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# Effect of PGPR on morphological properties of different varieties of wheat *(Triticum aestivum)*

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**Abstract**
Different plant growth-promoting rhizosphere (PGPR) bacteria, including associative bacteria such as *Azospirillum*, *Bacillus*, *Pseudomonas* and enterobacter group have been used for their beneficial effects on plant health. Several studies clearly showed the effect of PGPR on health of different crops at different climates, soils and temperatures. Rhizosphere bacteria *Pseudomonas spp*., *Azospirillum spp., Pantoea spp., Agrobacterium spp*., increases plant growth and nutrient uptake of wheat, maize and legumes in moderate climates. PBW274 when treated with isolated PGPR2 and PGPR3 showed non-significant effect over uninoculated control, while isolated PGPR1 and mixture of PGPR1, PGPR2 and PGPR3 showed significant effect (10.06 cm and 10.53 cm). In this study, all these three isolates showed better performance in comparison to un-inoculated control.

**Keywords:** PGPR, *Pseudomonas spp*., plant growth & un-inoculated control

## 1. Introduction
Plant growth-promoting rhizobacteria (PGPR) are highly diverse and superior sources of organic materials, which affect the plant growth by several mechanisms, produce a variety of compounds such as phytohormone, organic acid, and siderophores. The use of PGPR to boost plant health and crop yield is predicted to become an emerging trend in present-day agriculture practices. Rhizospheric soils of crop plants have flora and fauna due to availability of more organic compound, macronutrient and micronutrient. Rhizobacteria that exert beneficial effect on plant growth and development are referred to as plant growth promoting rhizobacteria. It's a group of free living soil bacteria which has ability to promote plant health by direct and indirect mechanism. PGPR is categorized in two types. Type I colonies are present inside plant cell called as intracellular PGPR (iPGPR) and Type II colonies outside plant in rhizosphere called as extracellular PGPR (ePGPR). Early studies on PGPR focused more on biological control of plant diseases than on plant health, and involved PGPR like *fluorescent Pseudomonas* and *Bacillus subtilis* that are antagonistic to soil-borne plant. Their use in agriculture favors a reduction in agro-chemical use and support eco-friendly crop production [15, 6]. PGPR can help the development of plant growth, plant nutrition, root growth pattern, plant competitiveness and responses to external stress factors. They can also inhibit soil-borne plant pathogens by producing growth-promoting chemical substances and inducing plant resistance [4, 17, 7].

This study was conducted to isolate PGPR from different soil samples and to evaluate their potential use for improving plant health, plant growth and nutrient uptake of wheat. Three PGPR strains were isolated and studied for their morphological characteristics. Efficiency of these three PGPR isolates and their mixtures (combinations) in different wheat varieties (low and high yielding) was evaluated at lab. A novel approach could be converted into a value added product such as an effective bio-fertilizer by blending with PGPR.

Wheat (*Triticum aestivum*) is the source of almost 20% of the total calories of the world's population. It is the most valuable staple food, which occupies a central position in forming agricultural policies because of its per hectare yield is far below the actual potential of the existing varieties. Keeping this in view, an experiment is performed to assess the effectiveness of the integrated application of seed inoculation with PGPR for improving plant, growth and health.

In the current research work was to isolation of plant growth promoting Rhizobacteria (PGPR) from different soil samples (leguminous plant rich) with study of the morphological effect of PGPR on different varieties of wheat plant.

## 2. Materials and methods

### 2.1 Materials

Peptone and beef extract were obtained from Sisco Research Lab. Pvt. Ltd., Mumbai (IN) & Qualigens Fine Chemicals respectively. Sodium chloride, Agar-agar, Sodium Carbonate, Acetone, Meta Phosphoric Acid, Ascorbic Acid, Glacial Acetic Acid, O-phosphoric Acid were procured from Fishers Scientific, Mumbai (IN) and other materials eg. Covac's Indole Reagent, DPIP, Anthrone, Ninhydrine Reagent were from Himedia Lab. Pvt. Ltd., Mumbai (IN). Folin Ciocalteau Reagent & Proline were obtained from Merck, Mumbai (IN).

