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COMPARATIVE EFFECTIVENESS OF PLANT GROWTH PROMOTING RHIZOBACTERIA AND VARIOUS ORGANIC CARRIERS ON WHEAT GROWTH, PHYSIOLOGY, ANTIOXIDATIVE ACTIVITIES AND RHIZOSPHERE PROPERTIES

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Abstract

Plant growth-promoting rhizobacteria (PGPR) plays a key role in soil fertility and crop production. Inoculation of PGPR and various organic manures are known to sustain plant growth. Organic carrier materials were meet the requirements of a biofertilizer carrier i.e porosity, lightweight and environmental friendly. The present study describes the synergistic effects of PGPR and different organic carrier materials on wheat growth, physiology, antioxidants and rhizospheric soil properties. Plant growth parameters were increased as plant height (18%) enhanced with the combined use of PGPR and cow dung, while shoot fresh biomass (34%) and leaf area (77%) increase due to the combined use of PGPR + fruits and vegetable wastes. Plant root characteristics including root surface area, root length and root volume were improved due to individual as well as combined inoculation of PGPR and different organic carrier materials. Anti-oxidant enzyme activities related to ascorbate peroxidase (APX) and peroxidase (POX) were also improved due to separate as well as combined inoculation of PGPR and applied organic carrier materials. Chlorophyll a, chlorophyll b, carotenoids, membrane stability index and relative water contents were also significantly improved in all applied treatments. For each parameter combined application showed more promising results. So it can be concluded that various nutrient-rich organic carrier materials and PGPR should be applied in agricultural soils to sustain productivity and support crop production.

Key words: Beneficial microorganism, Organic materials, Soil properties, Wheat, Growth, Nutrients.

Introduction

Different organic materials (press mud, cow dung, compost and fruits & vegetable wastes) are being used as carriers for plant beneficial microbes as they are available easily and no deleterious effects on the surrounding environment (Arora *et al*., 2014). Soil treated with these organic additives seems to change physical, chemical and biological properties notably. Like press mud, a by-product obtained during the sugarcane crushing process is a nutritive organic addible which has a direct effect on soil biological properties. On accumulation to the soil, it flourishes it with micronutrients like Fe, Zn, Cu as well as macronutrients like N, P, K, Ca and Mg (Boateng *et al*., 2006; Liu *et al*., 2008). Due to its soil conditioning ability, it improves structure by enhancing aeration, porosity and water holding capacity. Notable growth in soil microbial activities and catalytic reactions is observed on its decomposition. Press mud can be used as a carrier material for various bacteria. A heterogeneous group of bacteria that resides in the rhizosphere and enhances the growth of plants through direct or indirect mechanisms even under normal and stressed conditions are called as plant growth promoting rhizobacteria (Glick, 2014; Adnan *et al*., 2020; Danish *et al*., 2019; 2020). Like phosphorus solubilizing bacteria making phosphorus available, zinc solubilizing bacteria making zinc available to plant through a number of processes occurring in the rhizosphere with developed soil biological properties (Kumar *et al*., 2017; Ali *et al*., 2017; Danish *et al*., 2019). Such beneficial native microbes can be used in amulgam with these substances as they provide niche and conditions necessary for continuance survival.

For the better development of the plant, widely used organic amendment is compost that is also beneficial for enhancing soil quality and balancing nutrients homeostasis in agricultural crops (Niamat *et al*., 2019). Along with this, compost enhances the availability of various nutrients through increasing their available fractions making them available to plant (Wei *et al*., 2015). Soil treated with composts shows higher microbial activities providing the surrounding soil with balanced nutrient cycling. For a long period, cow dung has been also applied to soil as a conventional additive for nutrients and soil quality improvement (Singh *et al*., 2015). Another source of organic manures is fruits & vegetable wastes supporting microbial life during transfer to the soil for PGPR formulations which also maintains moisture contents along with pH and water holding capacity of soils (Banerjee *et al*., 2017; Danish & Zafar-ul-Hye, 2019; Zafar-ul-Hye *et al*., 2019). Organic carrier materials were helpful for inotial establishment of bacterial strains by providing nutrient and cover from pathogens. The soil conditioning effect improves soil physical, chemical and biological outcomes (Abrishamkesh *et al*., 2015). Most of the research until now has focused on composts and, animal manure as microbe carrier. While neglecting the use of press mud, fruits and vegetable wastes till now.

