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Biochemical analysis of PGPR and its effect on chlorophyl, ascorbic acid, starch & total polyphenolic content (TPC) of different varieties of wheat (*Triticum aestivum*)

Pukhraj Meena and Alok Kumar Rai

Abstract
The efficiency of plant growth-promoting rhizosphere (PGPR) in various conditions, observing that PGPR are effective under determined conditions only. Awareness of the structure of rhizosphere microbial communities and their diversity, as related to other essential processes within the system such as complexity, natural selection, inter populational relations (symbiosis, parasitism, mutualism or competence), succession or the effect of disturbances, is the key to a better understanding of the system and for the correct utilization of PGPR in biotechnology. PBW274 wheat varieties posses higher amount of Chl.a +b (13.67 mg g^{-1}), when treated with isolated PGPR1. Ascorbic acid was significantly increased in all the three selected varieties. PGPR3 isolates showed better response in comparison to PGPR1 and PGPR2 isolates. PGPR3 isolates treatment showed high ascorbic acid content (178.52 mg/g). PGPR1 treated plants showed better result in comparison to other isolated PGPR treated plants. TPC (Total phenolic compound) concentrations were increased significantly by the incorporation of PGPR isolate and showed wide variation (161.6 mg $GAEg^{-1}$ to 635.6 mg GAE g^{-1}). In this biochemical analysis, results were found to be significant ($p<0.05$).

Keywords: rhizosphere microbial communities, ascorbic acid, TPC & Chl. a+b.

1. Introduction
Organic matter is a very important part of the biogeochemical cycles and it affects various chemical, physical and biological properties of the plants that are related to plant behaviour and availability of nutrients [6]. Inorganic fertilizers (N or P) cannot supply all required nutrients to plants. So, the addition of organic materials was needed for sustainability. PGPR is 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.
Plant growth-promoting rhizobacteria (PGPR) can be described as bacteria having most of or all the following qualities - ability to colonize plant roots, adherence to soil in the rhizosphere, capacity to enter into root interior and establish entophytic populations with adaptability to the niche and benefit to the host plant [15, 18, 11, 4]. It is reported that some PGPR are able to reduce the negative impacts of irrigation after using high salty waters. The usefulness of PGPR in forestry as well as in environmental remediation has also been explored. In summary, PGPR have been reported as useful in many parts of the world (Shown in Table 1) and the beneficial effects are many, including bio-control and management of soil and plant health [16, 5, 10, 12, 4, 8, 1].

Table 1: *In vitro* production of plant growth regulators by rhizobacteria

PGPR	PGR
Arthrobacter mysorens 7,	Indole-3-acetic acid, ethylene
Flavobacterium sp.L30,	Indole-3-acetic acid, ethylene
Azotobacter chroococcum	Indole-3-acetic acid
Bacillus licheniformis	Ethylene
Pseudomonas sp.	Auxins
*Bacillus megaterium*BHUPSB14	Indole-3-acetic acid
Rhizobium sp.	Indole-3-acetic acid

A putative PGPR qualifies as a PGPR when it is capable to produce a positive effect on the plant leading inoculation, hence representing good competitive skills over the existing

rhizosphere communities. Generally, about 2–5% of rhizosphere bacteria are PGPR. Some PGPR have been produced commercially as inoculants for agriculture, but it must be borne in mind that the inoculation of these bacteria in soil may affect the composition and structure of microbial communities and these changes must be studied since they have, at times, been related to the incompetence of biofertilizer when applied to plant roots.

In this study, PGPR strains were isolated and cultural characteristics, indole acetic acid (IAA) production, auxin production, chlorophyll production, starch production and ascorbic acid production (Vitamin C) with study of the effect of PGPR on physiochemical properties of different wheat varieties.

2. Materials and methods

2.1 Materials

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). Other material procured from Sisco Research Lab. Pvt. Ltd., Mumbai (IN) and Fishers Scientific, Mumbai (IN).

2.2 Biochemical analysis

2.2.1 Estimation of Ascorbic acid content

Dye solution -50mg of sodium salt of 2, 6-DPIP (Dichlorophenol indol phenol) was dissolved in appropriately 100ml.of hot distilled water containing Na_2CO_3. then it was cooled and diluted with distil water to 200ml.

2.2.2 Estimation of Chlorophyll content

1g of finely cut leaf sample was weighted and mixed well in a clean mortar and pestle. Grind the leaf with addition of 20ml of 80% acetone. Solution was centrifuge in cold centrifuge (5000 rpm for 15 minutes) and transfers the supernatant to a 100ml volumetric flask. The residue had grind with 20ml of 80% acetone, centrifuge and the supernatant was transferred to the same volumetric flask. This procedure had repeated until the residue was colourless, mortar and pestle was washed thoroughly with 80% acetone and the clear washing was collected in the volumetric flask. The final volume had make up to 100ml with 80% acetone. The observation of the solution had taken at 645,663 and 652nm against the solvent (80% acetone) blank.

2.2.3 Indol production test

1% tryptone broth was prepared and 10g peptone was dissolved in 1 litre distilled water. It was sterilized in autoclaved at 15 psi 121˚C for 15 minutes. Each tryptone broth was inoculated with bacterial strains and one tube was kept uninoculated cooperative control. Inoculated and uninoculated tubes were incubated at 35˚C for 48 hours. After 48 hours of incubation 1ml Kovac's reagent was added to each tube including control. Each tube was gently shaken for 10 -15 minutes. The tube was allowed to stand to permit the reagent to come to the top.

2.2.4 Estimation of Starch content

100 mg leaves were extracted with 5 ml hot 80% ethanol in clean mortar- pestle. The extract was centrifuged at 5000 rpm for 10 minutes, and the supernatant was removed and the residue was saved. The residue was re-extracted with 5 ml of ethanol, and the step 2 was repeated. The residue was washed with hot 80% ethanol and then it was dried over a water bath. Then the residue was extracted with 11.5 ml of perchloric acid and supernatant was collected and this step repeated. Supernatant was diluted with 25 ml percholoric acid. 0.2 ml of the supernatant was taken through pipette and makeup the volume 1 ml with double distilled water.1ml d H_2O was taken in another tube. This was act as a control to adjust zero O.D. Coloured was appeared with anthrone reagent and O.D was taken at 620 nm. Standard curve of glucose was used and the glucose content was calculated. Glucose content was multiplied by 0.9 to get starch content.