### 2.2 Isolation and characterisation of plant growth promoting rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR) were isolated from rhizospheric soil of wheat crop, Arhar (Pigeon pea) and Botanical garden of Banaras Hindu University, Varanasi by using a dilution plate technique. The rhizospheric soil sample obtained from different sources were autoclaved in vertical autoclave at 121˚C for 15 minutes. 10g soil sample was taken and suspended in 90ml. double distilled water. After, sedimentation of solid particle dilution made up to $10^{-1}$ to $10^{-8}$. Each dilution was spread on pre-prepared nutrient agar plate. (100µl in each). Then after the plate was incubated at 28˚C for 72 hours and records the morphology and texture of each colony was recorded. Selected colony was further purified by streaking on slant medium and incubated it for 48 hours at 28 ˚C.

Each strain was characterised by gram staining. The plants were grown in pots for a period of 1 month. After 4 weeks germination shoot and root were separated and dried at 72˚C for 3 days in hot air oven before determining the root and shoot dry weight. Before drying leaf samples were taken for some biochemical analysis viz., Starch content, chlorophyll content, ascorbic acid content and phenolic compound contents.

## 3. Results and Discussion

### 3.1 Isolation of PGPR

Three types of bacteria were isolated successfully these three strains were isolated from the rhizosphere soils of three different places of BHU. These strains were designated as PGPR1, PGPR2, and PGPR3. Shown in Table 1.

### 3.2 Morphological characteristic of PGPR isolates

As shown in Table 2 the morphological characteristics of PGPR isolates showed widely variation. Out of three, two isolated strain appeared round shaped and one isolated appeared star shaped. Fig. 4a showed isolated PGPR1, PGPR2 and PGPR3. All the developed colonies have smooth shiny appearance. Which resembles the *Pseudomonas Spp.* The three isolates differed in colour but out of three isolates two produced pungent odor and one isolates was odorless. Diameter of the colonies of different isolates designated as PGPR1, PGPR2 and PGPR3 varied in shape (diameter in the range of 0.2-2.0 mm) shown in Fig. 1.

**Table 1:** Location of Rhizosphere soil from different fields

| S. No. | Isolates | Location of Rhizosphere soil | Field |
|---|---|---|---|
| 1 | PGPR1 | Botanical garden, B.H.U | Rose plant |
| 2 | PGPR2 | International Agri. farm IAS B.H.U | Chilli(*Capsicum annum*)plant |
| 3 | PGPR3 | Horticulture, IAS, B.H.U | Wheat(*Triticum aestivum*) & Barley (*Hordium vulgare* ) |

**Table 2:** Morphological characteristics of PGPR isolates

| S. No. | Isolates | Surface | Colour | Odor |
|---|---|---|---|---|
| 1 | PGPR1 | Smooth Shiny | Whitish | -ve |
| 2 | PGPR2 | Smooth Shiny | Yellowish | +ve |
| 3 | PGPR3 | Smooth Shiny | Orange | +ve |