Therefore, keeping in view the above mentioned characters of organic materials showing potential applications in agricultural crop production, the present study was conducted to evaluate the comparative impacts of PGPR and various organic based microbe carriers on soil properties, growth and productivity of wheat grown as a reference crop on large acreage in Pakistan (Mustafa *et al*., 2019b). It is hypothesized that functioning of PGPRs can be more efficacious in the presence of organic carriers regarding improvement in soil characteristics.

Materials and Methods

Collection of bacteria and experimental site: To study the effectiveness of rhizobacteria and organic materials on wheat growth, previously isolated PGPR strains *Alcaligenes* sp. AZ9 having accession number KU494828 (Hussain *et al*., 2015), was acquired from Environmental Sciences Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. Wheat variety Johar 2016 was kindly donated by Regional Agriculture Research Institute (RARI), Bahawalpur. Seeds were soaked in 2% sodium hypochlorite solution for 10 min in order to sterilize them, washed multiple times with tap water and again with ultra-pure water in order to remove any adhering contaminants. Physico-chemical properties of the test soil are given in (Table 1).

Table 1. Physio-chemical characteristics of soil before sowing of wheat.

Characters	Values
Soil textural class	Sandy loam
EC_e (dSm^{-1})	1.4 ± 0.04
pHs	8.1 ± 0.02
Organic matter (%)	0.4 ± 0.03
Available phosphorus (mg kg^{-1})	6.4 ± 0.17
Available potassium (mg kg^{-1})	78.3 ± 2.59
Saturation percentage (%)	35.7 ± 0.19
Nitrogen percentage (%)	0.02 ± 001

The data are presented as mean values of three replications; The values ± are standard error (SE)

Culture preparation and seed inoculation: General purpose nutrient broth was prepared and autoclaved at 121°C temperature and 15 psi for 20 minutes. Afterwards, placed in the laboratory to cool down at room temperature. Later on, a loop full of strain was dipped in the 100 ml conical flask and incubated in a rotary shaker (S19R-2, Sheldon manufacturing, INC. USA) at 130 rpm, 28±2°C for 48 hrs. The culture was centrifuged at 6000 rpm for 20 minutes (Saeed *et al*., 2019). The bacterial population was maintained by the turbidity metric method set at an optical density (OD_{540}) of about 10^8 cells mL^{-1} (Bhuvaneswari *et al*., 1980). For inoculation, five grams seeds were dipped in bacterial suspension for 2 hours along with the addition of 2.5g respective organic carrier material (cow dung, fruit and vegetable waste, compost and press mud) and 2.5 ml of 10% sugar solution. Seeds were mixed firmly in order to ensure fine homogeneous coating of inoculum to appear on seeds (Saeed *et al*., 2019). For control seeds, the sterilized broth was used instead of bacterial culture.

Seed inoculation and organization of experiment: A pot experiment was conducted to reach the experimental goals and each pot was filled with 4 kg of well sieved and air-dried soil collected from research area of Department of Soil Science, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, located at Lat: 29.40°N, Lon: 71.68°E and 116 meters elevation above the sea level. The experiment was laid out in completely randomized design (CRD) with 10 treatments *viz*., (Control, Compost, Fruits & vegetable wastes, Press Mud, Cow Dung, PGPR, PGPR + Compost, PGPR + Fruits & vegetable wastes, PGPR + Press Mud, PGPR + Cow Dung) and three replications. Ten (10) seeds were sown in each pot and six (6) plants per pot were maintained after germination. One third (1/3) of the recommended dose of nitrogen and full dose of phosphorus and potassium at the rate of (120, 90 and 60 kg/ha^{-1}) were applied in each pot before sowing in the form of Urea, DAP and SOP, respectively. However, the remaining dose of nitrogen was applied in two splits after germination at 25 days interval. All agronomic practices were performed as required.

At flowering stage, young leaves were obtained and stored for the measurement of antioxidant enzyme activities. At, maturity growth parameters were recorded and crop was harvested for yield parameter.

Biomass measurement and physiological recordings: Plant growth parameters like plant height, shoot fresh biomass, root fresh biomass were recorded at maturity. Dry shoot biomass and dry root biomass was recorded by keeping the samples in Memmert Loading Oven (100-800) for 48 hours at 65.4 ± 2ºC. Root volume, root surface area, total root length, leaf area and number of root tips were determined by using leaf and root scanner (STD, 4800). For membrane stability index, about 100 mg leaf sample was taken in a test tube having 10ml distilled water, keeping the sample for 30 minutes at 40°C in a water bath. The electrical conductivity of samples were measured and denoted as C_1. After heating the sample for 10 minutes at 100ºC, electrical conductivity ws again measured and denoted as C_2. Membrane stability index (MSI) was calculated by using the following formula (Sairam & Saxena, 2000).

