Original Research Article

Carrier Based Formulation of Plant Growth Promoting *Bacillus* Species and their Effect on Different Crop Plants

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**Abstract**

Rhizospheric microbes have immense potentiality to synthesize and release various compounds, that are regulating plant growth as well as physical and chemical texture of the soil. In this small piece of research, we evaluated the plant growth promoting activity of two different carriers such as charcoal and talc based formulation of *Bacillus* species. It was observed that, the bio-inoculants were able to enhance the organic carbon, nitrogen, phosphorous and potassium in soil, there by promoting growth of test crop plants such as mung bean (*Vigna radiata* L.) and rice (*Oryza sativa* L.). Charcoal based formulation depicts higher plant growth promoting activity in comparison with other carrier. Moreover, the *Bacillus* specie showed antagonistic effect against different phytopathogens including *Rhizoctonia solani* (ITCC-186) and *Fusarium oxysporum* (ITCC-578). Thus, the charcoal based formulation of *Bacillus* specie can be used for plant growth promoting activity of various crops. Before field application extensive research is highly indispensable in this regard.

**Keywords**

*Bacillus*, Formulation, Plant growth, Phytopathogen, Charcoal.

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**Introduction**

With advent of civilization, population explosion has demanded more space for industrialization, urbanization resulting decrease in agricultural land. The present day problem is to produce food grain with the available land without affecting soil health has become a great challenge to scientists. Soil harbors a wide array of microbes, among them several beneficial bacteria are colonizing in the rhizospheric region their by promoting growth of plant. Such type bacteria are generally affiliated as PGPR (Plant Growth-Promoting Rhizobacteria). Plant growth promotion by the PGPR can be either through stimulating plant growth by the production of phytohormones or by the application of bio inoculants to control various plant diseases (Glick, 1995; Bashan and de-Bashan, 2005; Bloemberg and Lugtenberg, 2001; Sivakumar et al., 2014).

In the present scenario development of carrier based formulation of bio-inoculant is an industrial skill to renovate a promising laboratory documented bacteria to a commercial profitable field product (Bashan, 1998). Formulation characteristically should contain active constituent or ingredient in a suitable carrier with additives that will assist in the stabilization and perform as protective shield of the bacterial cells during storage, transportation and at the target region. It is
easy to handle, increase the activity of the organism in the field, cost-effective and convenient for field applications. For this bio-agent dependent technology, screening of microbes for desirable traits, selection of potential strains and inoculum development are important steps.

Bashan (1998) reviewed that, viability of inoculum in a suitable formulation for a definite duration is vital for the commercialization of the product. Importance of formulation is to obtain the desired benefit when applied to soil by maintaining the bacterial cell and the active constituent to be in a metabolically and physiologically competent state.

According to Cassidy et al., (1996), immobilization of bacterial cells into polymer matrix has confirmed to be beneficial over direct inoculation to the soil.

A major purpose of bacterial inoculant formulation is to offer more suitable micro-habitat for survival in the soil ecosystem. Moreover, for field applications use of encapsulated cells has several advantages over free cell formulations namely, protection from biotic stresses (Smit et al., 1996) and abiotic stresses such as the inhibitory effect of toxic compounds (Cassidy et al., 1997), enhanced survival and improved physiological activity (Weir et al., 1995), supply of encapsulated nutritional additives (Trevors et al., 1993), increased cell densities and preferential cell growth in various internal aerobic and anaerobic zones of encapsulating gel. In view of this, the small piece of research is focused towards evaluation of biocontrol efficacy of the potential PGPR isolate against different phyto-pathogens and development of carrier based formulation of PGPR isolates and study their effect on growth of Mung bean and Rice plant by pot culture method.

Materials and Methods

Inoculum preparation for green house study

Previously isolated Bacillus species (Pradhan et al., 2015) was taken from the glycerol stock and streaked onto nutrient agar. Single colony of the bacteria was inoculated and grown in tryptone yeast extract broth with constant shaking at 150 rpm for 48 h at room temperature. The culture obtained at stationary phase was centrifuged at 6000 rpm for 10 min and bacterial cells re-suspended in phosphate buffer (100 mM, pH. 7.0). The cell concentration was adjusted to 9 × 10^8 cfu/ml. (0.3 OD at 595 nm = 10^8 cfu/ml).