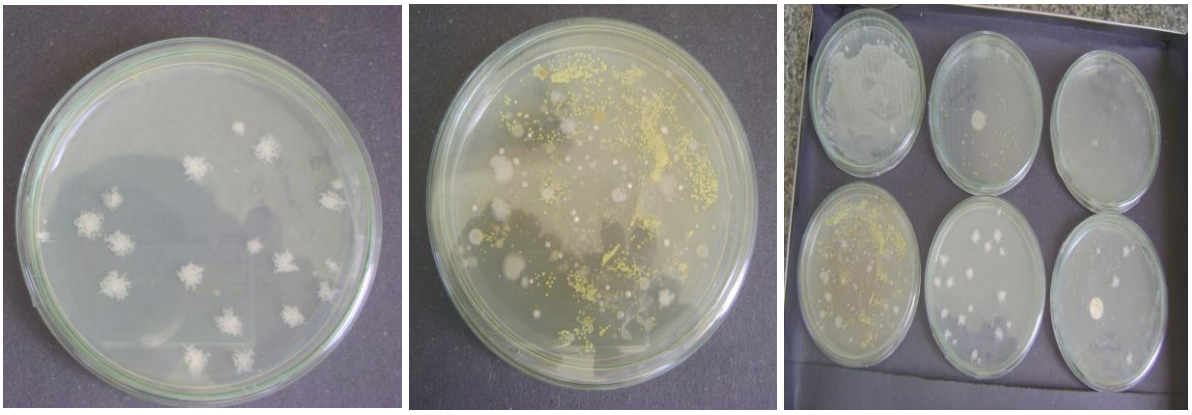

**Fig 1:** Isolated PGPR1, PGPR2 and PGPR3

### 3.3 Production of Indole Acetic Acid (IAA)

PGPR isolates were tested for IAA production. All the isolates induced the production of IAA. Isolates PGPR2 and PGPR3 were found to be excellent producers of IAA while the PGPR1 was showed moderate activity of IAA in comparison to PGPR2 and PGPR3.

### 3.4 Effect of PGPR isolates on plant height

The isolated PGPR significantly affected the height of wheat seedlings it can be clearly decided from (Fig. 2, 3 and 4) that the plant height increased in PGPR treated plants over un-inoculated control. HUW 510 wheat varieties showed maximum increase in height (10.76 cm) when treated with

PGPR2 isolates (Fig.5) in comparison to other wheat varieties. Similarly PBW343 showed maximum increasement in wheat varieties showed height (17.63 cm) when treated with marked increase in height PGPR3 isolates. When treated with mixture of three PGPR isolates (PGPR1, PGPR2 and PGPR3) in equal concentration over un-inoculated control three wheat varieties showed significant effect on plant height (8.50 cm). PBW274 when treated with isolated PGPR2 and PGPR3 showed non-significant effect over un-inoculated control, while isolated PGPR1 and mixture of PGPR1, PGPR2 and PGPR3 showed significant effect (10.06 cm and 10.53 cm) [11]. This might be due to the reason that the isolated PGPR help to receive light resulting in more photosynthesis.


