate the microbial consortia with sodium alginate and testing its plant growth promoting parameters on *Cajanus cajan*.

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MATERIALS AND METHODS

Microbes and culture maintenance

All the plant growth promoting microbial cultures were sourced from Microbial type culture collection, Chandigarh. Bacterial cultures *viz. Bacillus megaterium* MTCC 2412, *Azotobacter chroococcum* MTCC 3853 and *Pseudomonas flourescens* MTCC 103 were subcultured on Soyabean casein digest agar slants and incubated at 30°C for 24-48 h. *Trichoderma viride* MTCC 793 was subcultured on Potato Dextrose agar slants and incubated at 28°C for 4-7 days. All the subcultured microbial cultures were stored in refrigerator at 4°C [8].

Development of microbial cultures

100 ml of Soya bean casein digest broth for each bacterial culture and 100 ml of Potato dextrose broth for fungal culture were prepared. Liquid medium was autoclaved at 121°C for 15 min and cooled. One loopful of each bacterial culture was inoculated into separate 100 ml Soya bean casein broth and kept on orbital shaker at 120 rpm for 24-48 h. Similarly, 4 mm of disc was cut from the fungal culture plate, inoculated into Potato dextrose broth and kept on orbital shaker at 120 rpm for 7 days. Liquid microbial samples were collected after the completion of incubation period to assess the microbial load. The broth samples were centrifuged by using the REMI C-24 BL and the cell pellet was collected. Total microbial cell count in the final broth and cell pellet were assessed by using the standard spread plate technique and the cell count was expressed in CFU.

Encapsulation of microbial cultures and formulation

100 ml of distilled water was taken, and 2 g of pre-gelatinized starch was added and boiled till it completely dissolves. 1g of inulin and 4 g of sodium alginate were added to the above starch solution and autoclaved at 121°C for 15 min. To this mixture 1 g of cell pellet from each microbial culture was added after cooling under aseptic condition and kept on orbital shaker for 30 min. 100 ml of flax seed oil with 0.01% Tween 80 was taken and pre sterilized. Flax seed oil was added to the sodium alginate mixture under aseptic condition and kept on orbital shaker for 15 min [8]. Formulated sample was drawn and total microbial count was assessed by using the standard spread plate technique. The formulated microbial consortium was further used for testing the plant growth properties in *Cajanus cajan*.

Bio-efficacy trials on *Cajanus cajan*

The encapsulated PGP microbial consortium was tested for its bio-efficacy on *Cajanus cajan*. Garden soil was sterilized 3 times consecutively for 3 days at 121°C for 30 min. Pots were segregated into 2 sets *i.e.* treated and control and drain holes of the pots were covered with the Whatmann filter paper No.1 discs. Pots were filled with sterilized garden soil. The *Cajanus cajan* seeds were surface sterilized with 0.1% $HgCl_2$ and 2 seeds per pot were sown. The seeds were coated with the formulated microbial consortia and were taken as treated and the uncoated seeds were taken as control. Treated and control seeds were sown in the respective pots. Whole experiment was carried out in green house under control environmental conditions. In the pot experiment, parameters like germination rate, root length, shoot length, root weight, shoot weight and no. of pods were assessed [5,7].

RESULTS AND DISCUSSION

In rhizosphere, the beneficial non-pathogenic microbial communities and plant growth promoting rhizobacteria will be in symbiotic relationship with the plants. Based on this symbiotic relation, microbial consortia-based formulation became well popular these days. The microbes in the consortia need to be compatible without showing any negative effect on each other [9].

The microbial cultures *viz. Bacillus megaterium* MTCC 2412, *Azotobacter chroococcum* MTCC 3853 and *Pseudomonas flourescens* MTCC 103 and *Trichoderma viride* MTCC 793 were subcultured and the agar slants were stored at 4°C. The microbial cultures were inoculated into the respective media and were incubated at the desired temperatures. The final broth samples and cell pellet samples were assessed for no. of Colony Forming Units (CFU) to check the microbial load Table 1. All the results are the average of 3 replicates performed.

As per the protocol mentioned above, the microbial cultures were encapsulated with the sodium alginate, formulated and microbial load was assessed to be $2.9x10^{9.}$ The formulated microbial consortium was further used for the bio-efficacy trials. The plant growth promotion trials were carried out on *Cajanus cajan* under standard conditions in the green house by using the sterilized soil in the pots. As per the previous research reports, highest germination percentage and less contamination was observed in the pots with sterilized soil [10]. The seeds were coated with the Encapsulated Microbial Consortia (EMC) at a dosage of 10 g/Kg of seeds and uncoated seeds were taken as control. Different parameters like shoot length shoot weight, root length, root weight, no. of branches and no. of pods were assessed. The plants were observed till the completion of crop period and the data was recorded. When shoot data was compared the encapsulated microbial consortia showed high shoot length and shoot weight *i.e* 125.5 cm with 264.2 g of weight (Figure 1).

Similarly, the root length and root weight of the *Cajanus cajan* plants were compared with high root length and root weight in EMC (Height - 42.4 cm and Weight - 89.4 g) when compared to the uncoated control (Figure 2).

No. of branches were counted to assess the plant growth and the results of both EMC and PGP were compared. Plants treated with EMC showed 18 branches whereas control plants showed 10 branches (Figure 3).

Similarly, no. of pods was counted for the treated and control which showed 148 pods for EMC and 79 pods for control (Figure 4). The *Bacillus spp.* are proven to be showing the plant growth promoting compounds viz. indole acetic acid, gibberellins, antifungal compounds, extracellular chitinase and phytase. Seed treatment formulations which have *Bacillus spp.* proven to be showing high yields in a wide variety of crops [11].