Talc-based formulation of Bacillus species

The talc-based formulation was prepared by following the method described by Vidhyasekaran and Muthamilan (1995). A loopful bacterial culture was inoculated into the tryptone yeast extract broth and incubated in a rotary shaker at 150 rpm for 48 h at room temperature (25 ± 2°C). One kg of sterilized talc powder was taken in a metal tray and its pH was adjusted to neutral by adding CaCO₃ at the rate of 15 g/kg. 10 gm of CMC was added to 1 kg of talc powder and mixed well. This mixture was autoclaved for 30 min on each of two consecutive days. The 400 ml of 48 h grown bacterial suspension containing 9 × 10^8 cfu/ml was mixed with carrier-CMC mixture under aseptic conditions. After drying overnight in laminar air flow hood, it was packed in polypropylene bag, sealed and stored at room temperature (25 ± 2°C).

Charcoal-based formulation of Bacillus species

Charcoal-based formulation was developed as described by Trivedi et al., 2005. The bacterial culture was grown in on tryptone
yeast extract (TYE) broth at 28 ± 2°C for 24–48 h rising the final concentration of 9×10⁹ cfu/ml. 150 gm of sterile charcoal was mixed with 150 ml bacterial suspension and 10 gm of gur (local sugar) was added. The slurry was mixed properly under aseptic conditions and air dried at 28 ± 2°C overnight in a laminar flow hood.

**Greenhouse study**

The seeds of Mung bean (OUM-11-5) and Rice (Lalat) were obtained from the Department of Agronomy, OUAT and these seeds were soaked overnight in water, surface sterilized with 0.2% HgCl₂ solution for 2-3 min and air dried for 15 min. The seeds were soaked in double volume of sterile distilled water containing different formulation (10 gm/L) (Salaheddin et al., 2010) and the treated seeds were shade dried for 30 min. Total 4 kg of sterilized soil was taken in each pot and the holes of the pots were closed to prevent of drainage of water. The bacteria treated seeds were showed in soil (diameter 0.25 m; height 0.3 m) at the rate of 7 seeds per pot and un-inoculated seeds were served as control. After 45 days the total chlorophyll content in leaf was measured by using the method stated by Arnon (1949) and growth parameters such as root length, shoot length and Biomass were recorded after harvesting. Physico-chemical parameters such as organic carbon, pH, Electrical conductivity (ds/m), available N (kg/ha), P₂O₅ and K₂O (kg/ha) of the soil and total bacterial population in each pot were also studied in regular interval.

**Testing of *in vitro* antagonism**

The antagonistic effect of *Bacillus* sp. was tested for by duel culture method against two common plant pathogen *Rahizoctonia solani* (ITCC-186) and *Fusarium oxysporum* (ITCC-578). Spores of fungal cultures grown on patato dextrose agar medium (PDA). A 5mm diameter mycelial agar disc was cut from the margin of 7-day-old fungus culture and placed on one side of a 9 cm Petri dish containing PDA medium and test bacteria was streaked on the other end of the Petri dish. Plates were incubated at 28ºC±2ºC for 5 to 8 days. Dishes inoculated only with test pathogens served as controls. The percent of inhibition of each fungus was measured using the formula (Vincent, 1927): Inhibition percentage (%) = (R1-R2) / R1 X 100 where R1 is radial growth of mycelia in control and R2 is radial growth of mycelia in treatment.

**Statistical analysis**

All the experiment was done in triplicate and the data was analyzed statistically by one way ANOVA at p<0.05 significant level.