**Fig 2:** PGPR1


**Fig 3:** PGPR1


**Fig 4:** PGPR1

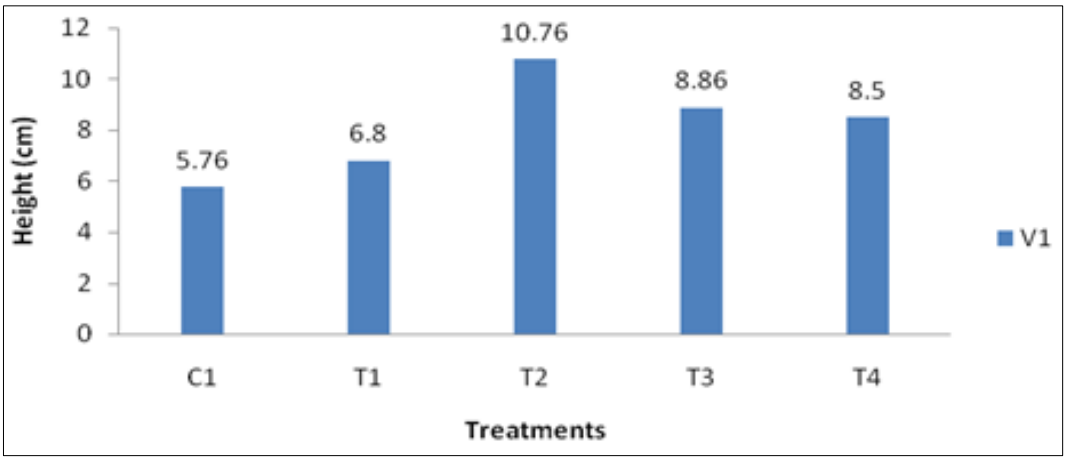


**Fig 5:** Effect of isolated PGPR on HUW-510

**Table 3:** ANOVA table for effect of isolated PGPR on HUW-510

| SOV | df | SS | MSS | F cal | F tab 5% | 1% |
|---|---|---|---|---|---|---|
| TREAT | 4 | 44.956 | 11.239 | 46.06148 | 3.48 | 5.99 |
| ERROR | 10 | 2.44 | 0.244 | | | |
| SE(m) | 0.220907 | | | Significant | | |
| CD 5% | 0.695944 | | | | | |
| CD 1% | 0.989878 | | | | | |
| CV | 0.404557 | | | | | |

**There:** C1:Uninoculated control (HUW-510), C2:Uninoculated control (PBW-343), C3:Uninoculated control (PBW-274),T1:Treated plant with PGPR1,T2:Treated plant with PGPR2,T3:Treated plant with PGPR3,T4:Treated

plant with mixture of PGPR1, 2 and PGPR3, CD: Critical differences, CV: Coefficient. Of Variance, df: Degree of freedom, SS: Sum of squares, MSS: Mean of Sum of Squares, SOV: Sum of variance and SE: Standard error.

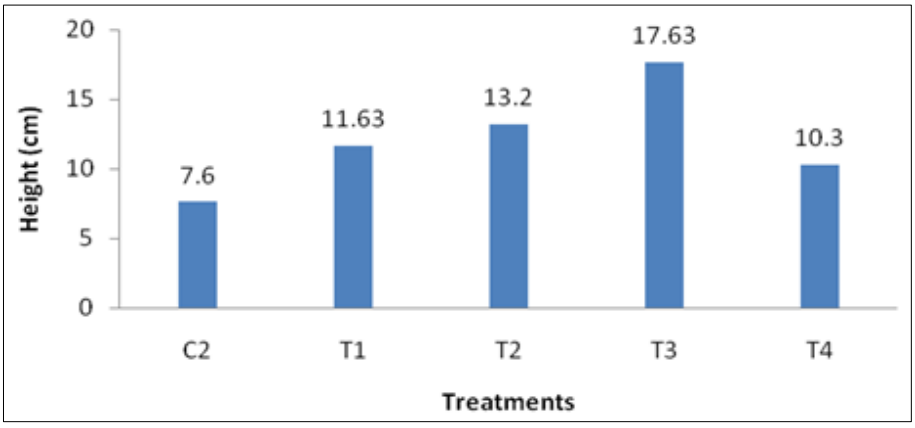


**Fig 6:** Effect of isolated PGPR on PBW-343

**Table 4:** ANOVA table for effect of isolated PGPR on PBW-343

| SOV | df | SS | MSS | F cal | F tab 5% | 1% |
|---|---|---|---|---|---|---|
| TREAT | 4 | 166.596 | 41.649 | 701.9494 | 3.48 | 5.99 |
| ERROR | 10 | 0.593333 | 0.059333 | | | |
| SE(m) | 0.108934 | | | Significant | | |
| CD 5% | 0.343186 | | | | | |
| CD 1% | 0.488131 | | | | | |
| CV | 0.134503 | | | | | |