2.2.5 Assay for Total Polyphenols

The total polyphenols was determined as described in the method respectively for TPC, each extract (1mg/ml) folin ciocalteau reagent (1N,ml) and sodium carbonate (20%,2 ml) were added subsequently,the test mixture was mixed properly on cyclomixer, left at room temperature for 30 minutes and maintained to 25 ml with deionised water. The absorbance of test mixture measured at 725 nm using "Varian Cary 50 vis UV spectrophotometer. TPC was determined using standard curve with gallic acid 0-100 gm as the standard TPC was expressed as mg of gallic acid equivalents (GAE) gl for extract.

3. Results and discussion

3.1 Biochemical analysis

3.1.1 Effect on Chlorophyll content

Upon treatment significant increase in chlorophyll contents of *Triticum aestivum* leaves with PGPR isolates. From Fig. 1 & Fig. 2. From Table 2, 3 & 4, it can be clearly decided HUW510 and PBW343 wheat varieties posses higher amount of Chl.a when treated with isolate PGPR2. However PGPR3 and PGPR4 showed significant increase in Chl.a content. Fig. 3 clearly showed marked increased in Chl.a content (9.84 mg $^{g-1}$ 6.20mg g $^{-1}$ respectively) in comparison to uninoculated control PBW274 wheat varieties.

However, treatment T3 showed less increase in Chl.a content (4.50 mg g-1) All their results found as significant (p<0.05).

From Table 5, it can it can be clearly decided that HUW510 wheat varieties posses higher amount of Chl.b (6.79 mg $g^{-1)}$ when treated with isolate PGPR2. However PGPR3 and PGPR4 showed significant increase in Chl.b content. Fig.4.14 clearly showed marked increased in Chl.b (5.40 mg g $^{-1}$ and 5.64 mg g $^{-1}$respectively) content in PGPR4 and PGPR3 treated varieties in comparison to uninoculated control HUW510 wheat varieties. However, treatment T1 showed less increase in Chl.b content (4.05 mg g^{-1}) All their results found as significant (p<0.05).

From Table 6, it can be clearly decided that PBW343 wheat varieties posses higher amount of chl.b (8.00 mg g^{-1} 7.48 mg g^{-1}) when with isolate PGPR2 and mix culture of PGPR1 PGPR2 and PGPR3. However, PGPR1 and PGPR3 showed significant increase in Chl.b (3.74 mg g^{-1} 5.30 mg g^{-1}) in comparison to uninoculated control PBW343 wheat varieties. As shown in Fig. 8.

From Table 7, it can it can be clearly decided that PBW274 wheat varieties posses higher amount of Chl.b (5.40 mg g^{-1}) when treated with isolate PGPR2. Fig. 6 clearly showed marked increased in Chl.b contents (5.30 mg g $^{-1}$, 5.35 mg g $^{-1}$respectively) in PGPR4 and PGPR3 treated varieties in comparison to uninoculated control PBW274 wheat varieties. However, treatment T1 showed less increase in Chl.b content (3.85 mg g^{-1}) All their results found as significant (p<0.05).

From Table 8, it can it can be clearly decided that HUW510 wheat varieties posses higher amount of Chl.a +b (9.05 mg g$^{-1)}$ when treated with isolate PGPR2. Fig. 7 clearly showed marked increased in Chl. a+b contents (6.25 mg g $^{-1}$, 7.57 mg g $^{-1}$respectively) in PGPR3 and PGPR4 treated varieties in comparison to uninoculated control HUW510 wheat varieties. However, treatment T1 showed less increase in Chl.a+b content (4.59 mg g-^{1}) All their results found as significant (p<0.05).

From Table 9, it can it can be clearly decided that PBW343 wheat varieties posses higher amount of Chl.a +b (13.53 mg g^{-1}) when treated with isolated mix culture of PGPR1 PGPR2 and PGPR3. Fig. 8 clearly showed marked increased in Chl. a+b contents (12.15 mg g $^{-1}$, 9.60 mg g $^{-1}$respectively) in PGPR2 and PGPR3 treated varieties in comparison to uninoculated control PBW343 wheat varieties. However, treatment T1 showed less increase in Chl.a+b content (8.33mg g-^{1}) All their results found as significant (p<0.05).

From Table 10, it can it can be clearly decided that PBW274 wheat varieties posses higher amount of Chl.a +b (13.67 mg g^{-1}) when treated with isolated PGPR1 Fig. 9 clearly showed marked increased in Chl.a+b contents (11.59mg g $^{-1}$, 9.13 mg g $^{-1}$respectively) in PGPR2 and PGPR3 treated varieties in comparison to uninoculated control PBW274 wheat varieties. However, treatment T4 showed significant increase in Chl.a+b content (8.33mg g-^{1}) All their results found as significant (p<0.05).

The amount of chlorophyll b was also significantly increased. In HUW510 the PGPR2 treated plants showed higher amount of chlorophyll b. in comparison to other treated and control plants. In PBW 345 mixture of PGPR's showed higher amount of chl.b and in PBW-274 PGPR2, 3, and the mixture of these three showed good response in comparison to uninoculated control. That means, it might be happened that the competition occurred between the PGPR's which influences the amount of chlorophyll.

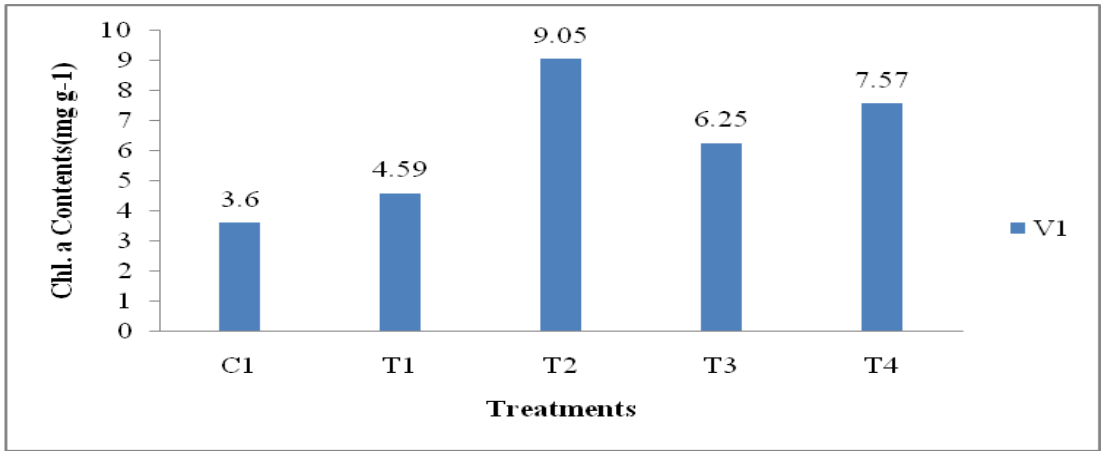

Fig 1: Effect of PGPR on Chlorophyll-a content in HUW-510

Table 2: ANOVA table for effect of PGPR on Chlorophyll-a content in HUW-510

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	58.14713	14.53678	29073.57	3.48	5.99
ERROR	10	0.005	0.0005			
SE(m)	0.01			Significant		
CD 5%	0.031504					
CD 1%	0.04481					

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.

