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# Bio-control Activity of Composts for Supporting Growth of Pearl millet

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***Abstract:*** *The use of compost is very important to sustainable crop growing. Plant growth-promoting bacteria (PGPB) were studied in this work to affect the enlargement, yield and nutrient uptake by a selection of methods. Seven various plant growth-promoting characters and antagonistic capacity was selected to screen bacteria isolated from various composts. Growth supporting capability of bacteria was studied on seed germination and biomass of plant. Ten isolates stand on various plant growth-promoting characters and seed vigor index were estimated at greenhouse for plant growth-promoting action on pearl millet. Maximum increase in plant weight was by P. fluorescens ISR 33 (1110 mg), S. marcescens ISR75 (772 mg). Plant growth-promoting action of composts and bacteria was considered mutually. Application of composts with bacteria improved plant growth that P.fluorescens ISR 33 increased the shoot length maximum (53cm) and B. diffusa ISR 35 showed the minimum increment (44cm). Leaf area was found maximum (51cm$^2$) in case of Bacillus circulans ISR 35 and minimum (41 cm$^2$) in Boerhaavia diffusa ISR 35. Root length density and Plant dry weight was found maximum in P. fluorescens ISR 33 and minimum in S. marcescens ISR75.*

***Keywords:*** *PGPB, Compost, Seed germination, Biomass, Greenhouse, Pearl millet*

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## I. Introduction

Plants are broadly occupied by a series of useful microorganisms and obtain a range of plant–microbe relations. Few of these interactions are useful, whereas few are harmful for the plant. Mycorrhizal association helps the plant to get nutrients from the soil [1, 2]. Crop deposits are the chief by-products in gardening [3]. The majority of microorganisms use plant biomass like crop residues in the form of food or energy source. Some microorganism can grow on solid substrate in absence of moisture; they can be used in degradation of crop residues [4]. Mycorrhizal associations are significant in make positive plant growth through escalating nutrient and water uptake and enhancing plant struggle to abiotic and biotic hassle [5]. Special bacterial species have been recognized taking over plant tissues from plant parts. Rhizobium bacteria are capable to increase nutrient uptake from the soil by the plants that they settle. The species like Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Klebsiella, Pseudomonas, and Serratia are growth-promoting bacteria [6]. Special observations exist to recognize what comprise a sustainable management method. If suitably distinguished, soil quality can serve as an indicator of on-going conservation and degradation process. It depends exceedingly on the nutrient substance, biological and microbiological element of the soil eco-system and manipulates crop yield and quality [7]. Soil biological factor can has potential as before time and susceptible indicators of soil ecological pressure [8]. Soil microorganisms are strongly and responsive biological markers existing and must be helpful for classification of troubled or infected systems [9]. In this study, microorganisms were isolated and screened from three unlike composts for plant growth-promoting features. Preferred bacterial isolates were assessed for their growth support of pearl millet along with composts.

## II. Materials and Methods

Composts were organized by three unlike undeveloped wastes at different farms of Bulandshahr, UP, India. Compost was ready in an above-soil surface brick chamber (150 cm×90 m×100 cm) that received farm and kitchen waste. A little population of macro fauna (10–15/kg compost) such as centipedes, earthworms, snails, and slugs were already present in compost. Macro fauna were positioned unconnectedly in sterilized high-density polyethylene bags (20 cm in width and 11 cm in length) after rinse with distilled water to isolate bacteria from their excreta. One gram of excreta was mixed in 9 ml saline (0.9% NaCl) and suitable dilutions were poured on nutrient agar (NA). Ten macro fauna were placed in Petri dish, dehydrated to death, and appropriate dilutions of surface washings in saline were poured on nutrient agar for isolating bacteria from surface. Rice-straw compost (RSC) was prepared in heaps unlike the old method of using digesters for compost preparation [10]. Multiple heaps (5 m long x 1.5 m wide x 1.5 m high) of 500 kg capacity were prepared for