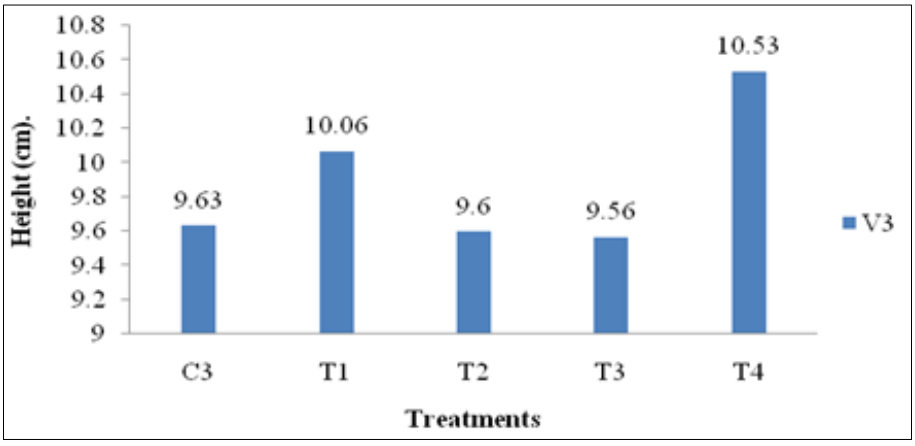


**Fig 7:** Effect of isolated PGPR on PBW-274

**Table 5:** ANOVA table for effect of isolated PGPR on PBW-274

| SOV | df | SS | MSS | F cal | F tab 5% | 1% |
|---|---|---|---|---|---|---|
| TREAT | 4 | 2.097333 | 0.524333 | 9.591463 | 3.48 | 5.99 |
| ERROR | 10 | 0.546667 | 0.054667 | | | |
| SE(m) | 0.104563 | | | Significant | | |
| CD 5% | 0.329413 | | | | | |
| CD 1% | 0.468541 | | | | | |
| CV | 0.157766 | | | | | |

### 3.5 Effect of PGPR isolates on shoot biomass

Fig. 8, 9 and 10 represents a significant increase in shoot biomass of *Triticum aestivum* varieties (HUW510, PBW343and PBW274). Table 6, 7 and 8 represents effect of different PGPR isolates on shoot biomass of HUW510. it can be clearly decided from Table 6 that treatments T1 and T2 showed marked increased in shoot biomass (1.50g and 1.60g, respectively) in comparison to un-inoculated HUW510 varieties (control). However T3 and T4 showed less increase in shoot biomass (1.15 and 0.91g, respectively) All three results were found to be significant ($p$<0.05) as represented in ANOVA Table 6.

Table 4.7 represent effect of different PGPR isolates on shoot biomass of PBW343 it can be clearly decided from Fig. 9 that treatments T1 and T2 showed marked increase in shoot biomass (1.25 cm and 1.35 cm respectively ) in comparison to un-inoculated PBW343 varieties (control ). However, treatment T3 and T4 showed less increase in shoot biomass (1.16cm and0.83 cm respectively). All three results were found to be significant ($p$<0.05) as represent in ANOVA table

7.

Table 4.8 represent effect of different PGPR isolates on shoot biomass of PBW274 it can be clearly decided from Fig. 10 that treatments T1 & T3 showed marked increase in shoot biomass (2.98 cm and 1.94 cm respectively ) in comparison to un-inoculated PBW274 varieties (control). However, treatment T2 & T4 showed less increase in shoot biomass (1.37cm and 0.83 cm respectively).All three results were found to be significant ($p$<0.05) as represent in ANOVA table 8. So, we saw that all these three isolates showed better performance in comparison to un-inoculated control.