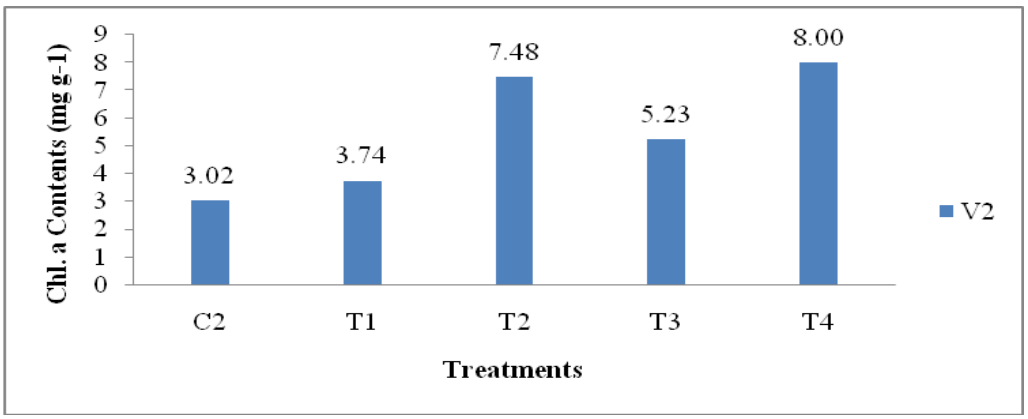

Fig 2: Effect of PGPR on Chlorophyll-a content in PBW-343

Table 3: ANOVA table for Effect of PGPR on Chlorophyll-a content in PBW-343

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	58.44869	14.61217	52.99642	3.48	5.99
ERROR	10	2.7572	0.27572			
SE(m)	0.234828			Significant		
CD 5%	0.739799					
CD 1%	1.052254					

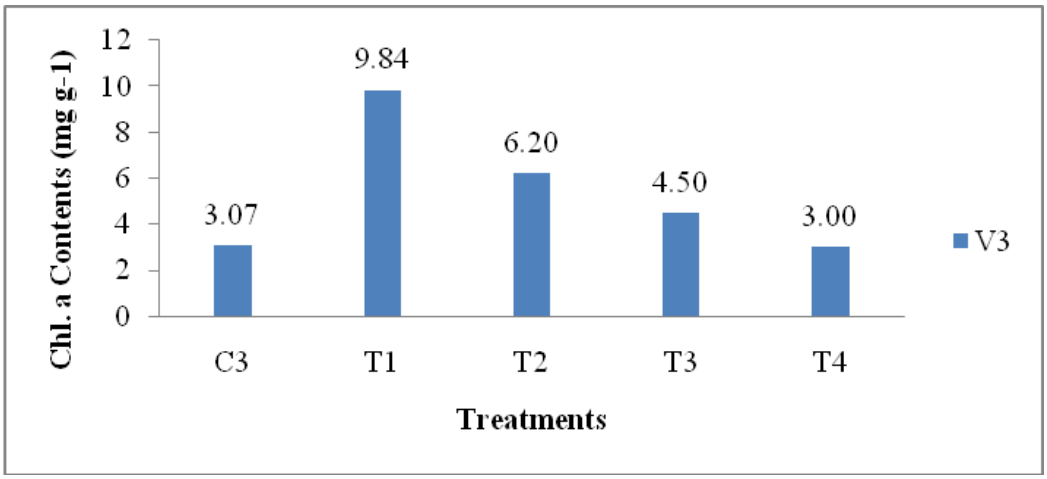

Fig 3: Effect of PGPR on Chlorophyll-a content in PBW-274

Table 4: ANOVA table for Effect of PGPR on Chlorophyll-a content in PBW-274

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	96.87737	24.21934	3784.272	3.48	5.99
ERROR	10	0.064	0.0064			
SE(m)	0.035777			Significant		
CD 5%	0.112712					
CD 1%	0.160316					

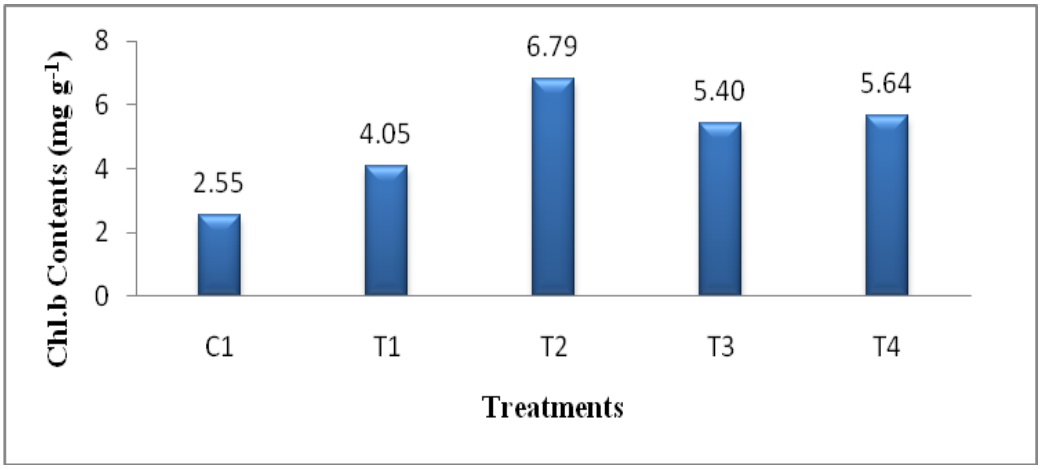

Fig 4: Effect of PGPR on Chlorophyll-b content in HUW-510

Table 5: ANOVA table for effect of PGPR on Chlorophyll-b content in HUW-510

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	31.95143	7.987857	6020.997	3.48	5.99
ERROR	10	0.013267	0.001327			
SE(m)	0.016289			Significant		
CD 5%	0.051317					
CD 1%	0.072991					

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.