composting. The weight of 5-10 kg bundles of air-dried rice-straw were tied up in plastic nets or twine and dipped in water (every 1 kg dry straw soaked in 1.5 L water) for 2 to 4 minutes and then allowed to drain for 6 minutes. The dripping were captured on a tarpaulin sheet and recycled. Moistened straw was allowed to stay in a heap and covered with polythene sheet to reduce evaporation losses and to prevent it from getting extra moisture from rain during the composting period. Each heap was covered on all sides with at least 10 cm thick layer of moistened rice-straw (non-experimental). Care was taken to maintain wetness of contents and water was applied using a specially designed lance. Temperature and relative humidity was recorded using Hobo data logger (from Onset Computer Corporation, USA), which helped to decide the time for watering. After maturity period of compost, contents of the heap were passed through a pulverizer and used. Compost was prepared in cement cylinder (100 cm diameter x 45 cm height) with foliage and branches of Saranga *(Gliricidia sepium)* , by soaking in 1.5 % cow dung slurry. After 15 days, approx 600 earthworms were released into the same container. Seven layers of foliage of 12 cm thickness of each were added after decaying of lower layer. Compost formation obtained about 50–60 days at 65-70% moisture. After 1-2 months, 10 g of the compost was used and suitable dilutions were poured on nutrient agar to isolate bacteria. These colonies were re-cultured from all samples and conserved as glycerol stock at −15°C. These isolates from Farm waste compost and soil organisms associated, *Gliricidia* vermicompost and Rice-straw compost were selected as *Bacillus* sp., *Boerhaavia* sp. and *Pseudomonas* sp.

**Plant growth promoting bacteria in different medium**

About 100 different bacteria were tested for gram staining. About 200 µl of inject of every isolate grown in LB broth was added to the well of sterilized inoculator that transferred ~1000 cells to media plates for instantaneous inoculation of 20 isolates [11]. Modified M9 medium g/L [12] for cellulose utilization, Pseudomonas isolation agar (g/L) for fluorescent pseudomonads, Phosphate solubilization by [13], Phytase production with phytic acid [14] Dworkin foster minimal medium (g/L) by [15] for ACC deaminase, Indole acetic acid (IAA) production [16], Siderophore production [17] and Hydrolysis of chitin by [18]. Hydrogen cynide making was analyzed on petri dish having Kings B agar medium modified with glycine (4.4 g /l) with filter paper dipped in picric acid in the upper cover and closed with Parafilm. After poring, plates were kept warm at ~31°C for 1- 6 days as essential support on the trait. On evaluation the positive bacterial separates for plant growth was measured. Selection was completed thrice with two replications each.

**Assessment of antagonistic fungi**

About 100 separates were monitored in test-tube against *Botrytis cinerea***,** *Macrophomina phaseolina***,** *Rhizoctonia solani* and *Fusarium fujikuroi* on PDA medium using dual culture technique. An agar block was cut from an actively growing (4 days old) fungal culture and poured it on the surface of freshly prepared agar medium in the middle of Petri dish. One day old culture of each bacterium was streaked on surface of medium. Plates inoculated with the similar fungus with no bacteria were used as control. For every action only three replications were continued. Plates were placed at 31ºC and the inhibition zone between the two cultures was calculated **5** days after inoculation. Percent inhibition of fungi was calculated as:

$$\text{Percent inhibition (I)} = \frac{(C - P)}{C} \times 100$$

Where**,**

C is growth of fungus in the control plate (mm), and

P is growth of fungus on the plate inoculated with bacterium (mm).

**Recognition of selective bacteria**

Ten separates recognized as per Bergey's manual [19] and 3 isolates identified at Standard Analytical Laboratory, New Delhi, India for confirmation.

**Seed bacterization**

*Pennisetum glaucum* nuts were cleaned outside with 1% sodium hypochlorite for 4-5 min and cleaned 3-4 times with distilled water. Seeds were layered with peat based inoculum of bacteria (105−106/g peat) using 1% carboxymethylcellulose as adhesive, dehydrated in air and the cell count was $10^6$–$10^7$ CFU/ seed prior to sowing.