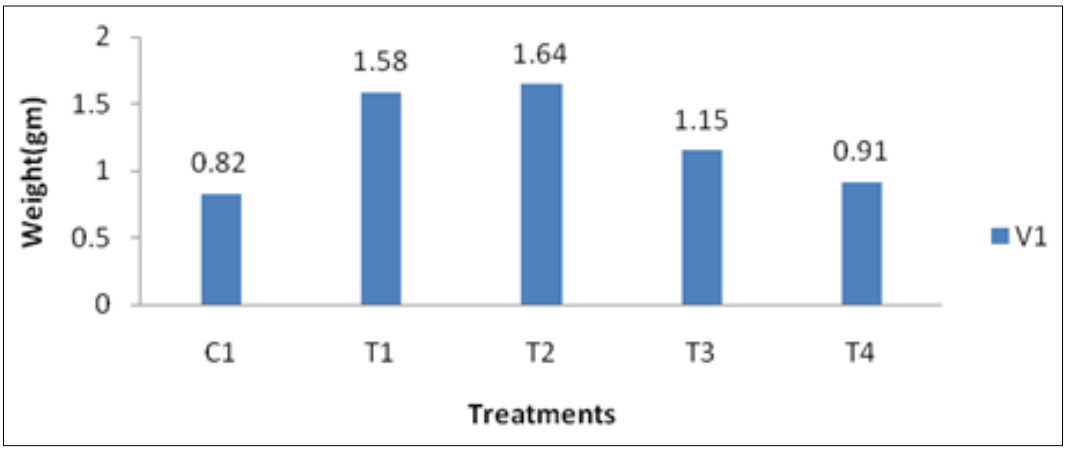


**Fig 8:** Effect of PGPR on shoot biomass of HUW-510

**Table 6:** ANOVA table for effect of PGPR on shoot biomass of HUW-510

| SOV | Df | SS | MSS | F cal | F tab 5% | 1% |
|---|---|---|---|---|---|---|
| TREAT | 4 | 1.701693 | 0.425423 | 278.6616 | 3.48 | 5.99 |
| ERROR | 10 | 0.015267 | 0.001527 | | | |
| SE(m) | 0.017474 | | | Significant | | |
| CD 5% | 0.055049 | | | | | |
| CD 1% | 0.078299 | | | | | |
| CV | 0.212814 | | | | | |

**There:** C1:Uninoculated control (HUW-510),C2:Uninoculated control (PBW-343), C3:Uninoculated control (PBW-274),T1:Treated plant with PGPR1,T2:Treated plant with PGPR2,T3:Treated plant with PGPR3,T4:Treated plant with mixture of PGPR1, 2 and PGPR3, CD: Critical differences, CV: Coefficient. Of Variance, df: Degree of freedom, SS: Sum of squares, MSS: Mean of Sum of Squares, SOV: Sum of variance and SE: Standard error.

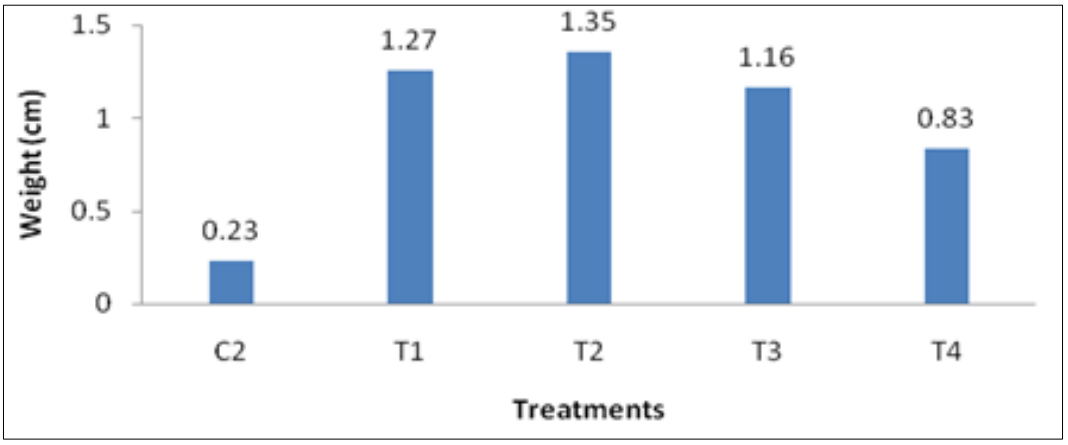


**Fig 9:** Effect of PGPR on shoot biomass of PBW-343

**Table 7:** ANOVA table for Effect of PGPR on shoot biomass of PBW-343

| SOV | df | SS | MSS | F cal | F tab 5% | 1% |
|---|---|---|---|---|---|---|
| TREAT | 4 | 2.492467 | 0.623117 | 983.8684 | 3.48 | 5.99 |
| ERROR | 10 | 0.006333 | 0.000633 | | | |
| SE(m) | 0.011255 | | | Significant | | |
| CD 5% | 0.035456 | | | | | |
| CD 1% | 0.050432 | | | | | |
| CV | 0.172963 | | | | | |