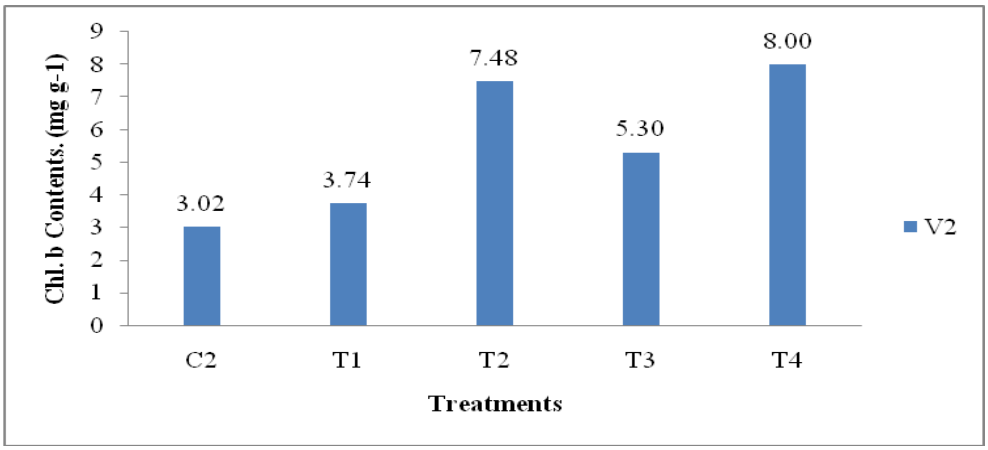

Fig 5: Effect of PGPR on Chlorophyll-b content in PBW-343

Table 6: ANOVA table for effect of PGPR on Chlorophyll-b content in PBW-343

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	68.07749	17.01937	7.931777	3.48	5.99
ERROR	10	21.4572	2.14572			
SE(m)	0.655091			Significant		
CD 5%	2.063793					
CD 1%	2.93544					

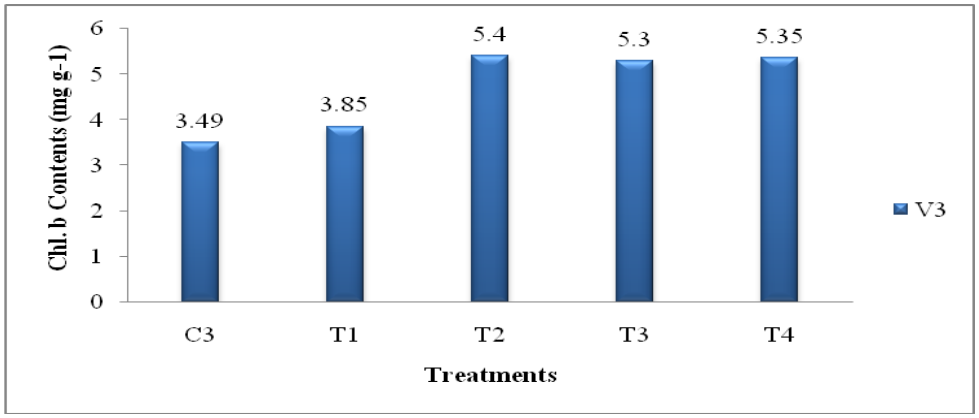

Fig 6: Effect of PGPR on Chlorophyll-b content in PBW-274

Table 7: ANOVA table for effect of PGPR on Chlorophyll-b content in PBW-274

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	10.31949	2.579873	948.4828	3.48	5.99
ERROR	10	0.0272	0.00272			
SE(m)	0.023324			Significant		
CD 5%	0.073479					
CD 1%	0.104513					

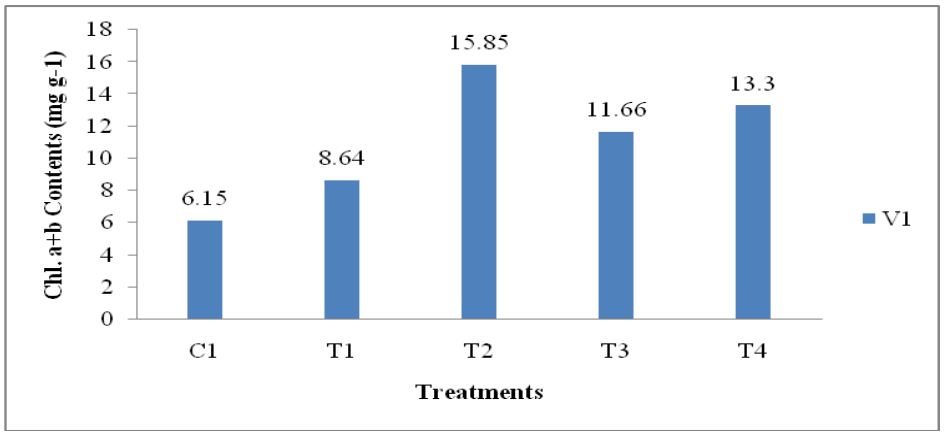

Fig 7: Effect of PGPR on Chlorophyll (a+b) content in HUW-510

Table 8: ANOVA table for effect of PGPR on Chlorophyll (a+b) content in HUW-510

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	174.8496	43.71241	4382.929	3.48	5.99
ERROR	10	0.099733	0.009973			
SE(m)	0.044662			Significant		
CD 5%	0.140702					
CD 1%	0.200128					

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.

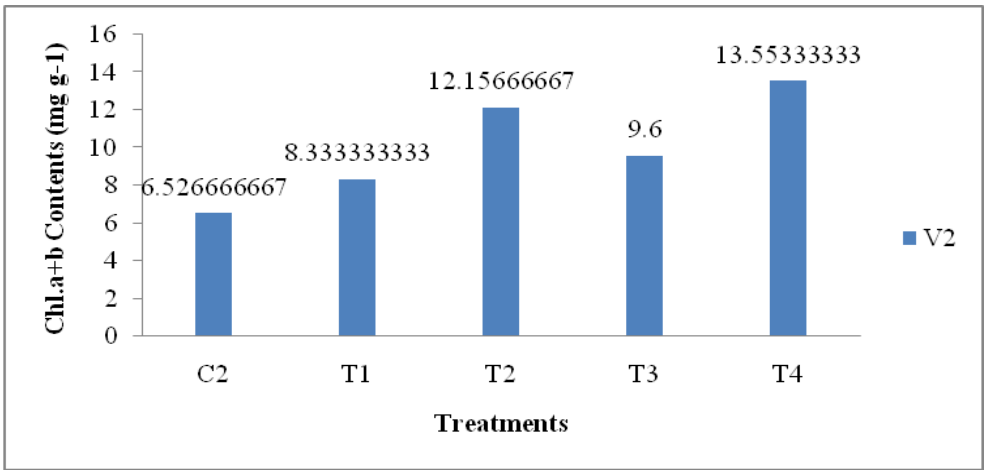

Fig 8: Effect of PGPR on Chlorophyll (a+b) content in PBW-343

Table 9: ANOVA table for effect of PGPR on Chlorophyll (a+b) content in PBW-343

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	96.82029	24.20507	88.38269	3.48	5.99
ERROR	10	2.738667	0.273867			
SE(m)	0.234037			Significant		
CD 5%	0.737308					
CD 1%	1.048712					