**Assessment of composts on plant growth in greenhouse conditions**

Based on plant growth-promoting features germination % and seed vigor, 10 bacterial isolates were preferred and assessed separately for growth of pearl millet cultivar ICMV 221. Normal soil was used as potting medium in cemented pots. Ten bacteroid, nuts were disperse in every pot and thinning was prepared to 5 per pot 7 days after germination. Seeds coated with *Azotobacter chroococcum* Mac-27 were used as positive control [20] and only with peat served as control. RSC, FWC, and GVC each separately and in mixture with

three bacterial strains, EB 35, EB 67, and CBD 35 were independently assessed for growth of pearl millet. Compost was mixed to the soil prior to filling the pots in a ratio 5 tons per hectare and ten bacteroid nuts were disperse in each pot and thinned to five per pot.

**Measurements for Plant growth**

Plants were watered once every 2-3 days with 20 ml distilled water. All pots were watered by weight once a week to achieve field capacity of the potting mix. Temperature in the greenhouse ranged from 24 - 32°C. Subsequent to one month, stem length, leaf size, length of root and dry matter of both root and stem models and oven-dried at 70°C to constant weight were measured.

**Analytical characterization of compost**

The pH and electrical conductivity (EC) were determined by using pH-meter and EC-meter respectively. Total nitrogen, $NO_3^-$, $NH_4^+$, $SO_4^{2-}$, Phosphorus ($P_2O_5$), potassium ($K_2O$) was determined. Many elements like Mg, Ca, Fe, Cu, Zn, B, etc. were determined by using inductively coupled plasma atomic emission spectroscopy. The nine samples of AC and AGVC were analyzed and the means of the parameters were calculated. T-test was carried out to validate the significance of the differences among compost and vermin-compost composition. The SPSS 24.0 program was used for the calculations.

## III. Result And Discussion

Population of bacteria was log 7.5, 6.5 and 5.5 CFU $g^{-1}$ in FWC, RSC and GVC respectively. Bacterial inhabitant on body surface of macrofauna was log 4.3 for centipedes, log 5.1 for earthworm and log 5.3 for slugs CFU macrofauna$^{-1}$. Bacteria in dropping of macrofauna were log 3.7 for centipedes, log 5.6 for earthworms, log 6.3 for slugs and log 5.0 for snails CFU macrofauna$^{-1}$ $h^{-1}$. Population of fungi was low compared to bacteria in all the sources characterized and ranged from log 2.2 in GVC, 2.4 in FWC and 3.6 in RSC $g^{-1}$ (Table no 1). There were no fungi found on body surface of centipede and slugs and excreta of centipedes, slugs and snails (at $10^{-1}$ dilution) in this work. This research examined only initial detection of these macrofauna and only three of them were identified, earthworm (*Eisenia foetida*), garden centipede (*Lithobius* forficatus and garden slug (Limnx sp.).

**Table no 1:** Inhabitants of microorganisms in composts and macrofauna

| Resource | Bacteria ($Log_{10}$ $g^{-1}$)[a] | Fungi ($Log_{10}$ $g^{-1}$)[a] |
|---|---|---|
| FWC | 7.5 | 2.4 |
| RSC | 6.5 | 3.6 |
| GVC | 5.5 | 2.2 |
| Centipede body surface | 4.3 | NF |
| Earthworm body surface | 5.1 | 2.6 |
| Slug body surface | 5.3 | NF |
| Centipede excreta | 3.7 | 3.1 |
| Earthworm excreta | 5.6 | 3.4 |
| Slug excreta | 6.3 | NF |
| Snail excreta | 5.0 | NF |

Gram negative bacteria were least at 34 % in RSC and maximum at 56% in macrofauna and Gram-positive were least at 44% in macrofauna and maximum at 62% in RSC. RSC had cellulose-degrading bacteria as a dominant class (88%) while in the other three sources, this trait was present in 22 to 32% of the bacteria. *Pseudomonas fluorescens* as model organism was found maximum in GVC (14%) RSC and while in the other two sources, *P. fluorescens* was absent (Table no 2).