$$MSI = [1 - \frac{C1}{C2}] \times 100$$

The relative water content of young leaves was measured as described by (Lazcano-Ferrat & Lovatt, 1999). Leaves were soaked in the distilled water for 16-18 hours until they become turgid. Leaves were dried with tissue paper before weighing. Dry weight was obtained by putting soaked leaves in an oven at 65ºC until constant dry weight. Following formula were used to calculate relative water content (RWC);

$$RWC\ (\%) = \frac{\text{Fresh weight (FW)} - \text{Dry weight (DW)}}{\text{Turgid weight (TW)} - \text{Dry weight (DW)}} \times 100$$

About 0.05 g fresh leaf sample was taken in test tubes and extracted in 10 ml dimethyl sulfoxide by heating it in a water bath at 64 ± 2ºC for 4 hours. The absorbance of extraction was recorded at 663, 645 and 470 nm for chlorophyll a, b and carotenoid, respectively (Arnon, 1949) and calculations were done by using Wellburn (1994) equation;

Chlorophyll 'a' (µg ml^{-1}) = (12.7 x O.D. at 663 nm) – (2.69 x O.D. at 645 nm)
Chlorophyll 'b' (µg ml^{-1}) = (22.9 x O.D. at 645 nm) - (4.08 x O.D. at 663 nm)
Carotenoids (µg ml^{-1}) = OD_{470} × total volume × dilution factor × 10 ÷ 2500

Measurement of antioxidant enzyme activity: For determination of enzymatic activity, about a half gram of plant sample was taken in pre-cooled morter surrounded by ice. The sample was crushed in solution with pH 7.8 having almost 2-3 ml of already prepared and cooled buffer until paste-like material appeared. The solution was shaken thoroghly by adding 5ml more phosphate buffer. The sample was centrifuged at 8000-13000 rpm for 20 minutes at 4ºC. and preserved in eppendorf tubes at 4ºC. Anti-oxidant enzyme activities like catalase activity (CAT), ascorbate peroxidase activity (APX), peroxidase dismutase activity (POD) and peroxidase activity (POX) were determined by taking absorbance on spectrophotometer and calculating them by equation described by Chance & Machly (1955), Asada & Takahashi (1987) and Chakraborty & Pradhan (1993). Serial dilution and pour plate techniques were used to find out bacterial population in LB agar plates described by Somasegaran & Hoben (1994) using Digital Colony Counter.

Statistical analysis

Statistical analysis was done using the software Statistix 8.1. Data was analysed using one-way analysis of variance (ANOVA). Tukey's highest significance difference (HSD) was used to attain if the differences between the treatments were significant at $p \leq 0.05$ level (Steel *et al*., 1997).

Results

PGPR and different organic carrier material had a significant effect on the physiology of plant: Data presented in (Table 2), revealed that plant physiological parameters were improved due to the inoculation of PGPR with the different organic carrier material. Separate application of different organic carrier materials and PGPR significantly improved physiological parameters of wheat. However, the combined inoculation of PGPR with different organic carrier materials showed distinct outcomes compared to a separate application. Separate application of press mud showed maximum improvement in membrane stability index 45% than control followed by the sole inoculation of PGPR (34%). Chlorophyll a & b were increased primly 33% and 41%, respectively by combined inoculation of PGPR + press mud. Relative water contents were observed 15% higher than control in the combined inoculation of PGPR + cow dung. Combined inoculation of PGPR + cow dung caused 24% rise in carotenoids than control following separate application of fruit & vegetable waste with 27% higher values than control.

Anti-oxidant enzyme activities were affected due to PGPR and different organic carrier material: The efficiency of PGPR and different organic carrier materials on plant enzymatic properties like peroxidase dismutase activity (POD), catalase activity (CAT), ascorbate peroxidase activity (APX) and peroxidase activity (POX) activity are presented in (Table 3). All applied treatments had a nonsignificant effect on peroxidase dismutase activity (POD) over control. However, the other three enzymatic activities were significantly improved by the inoculation of PGPR and different organic carrier material. Combined inoculation of PGPR + compost, PGPR + fruit & vegetable waste and PGPR + cow dung showed nonsignificant results to each other regarding catalase activity (CAT). While comparing these results with all other treatments were significant where 15% increase was recorded compared to control. Separate application of fruit and vegetable waste exhibited highest enhance up to 48% in ascorbate peroxidase activity. Combined inoculation of PGPR + compost, PGPR + press mud and PGPR + cow dung are the treatments in which highest peroxidase activity was recorded.