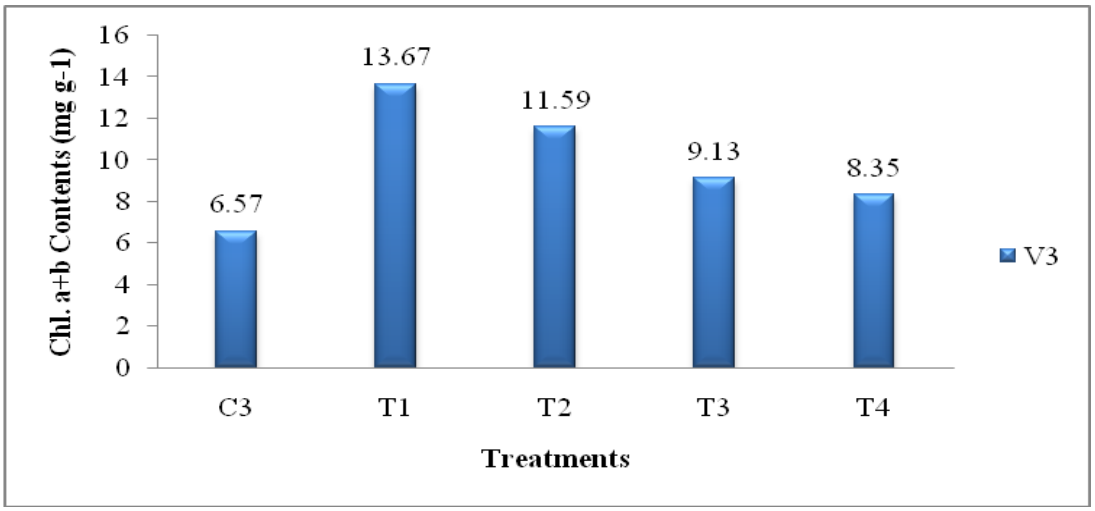

Fig 9: Effect of PGPR on Chlorophyll (a+b) content in PBW-274

Table 10: ANOVA table for Effect of PGPR on Chlorophyll (a+b) content in PBW-274

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	93.42363	23.35591	81.59744	3.48	5.99
ERROR	10	2.862333	0.286233			
SE(m)	0.239263			Significant		
CD 5%	0.753771					
CD 1%	1.072128					

3.1.2 Effect on Ascorbic acid content

Ascorbic acid plays a diverse role in several physiological processes in plants, including growth differentiation and metabolism. It also required for progression of cell cycle, cell elongation, and expansion it modulate the cell growth by controlling the biosynthesis of hydroxyproline rich proteins required for progression of G1 and G2 phase of the cell cycle and redox reaction of plasma membrane involved in elongation mechanism.

Ascorbic acid was significantly increased in all the three selected varieties. PGPR3 isolates showed better response in comparison to PGPR1 and PGPR2 isolates. Fig. 10 clearly indicated that HUW510 varieties showed more ascorbic acid content (1462.83 mg/g dry weight.) when treated with PGPR3 isolates. Ascorbic acid content showed increasing pattern in all the treatments over untreated HUW510 variety.

In case of PBW 343 wheat variety PGPR3 isolates treatment showed high ascorbic acid content (178.52 mg/g) in comparison to treatment T1,T2 and T4.Another of thing observed was that PBW343 wheat varieties ascorbic acid content was(19.8 %) higher than HUW510 in similar environmental condition and treatment (T3). PBW274 wheat varieties showed similar result. In PBW274 ascorbic acid content was hgher in treatment T3 quite similar to HUW510 and PBW343 isolates treatment enhance ascorbic acid content to greater extent in corporation to other PGPR isolates Table 9 represented that all the results were significant (p<0.01).

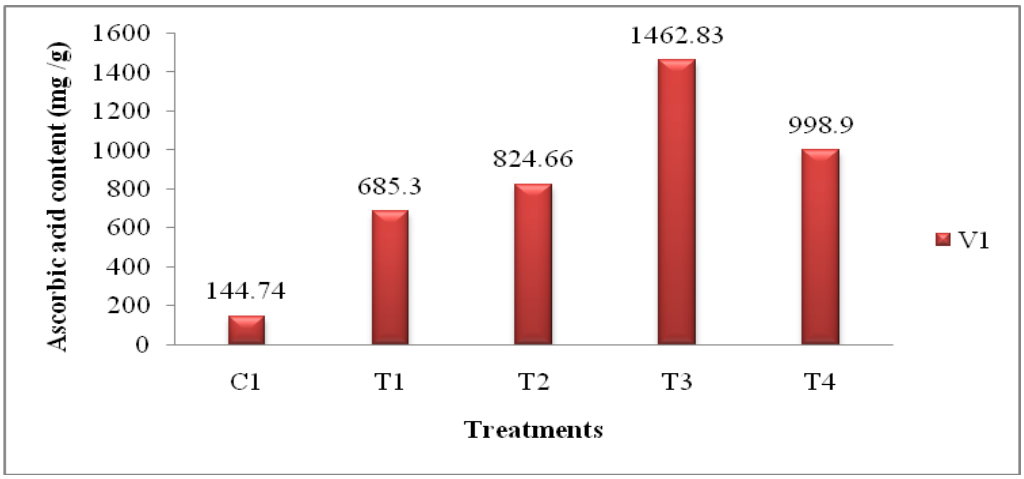

Fig 10: Effect of PGPR on ascorbic content in HUW-510

Table 11: ANOVA table for effect of PGPR on ascorbic content in HUW-510

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	4784927	1196232	309.4623	3.48	5.99
ERROR	10	38655.17	3865.517			
SE(m)	27.80474			Significant		
CD 5%	87.59582					
CD 1%	124.5921					

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.