**Table no 2**: Bacterial % with different Characters isolated from RSC, FWC, GVC and macrofauna

| Characters | RSC (n=25) | FWC (n=12) | GVC (n=22) | Macrofauna (n=41)* |
|---|---|---|---|---|
| Gram-positive | 62 | 55 | 50 | 44 |
| Gram-negative | 34 | 41 | 46 | 56 |
| *P. fluorescens* | 8 | 0 | 14 | 0 |
| Cellulose degradation | 88 | 30 | 22 | 32 |

n =Number of bacterial isolates,

Population of P-solubilizers (1– 4%), chitinase producers (7–8%), fluorescent Pseudomonas (10-17%), HCN (10–15%), and phytase producers (15–24%) were present in the resources used in this research and relatively lesser than the other sets of bacteria. Siderophore producers were marginally similar in RSC (45%),

GVC (42%), 30% in macrofauna and minimum in FWC (14%). Considerable population of siderophore producers in this work also exposed that composts supply a favorable location for antagonistic bacteria that promote plant growth. IAA producers were 55% in GVC followed by 34% in RSC, 24% in macrofauna and 5% in FWC. ACC deaminase producers were 54% in RSC, 48 % in GVC, 24% in macrofauna and minimum 14% in FWC (Table no 3). ACC deaminase producers support root and plant growth by hydrolyzing ACC and reducing ethylene biosynthesis in roots.

**Table no 3**: Plant growth-promoting characters (%) isolated from RSC, FWC, GVC and macrofauna

| Characters | RSC (n=25) | FWC (n=12) | GVC (n=22) | Macrofauna (n=41)* |
|---|---|---|---|---|
| P- solubilization | 2 | 1 | 2 | 4 |
| Chitinase production | 0 | 0 | 8 | 7 |
| *Fluorescent Pseudomonas* | 10 | 0 | 17 | 0 |
| HCN production | 10 | 0 | 15 | 0 |
| Phytase production | 15 | 20 | 21 | 24 |
| Siderophore production | 45 | 14 | 42 | 30 |
| IAA production | 34 | 5 | 55 | 24 |
| ACC deaminase production | 54 | 14 | 48 | 20 |

IAA = Indole acetic acid, ACC 1- Aminocyclopropane 1-carboxylic acid, HCN = hydrocyanic acid

Percent of bacterial isolates that had antagonistic activity against *B. cinerea* was 40% in GVC, 30 % in RSC, 24% in macrofauna and minimum 20% in FWC. M. phaseolina was 39% in GVC, 36% in RSC, 20% in macrofauna and minimum 18% in FWC. *R. solani* was maximum 45% in RSC, 42% in GVC, 27% in macrofauna and minimum 22% in FWC. *F. fujikuroi was maximum in* 43% in RSC and minimum 18 % in FWC (Table no 4).

**Table no 4:** Antagonistic Characters against different fungi isolated from RSC, FWC, GVC and macrofauna

| Characters | RSC (n=25) | FWC (n=12) | GVC (n=22) | Macrofauna (n=41)* |
|---|---|---|---|---|
| *Botrytis cinerea* | 30 | 20 | 40 | 24 |
| Macrophomina phaseolina | 36 | 18 | 39 | 20 |
| *Rhizoctonia solani* | 45 | 22 | 42 | 27 |
| *Fusarium fujikuroi* | 43 | 18 | 39 | 25 |

Pseudomonas species was leading among antagonistic bacteria in our work (Table no 6) and earlier research discovered that they make a wide range of antibiotics and cell wall-degrading enzymes and encourage plant growth [21].

**Greenhouse evaluation of plant growth-promoting activity**

Plant growth promoting traits of 10 isolates (Table no 5) demonstrated considerable boost in germination and seed-vigor index of bajra tested using paper towel technique in vitro.