**Results and Discussion**

Carrier based formulation protect the bacteria against many environmental stress; release to the soil, slowly but in large quantities. In the present study it was found that, talc based and charcoal based formulations of *Bacillus* species effectively increase the growth of Mung bean and rice when it was applied as seed treatment. Increased root and shoot elongation was apparent in PGPR treated seeds compared to control. Several strains of *B. subtilis* have proven to be efficient in plant growth promotion (Bai et al., 2003). In case mung bean and rice, the highest root elongation, shoot elongation and increase in total biomass in respect to the control observed when the seeds were treated with different carrier based formulation (Tables 1 and 2). Highest root (22.83 cm) and shoot elongation (43.53 cm) was recorded in case of mung bean and 22.67 cm root length and 75.97 cm shoot length was observed in case of rice when seeds were pre-treated with Charcol-based formulation. The total chlorophyll content of rice and mung bean of different treatment were also recorded (Table 5).
Table 1: Effect of different bacterial formulation on shoot length, root length and biomass of mung bean

| Sample                  | Root length(cm) | Shoot length(cm) | Biomass(gm) |
|-------------------------|-----------------|------------------|-------------|
| Control                 | 17.37±0.56      | 35.73±0.45       | 22.17±0.47  |
| Charcoal based formulation | 22.83±0.52      | 43.53±0.67       | 31.58±0.89  |
| Talc based formulation  | 20.66±0.47      | 42.04±0.14       | 26.62±0.75  |

Values represent mean ±SE and highly significant at p <0.05

Table 2: Effect of different bacterial formulation on shoot length, root length and biomass of rice plant

| Sample                  | Root length(cm) | Shoot length(cm) | Biomass (gm) |
|-------------------------|-----------------|------------------|-------------|
| Control                 | 15.36±0.3       | 37.06±0.5        | 19.88±0.31  |
| Charcoal formulation    | 22.67±0.52      | 75.97±0.44       | 25.90±0.34  |
| Talc formulation         | 20.97±0.84      | 71.11±0.31       | 21.66±0.35  |

Values represent mean ±SE and highly significant at p <0.05

Table 3: Effect of formulated bacteria on soil physico-chemical parameter of Mung bean

| Soil parameter            | pH | E.C(ds/m) | O.C(%) | N(kg/ha) | P₂O₅(kg/ha) | K₂O (kg/ha) |
|--------------------------|----|-----------|--------|----------|-------------|-------------|
|                          | BS | AH        | BS     | AH       | BS          | AH          |
| Control                  | 6.81±0.01 | 6.81±0.01 | 0.011±0.01 | 0.011±0.01 | 0.211±0.01 | 0.223±0.01 |
| Charcoal formulation     | 6.81±0.01 | 6.85±0.01 | 0.011±0.01 | 0.014±0.01 | 0.211±0.01 | 0.529±0.01 |
| Talc formulation         | 6.81±0.01 | 6.82±0.01 | 0.012±0.01 | 0.011±0.01 | 0.211±0.01 | 0.423±0.01 |

Values represent mean ±SE and highly significant at p <0.05

Table 4: Effect of formulated bacteria on soil physico-chemical parameter of rice

| Soil parameter            | pH | E.C(ds/m) | O.C(%) | N(kg/ha) | P₂O₅(kg/ha) | K₂O (kg/ha) |
|--------------------------|----|-----------|--------|----------|-------------|-------------|
|                          | BS | AH        | BS     | AH       | BS          | AH          |
| Control                  | 6.81±0.01 | 6.81±0.01 | 0.011±0.01 | 0.011±0.01 | 0.211±0.01 | 0.218±0.01 |
| Charcoal formulation     | 6.81±0.01 | 6.85±0.01 | 0.011±0.01 | 0.014±0.01 | 0.211±0.01 | 0.538±0.01 |
| Talc formulation         | 6.81±0.01 | 6.82±0.01 | 0.012±0.01 | 0.011±0.01 | 0.211±0.01 | 0.422±0.01 |