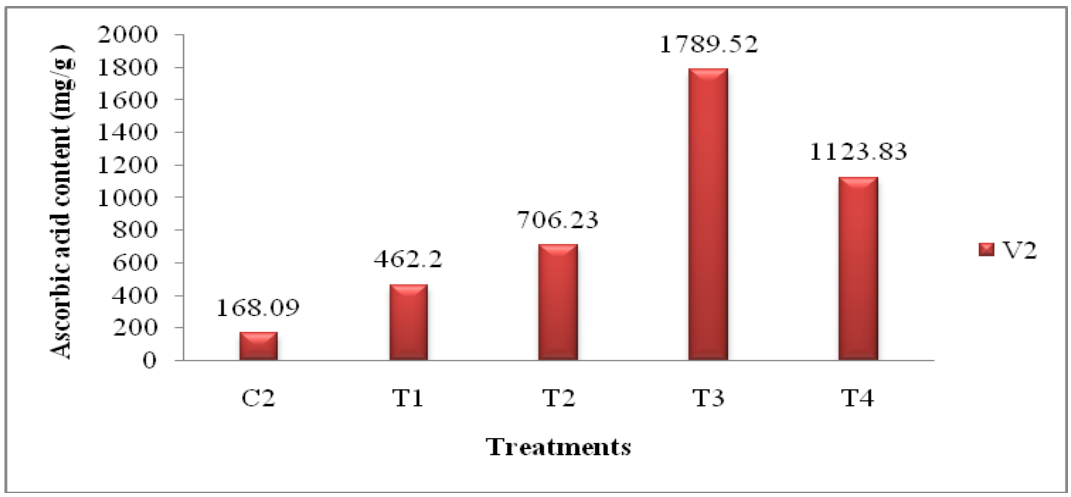

Fig 11: Effect of PGPR on ascorbic acid content in PBW-343

Table 12: ANOVA table for effect of PGPR on ascorbic acid content in PBW-343

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	4781243	1195311	309.1848	3.48	5.99
ERROR	10	38660.08	3866.008			
SE(m)	27.8065			Significant		
CD 5%	87.60139					
CD 1%	124.6					

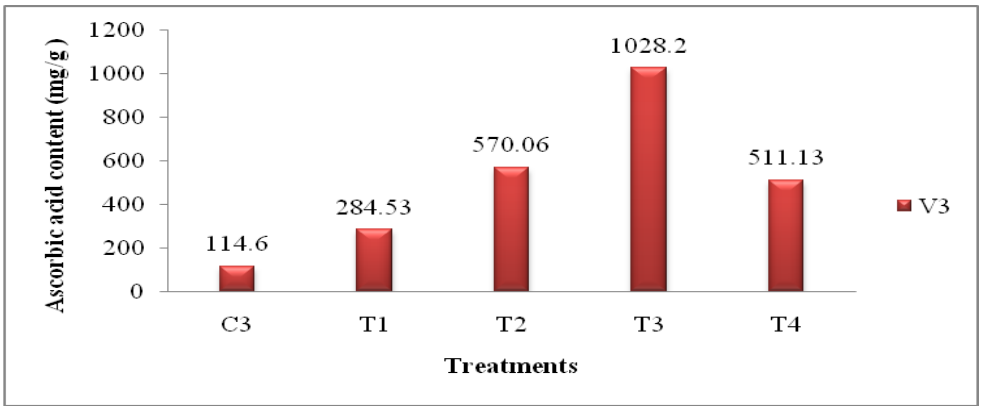

Fig 12: Effect of PGPR on ascorbic acid in PBW-274

Table 13: ANOVA table for effect of PGPR on ascorbic acid in PBW-274

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	1436919	359229.8	187.6677	3.48	5.99
ERROR	10	19141.8	1914.18			
SE(m)	19.5662			Significant		
CD 5%	61.64119					
CD 1%	87.67546					

3.1.3 Effect on Starch content

Current study finding suggest that the starch content was significantly increased in PGPR1, PGPR2 and PGPR3 treated plants. In case of HUW510 PGPR1 treated plants showed better result in comparison to other isolated PGPR treated plants. While in PBW343 and PBW274 wheat varieties PGPR1treatment showed more starch content PGPR2 also showed similar result. It clearly depicts that the isolated PGPR induced the activity of enzyme ADP-glucose pyrophosphorylase (AGPase) which catalyze the reaction glucose-1- phosphate with ATP to form ADP glucose. Then the ADP glucose used a substrate by starch syntheses enzyme.

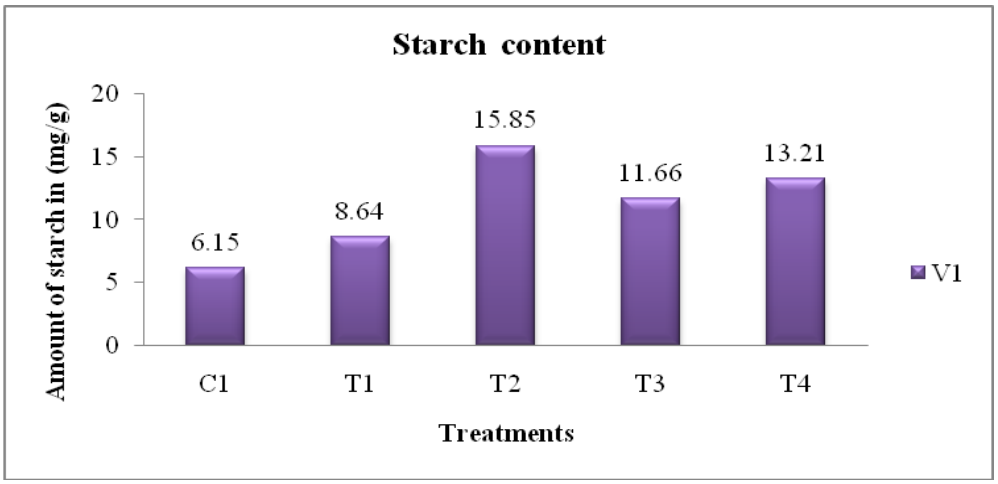

Fig 13: Effect of isolated PGPR on starch content in HUW-510

Table 14: ANOVA table for effect of isolated PGPR on starch content in HUW-510

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	0.001805	0.000451	125.3426	3.48	5.99
ERROR	10	3.6E-05	3.6E-06			
SE(m)	0.000849			Significant		
CD 5%	0.002673					
CD 1%	0.003802					

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.