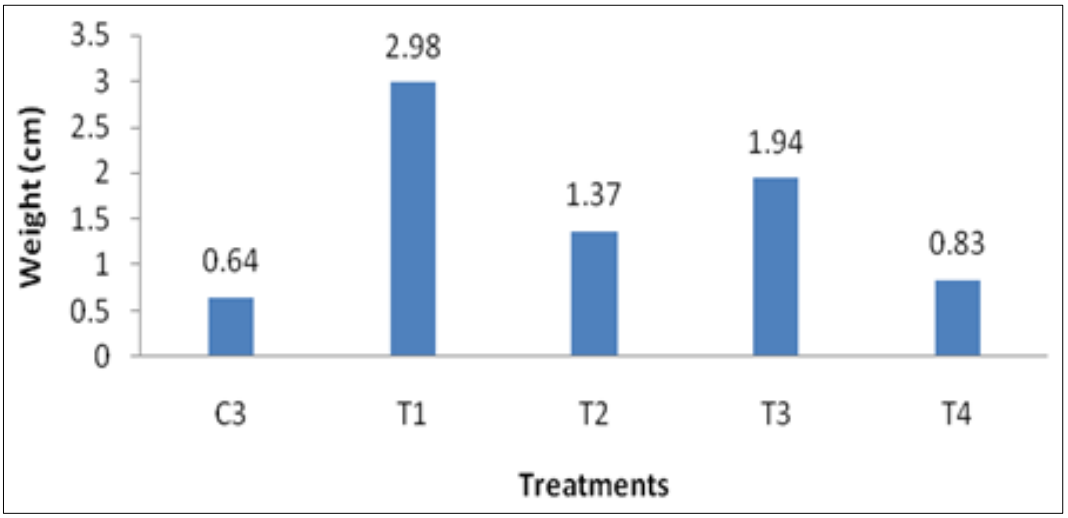


**Fig 10:** Effect of PGPR on shoot biomass of PBW-274

**Table 8:** ANOVA table for Effect of PGPR on shoot biomass of PBW-274

| SOV | DF | SS | MSS | F cal | F tab 5% | 1% |
|---|---|---|---|---|---|---|
| TREAT | 4 | 10.74956 | 2.68739 | 3445.372 | 3.48 | 5.99 |
| ERROR | 10 | 0.0078 | 0.00078 | | | |
| SE(M) | 0.01249 | | | Significant | | |
| CD 5% | 0.039348 | | | | | |
| CD 1% | 0.055967 | | | | | |
| CV | 0.119813 | | | | | |

## 4. Conclusion

In this study, the effect of Plant Growth Promoting Rhizobacteria (PGPR) on Morphological properties of different varieties of Wheat *(Triticum aestivum*). two isolated strain appeared round shaped and one isolated appeared star shaped. Fig. 4a showed isolated PGPR1, PGPR2 and PGPR3. All the developed colonies have smooth shiny appearance. Which resembles the *Pseudomonas Spp.* The three isolates differed in colour but out of three isolates two produced pungent odor and one isolates was odorless. Diameter of the colonies of different isolates designated as PGPR1, PGPR2 and PGPR3 varied in shape (diameter in the range of 0.2-2.0 mm). PBW274 when treated with isolated PGPR2 and PGPR3 showed non-significant effect over un-inoculated control, while isolated PGPR1 and mixture of PGPR1, PGPR2 and PGPR3 showed significant effect (10.06 cm and 10.53 cm). All three results were found to be significant ($p$<0.05) as represent in ANOVA.

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