Values represent mean ±SE and highly significant at p <0.05; BS= Before Sowing Ah= After harvest
Table 5 Estimation of total chlorophyll content in Rice and Mung bean plant

| Sample    | Control (mg/g) | Talc formulation (mg/g) | Charcoal formulation (mg/g) |
|-----------|----------------|-------------------------|----------------------------|
| Mung bean | 3.37±0.04      | 4.65±0.04               | 4.76±0.11                  |
| Rice      | 1.65±0.23      | 3.63±0.10               | 4.18±0.30                  |

Values represents mean ±SE and highly significant at p < 0.05

Table 6 Total bacterial population of Mung bean soil in different time interval

| Soil Parameter | Initial day | 30 Days (CFU/gm) | 60 Days (CFU/gm) | 90 Days (CFU/gm) | 120 Days (CFU/gm) |
|----------------|-------------|------------------|------------------|------------------|-------------------|
| Control        | 3.4±0.02×10^5 | 3.47±0.14×10^5   | 3.47±0.20×10^5   | 3.53±0.24×10^5   | 3.63±0.24×10^5    |
| Charcoal formulation | 3.4±0.02×10^5 | 5.87±0.12×10^5   | 6.23±0.24×10^5   | 6.47±0.2×10^5    | 6.63±0.29×10^5    |
| Talc formulation | 3.4±0.02×10^5 | 5.43±0.20×10^5   | 5.9±0.13×10^5    | 6.1±0.2×10^5     | 6.37±0.18×10^5    |

Values represents mean ±SE and highly significant at p < 0.05

Table 7 Total bacterial population of rice soil in different time interval

| Soil parameter | Initial | 15 days (CFU/gm) | 30 days (CFU/gm) | 45 days (CFU/gm) | 60 days (CFU/gm) |
|----------------|---------|------------------|------------------|------------------|------------------|
| Control        | 3.4±0.02×10^5 | 3.42±0.03×10^5  | 3.43±0.03×10^5   | 3.43±0.04×10^5   | 3.58±0.15×10^5   |
| Charcoal formulation | 3.4±0.02×10^5 | 4.0±0.11×10^5    | 4.3±0.12×10^5    | 4.4±0.18×10^5    | 4.6±0.11×10^5    |
| Talc formulation | 3.4±0.02×10^5 | 3.75±0.17×10^5   | 4.13±0.08×10^4   | 4.23±0.12×10^3   | 4.37±0.14×10^5   |

Values represents mean ±SE and highly significant at p < 0.05

Table 8 In vitro antagonistic effect of Bacillus sp. on mycelial growth of different plant pathogens

| Isolate No.   | Inhibition % |          |          |
|---------------|--------------|----------|----------|
|               | Rhizoctonia solani | Fusarium oxysporum |
| Bacillus species | 61.49 ± 0.69  | 59.33 ± 0.48 |

Values represents mean ±SE and highly significant at p < 0.05

It was also found that, the bio-inoculants were able to increase the organic carbon, nitrogen, phosphorous and potassium in soil, there by promoting growth of mung bean and rice (Tables 3 and 4) in respect to control. It was well established fact that the microbial members of soil communities are the most sensitive and rapid indicators for soil quality evaluation (Zelles, 1999; Zornoza et al., 2009). The pH of soil is one of the most important physicochemical parameter, which influence the mineral nutrient of soil quality and microorganism activity (Saseeswari et al., 2015). In the present investigation it was observed that the bacterial population in all the treatments was increased in respect to the control (Tables 6 and 7). The potential Bacillus species was tested for in vitro antagonism against Rhizoctonia solani and Fusarium oxysporum and showed positive result (Table 8). It was already proved that plant growth promoting rhizobacteria can protect the plant from different types of pathogen (Raupach and Kloepper, 1998). Result of the current study showed the positive impacts of Bacillus specie. on growth.
of mung bean and rice plant compared to the control. So as a simple and safe method, bacterization of seeds could be a promising technique for improvement of plant growth efficiency. Thus, the potential bacteria Bacillus sp. further investigated to increase productivity under field condition and use of PGPR as inoculants bio fertilizers is a novel approach to replace chemical fertilizers and pesticides for sustainable agriculture in India.

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