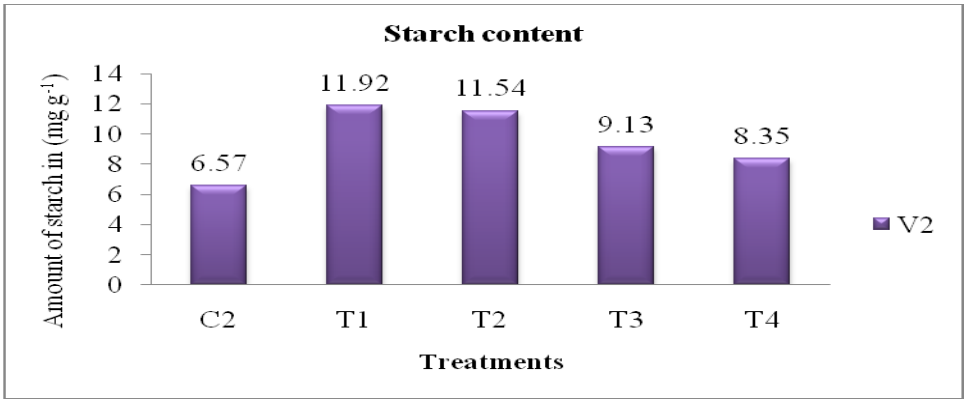

Fig 14: Effect of isolated PGPR on starch content in PBW-343

Table 15: ANOVA table for effect of isolated PGPR on starch content in PBW-343

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	0.001324	0.000331	47.75481	3.48	5.99
ERROR	10	6.93E-05	6.93E-06			
SE(m)	0.001178			Significant		
CD 5%	0.00371					
CD 1%	0.005277					

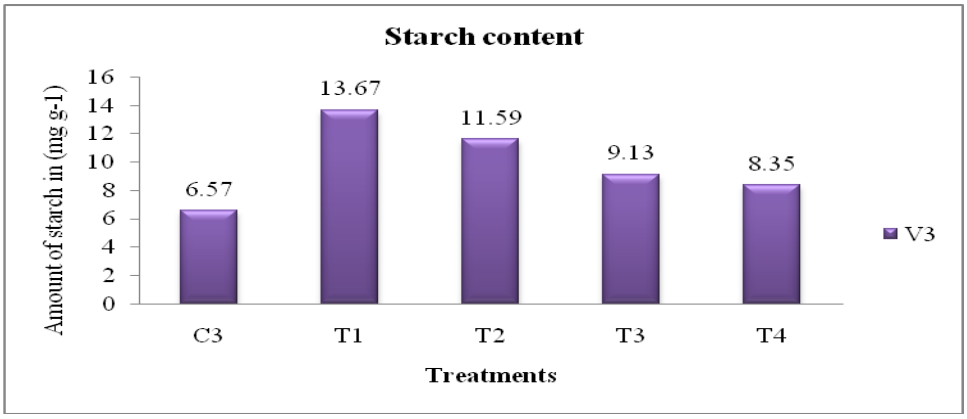

Fig 15: Effect of isolated PGPR on starch content in PBW-274

Table 16: ANOVA table for of isolated PGPR on starch content in PBW-274

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	0.00573	0.001432	15.97509	3.48	5.99
ERROR	10	0.000897	8.97E-05			
SE(m)	0.004235			Significant		
CD 5%	0.013341					
CD 1%	0.018976					

3.1.4 Effect on Total Polyphenolic Content (TPC)

As shown in Table 19 TPC (Total phenolic compound) concentration were increased significantly by the incorporation of PGPR isolate and showed wide variation (161.6 mg $GAEg^{-1}$ to 635.6 mg GAE g^{-1}). In HUW-510 the analytical data showed significant result which were 465.48mg $GAEg^{-1}$ in uninoculated control, PGPR1 treated plant showed 505.91mg$GAEg^{-1}$ PGPR2 treated plants showed 557.71mg$GAEg^{-1}$, and PGPR3 treated plant showed 557.71mg$GAEg^{-1}$ and the combination of these three showed 565.68 mg$GAEg^{-1}$ TPC. In PBW-343 TPC content was found to be 161.63 mg$GAEg^{-1}$ in control, 286.26 mg$GAEg^{-1}$ in PGPR1 treated plants, 253.27 mg $GAEg^{-1}$ in PGPR2 treated plants, 473.71 in mg$GAEg^{-1}$ PGPR3 treated plants and mixture treatment with PGPR isolates showed 358.29mg$GAEg^{-1}$ TPC content. In case of PBW-274 TPC content was 215.46mg$GAEg^{-1}$ in control plants, 375.78 mg$GAEg^{-1}$ in PGPR1 treated plants, 379.63mg$GAEg^{-1}$ in PGPR2 treated plants, 361.79 mg$GAEg^{-1}$ in PGPR3 treated plants and 383.41mg$GAEg^{-1}$ in mixture of PGPR isolates (PGPR1, PGPR2 and PGPR3).

In Fig.19 represents effect of different PGPR isolates on TPC of HUW510 it can be clearly defined from table 17 that T3 showed marked increase in TPC (635.60mg $GAEg^{-1}$) in

comparison to uninoculated HUW510 varieties (control) However, T4 and T2 showed less increase in TPC content (565.68 mgGAEg-^{1} and 557.71mgGAEg-^{1}) All there results were found to be significant (p<0.01) as represented in ANOVA table 17

In Fig. 17 represents effect of different PGPR isolates on TPC of PBW343 it can be clearly defined from table 18 that T3 showed marked increase in TPC (47 3.71 mgGAEg-^{1}) in comparison to uninoculated PBW343 varieties (control) However, T4 and T1 showed less increase in TPC content 358.29 mgGAEg-^{1} and 286.26mgGAEg-^{1}) T2 showed significant increase(286.26mgGAEg-^{1}) All there results were found to be significant (p<0.01) as represented in ANOVA table 18.

In Fig 18 represents effect of different PGPR isolates on TPC of PBW274 it can be clearly defined from table 19 that T4 showed marked increase in TPC (383.41 mgGAEg-^{1}) in comparison to uninoculated PBW274 varieties (control) However, T3, T2 and T1 showed less increase in TPC content(361.79 mgGAEg-^{1} 379.63mgGAEg-^{1}and 357.78mgGAEg-^{1}). All there results were found to be significant (p<0.01) as represented in ANOVA table 19.