**Table no 5:** Plant growth-promoting traits of selective bacterial isolates characterized in vitro

| Isolate | P solubilization | Phytase | Siderophore | Chitin | ACC deaminase | IAA | HCN | Biocontrol* |
|---|---|---|---|---|---|---|---|---|
| *Bacillus licheniformis* ISR13 | − | − | − | − | − | − | − | ++++ |
| *Bacillus circulans* ISR 35 | − | + | − | − | − | − | − | ++++ |
| Boerhaavia *diffusa* ISR 37 | − | − | − | + | − | − | − | ++ |
| Boerhaavia *diffusa* ISR 35 | + | − | + | + | + | − | − | ++ |
| *Serratia marcescens* ISR 67 | + | − | + | + | + | − | − | ++ |
| *Serratia marcescens* ISR75 | − | + | + | − | + | + | + | ++ |
| Pseudomonas *fluorescens* ISR 35 | + | + | + | − | + | − | − | ++ |
| *Pseudomonas fluorescens* ISR 36 | + | + | + | − | + | + | + | ++++ |
| *Pseudomonas fluorescens* ISR 37 | − | − | + | − | + | + | + | ++++ |
| *Pseudomonas fluorescens* | − | − | + | − | + | + | + | ++++ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ISR 33 | | | | | | | | |

*P.fluorescens* ISR 33, isolate increased the shoot length maximum (53cm) and *B. diffusa* ISR 35 showed the minimum increment (44cm). Leaf area was found maximum in case of *Bacillus circulans* ISR 35 and minimum in *Boerhaavia diffusa* ISR 35. From table-6, it is clear that Root length density and Plant dry weight was found maximum in *P. fluorescens* ISR 33 and minimum in *S. marcescens* ISR75. Most of the bacterial isolates tested for plant growth activity were at par or greater than *Azotobacter chroococcum* Mac-27 used as positive control in the study (Table no 6). Bacteria related with various composts and macrofauna had plant growth-promoting and antagonistic activity in vitro and supported growth of pearl millet in greenhouse circumstances.

**Table no 6:** Effect of seed treatment with bacteria isolated from compost on vegetative growth parameters of Pearl millet cultivar ICMV 221 using unsterilized soil in in greenhouse conditions

| **Isolate** | **Shoot length (cm)** | **Leaf area ($cm^2$)** | **Root length density (mg /$cm^3$ soil)** | **Plant dry weight (mg)** |
|---|---|---|---|---|
| Uninoculated control | 40 | 32 | 0.98 | 710 |
| *Azotobacter chroococcum* Mac-27 | 46 | 43 | 1.24 | 966 |
| *Bacillus licheniformis* ISR13 | 47 | 45 | 1.30 | 913 |
| *Bacillus circulans* ISR 35 | 50 | 51 | 1.48 | 1008 |
| *Boerhaavia diffusa* ISR 37 | 52 | 50 | 1.34 | 939 |
| *Boerhaavia diffusa* ISR 35 | 44 | 41 | 1.24 | 908 |
| *Serratia marcescens* ISR 67 | 48 | 49 | 1.21 | 940 |
| *Serratia marcescens* ISR75 | 45 | 48 | 1.05 | 772 |
| Pseudomonas *fluorescens* ISR 35 | 47 | 49 | 1.38 | 941 |
| *Pseudomonas fluorescens* ISR 36 | 50 | 48 | 1.60 | 1082 |
| *Pseudomonas fluorescens* ISR 37 | 47 | 48 | 1.31 | 926 |
| *Pseudomonas fluorescens* ISR 33 | 53 | 49 | 1.74 | 1110 |

* ISR= Induced systemic resistance, Mac = methyl ammonium chloride

## IV. Conclusion

RSC, FWC and GVC were used along with three bacterial strains *B. circulans* ISR 35, *P. fluorescens* ISR 36 and *P. fluorescens* ISR 33 demonstrated enhancement in root length and dry biomass. The increment in plant growth after the use of compost may be because of high percent of siderophore, ACC deaminase and IAA producers and nutrients present in them. The conclusion of this work showed that the effect of Rice-straw compost was better than *Gliricidia* vermicompost, which may be due to low percentage of IAA producers as the concentration of IAA more than the threshold levels is harmful for root growth and development. PGPB stimulated germination and support growth of plant and their use with composts interpedently improved plant growth. Such PGPB may be useful as supplementary inoculants along with composts and make a interactive treatment for improving plant growth.

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