All the above parameters tested showed good yield parameters when compared to the uncoated microbial consortia. When the

Table 1: Assessing the microbial load in final broth and cell pellet samples.

Name of the microbe	Final broth (CFU/ml)	Cell pellet (CFU/g)
Bacillus megaterium	$3.4x10^{9}$	$2.3x10^{11}$
Azotobacter chroococcum	$1.0x10^{9}$	$9.1x10^{10}$
Pseudomonas flourescens	$4.2x10^{9}$	$3.7x10^{11}$
Trichoderma harzianum	$8.0x10^{8}$	$1.2x10^{10}$

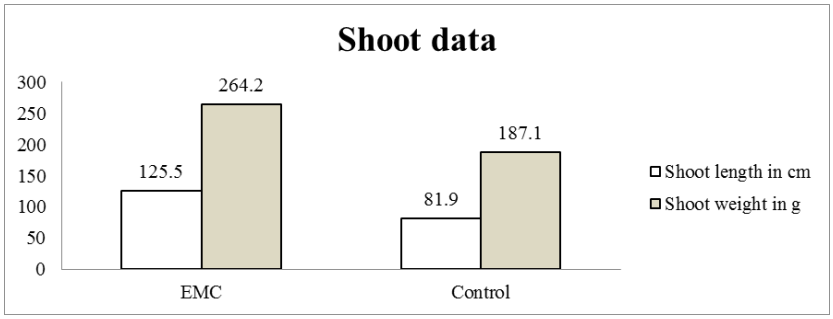

Figure 1: Shoot length and shoot weight data of *Cajanus cajan*.

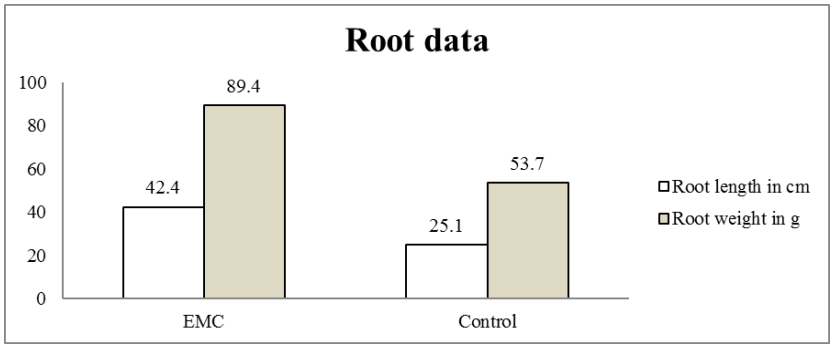

Figure 2: Root length and root weight data of *Cajanus cajan*.

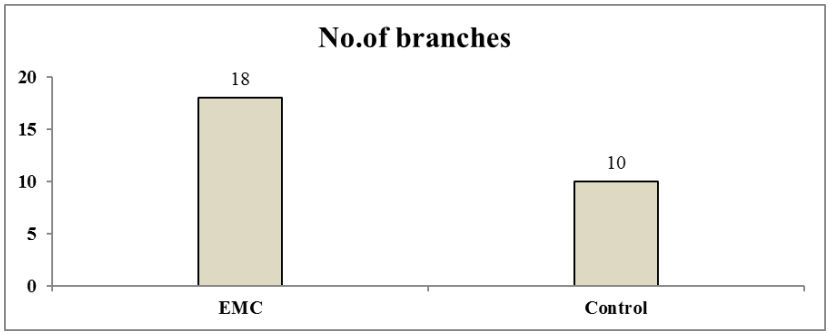

Figure 3: Graph showing no. of branches.

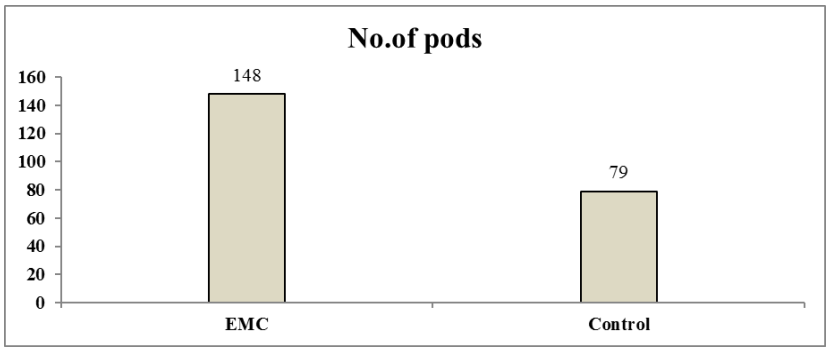

Figure 4: Graph showing no. of pods.

differences in both encapsulated and uncoated were compared the significance of variation is around 55-70%. This shows the enhancement of the yield when compared to the uncoated microbial consortia.

CONCLUSION

Based on the research study conducted, the encapsulated microbial consortium can be effectively used for the seed treatment as it was showing better efficacy when compared the untreated *Cajanus cajan* plants. As the PGP consortium is encapsulated with the biopolymers, the microbes present in the mixture can withstand even at harsh environmental conditions. Based on the above study, it can be stated that the microbes used in the formulation are compatible and has higher advantage in plant growth promotion when compared to the individual inoculants. So, the encapsulated microbial consortia can be effectively used in seed treatment. Further research studies need to be carried out to take this technology to the large scale. The major hurdle faced, will be

the stabilization of the formulation which will be focussed on the further line of research.

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