As it is belived that, phenolics are most abundant secondary metabolites in plants, and can be classified into non-soluble compounds such as lignins,tannins and phenolic compounds such as phenolic acid, phenylpropanoids, flavonoids and quinines. All these groups are involved in many processes in plants as well as animals, flavonoids is of particular interest because of its multiple role in plants and its impact on human health. In plants, flavonoids play a major role in flowers and seed pigmentation, fertility and reproduction and in various defense reaction to protect against abiotic stress like UV-light or biotic stresses such as predators and pathogen attack. It might be possible that PGPR regulate the synthesis of nod factor in legumes, which is responsible for the inception of the nodule.

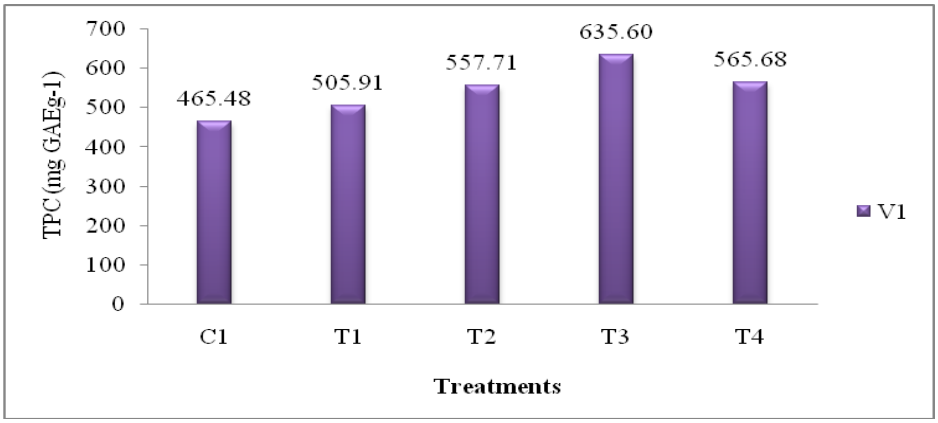

Fig 16: Effect of isolated PGPR on TPC content in HUW-510

Table 17: ANOVA table for effect of isolated PGPR on TPC content in HUW-510

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	46020.48	11505.12	10.37487	3.48	5.99
ERROR	10	11089.41	1108.941			
SE(m)	14.89256			Significant		
CD 5%	46.91739					
CD 1%	66.73304					

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.

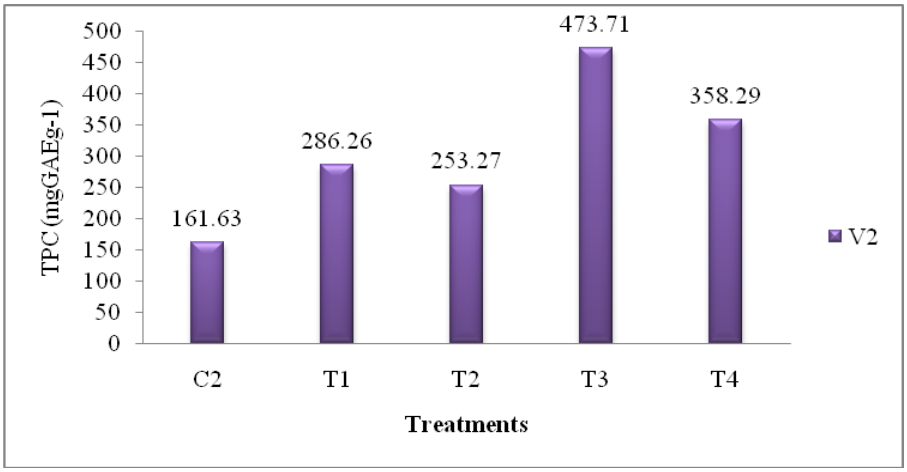

Fig 17: Effect of isolated PGPR on TPC content in PBW-343

Table 18: ANOVA table for effect of isolated PGPR on TPC content in PBW-343

SOV	df	SS	MSS	F cal	F tab 5%	1%
Treat	4	164618.9	41154.73	38.0421	3.48	5.99
Error	10	10818.21	1081.821			
SE(m)	14.70932			Significant		
CD 5%	46.34012					
CD 1%	65.91196					

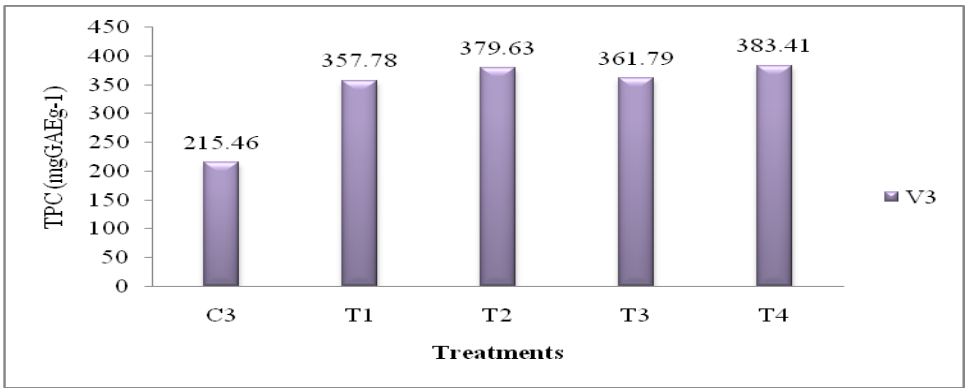

Fig 18: Effect of isolated PGPR on TPC content in PBW-274

Table 19: ANOVA table for effect of isolated PGPR on TPC content in PBW-274

SOV	df	SS	MSS	F cal	F tab 5%	1%
TREAT	4	59263.32	14815.83	6.340241	3.48	5.99
ERROR	10	23367.93	2336.793			
SE(m)	21.61848			Significant		
CD 5%	68.10668					
CD 1%	96.87166					

4. Conclusion

Biochemical properties investigation was carried out to study the effect of PGPR on the health of different varieties (HUW-510, PBW-347, and PBW-274) of wheat (*Triticum aestivum*) plant. Experiments were conducted using three morphologically different bacteria designated as PGPR1, PGPR2 and PGPR3 respectively. After preparation of inoculums seeds were treated in sterilized condition and with inoculum (5.0 ml inoculation) were at 7 days intervals. Experimental trials were performed in laboratory for one month (30 days). Results are discussed under the following sections. 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). All results in this biochemical analysis on effect of PGPR, Chlorophyl, Ascorbic acid, Starch & Total Polyphenolic Content (TPC) of different varieties of Wheat *(Triticum aestivum*) were found to be significant (p<0.05) as represent in ANOVA.

5. Acknowledgments

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6. References

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