The inoculation of PGPR with different organic carrier materials improved plant growth: The bacterial isolate AZ9 along with different organic carrier material exerted an influence on wheat growth as shown in (Table 4). All applied treatment had a statistically significant impact on wheat growth parameters. But the separate application of compost showed statistically nonsignificant results compared to control. Maximum leaf area was recorded 77% by the use of PGPR + fruit while the highest shoot fresh biomass was observed 34% by sole application of vegetable waste. The highest value of plant height was recorded with inoculation of PGPR + cow dung that was 18% more than control. Individual inoculation of PGPR was recorded with highest number of tiller pot^{-1} followed by combined inoculation of PGPR + compost. Shoot dry biomass enhanced 68% than control by combined inoculation of PGPR + compost. The maximum increase in shoot dry biomass observed was 54% with sole application of fruit and vegetable waste, PGPR and combined inoculation of PGPR + fruit and vegetable wastes.

Root characteristics such as, root fresh and dry biomass, root volume, total root length, root surface area and number of roots are demonstrated in (Table 5). Separate as well as combined inoculation of PGPR and different organic carrier material showed significant for all root growth parameters. The maximum increase in root fresh biomass was recorded 49% by combined inoculation of PGPR + composed. Separate application of compost and PGPR was observed with the highest gain in root dry biomass which was 38% more than control. Solely applying fruit & vegetable waste raised root tips and root surface area 10% and 17%, respectively. The maximum increase in total root length 29% was observed over control by the application of compost followed by the combined inoculation of PGPR + fruit & vegetable waste with 26% increase over control. Maximum root volume 9.4 cm^3 was observed due to the combined inoculation of PGPR + fruit & vegetable waste which was statistically similar to the sole application of PGPR.

Table 2. Comparative effects of PGPR and different organic carrier materials on physiological parameters of wheat crop.

Treatments	MSI	RWC	Chl a	Chl b	Carotenoid
	%		µg g^{-1}		
Control	26.3 f (±0.437)	71.3 h (±0.236)	0.54 d (±0.003)	0.44 i (±0.004)	0.68 i (±0.003)
Compost	35.1 a-c (±0.003)	75.7 d-f (±0.529)	0.68ab (±0.027)	0.54 de (±0.002)	0.78 de (±0.003)
F & V wastes	30.8 de (±0.003)	78.6 b-d (±0.384)	0.66 a-c (±0.007)	0.58 bc (±0.003)	0.86 a (±0.002)
Press Mud	38.2 a (±0.003)	77.1 c-e (±0.342)	0.58 cd (±0.003)	0.46 hi (±0.002)	0.70 hi (±0.003)
Cow Dung	30.0 d-f (±0.003)	72.2 gh (±0.312)	0.55 d (±0.002)	0.50 fg (±0.002)	0.74 fg (±0.003)
PGPR	36.0 ab (±0.003)	79.3 a-c (±0.200)	0.70 ab (±0.003)	0.60 ab (±0.003)	0.80 cd (±0.003)
PGPR + Compost	32.0 c-e (±0.003)	73.4 f-h (±0.362)	0.62 b-d (±0.003)	0.48 gh (±0.003)	0.72 gh (±0.003)
PGPR + F & V wastes	31.1 de (±0.003)	74.5 e-g (±0.308)	0.60 cd (±0.003)	0.56 cd (±0.003)	0.76 ef (±0.003)
PGPR + Press Mud	29.8 ef (±0.003)	80.1 ab (±0.337)	0.72 a (±0.003)	0.62 a (±0.003)	0.82 bc (±0.005)
PGPR + Cow Dung	33.8b-d (±0.003)	82.1 a (±0.348)	0.64 a-c (±0.003)	0.52 ef (±0.003)	0.84 ab (±0.003)
HSD (p≤ 0.05)	3.8991	3.0013	0.0807	0.0261	0.0299
CV	4.18	1.36	4.44	1.70	1.34

The data are presented as mean values of three replication; Means followed by same letter(s) within the column are statistically non-significant according to Tukey's HSD test at *p≤0.05*. Data in parenthesis represent standard error. F & V waste, fruit and vegetable waste; MSI, membrane stability index; RWC, relative water content; Chl a, chlorophyll a; Chl b, chlorophyll b

Table 3. Comparative effect of PGPR and different organic carrier materials on anti-oxidant enzyme activities in wheat crop in pot trial.

Treatments	POD	CAT	POX	APX
	Units mg^{-1} FW			Units min^{-1} mg^{-1} FW
Control	51.3 a (± 0.047)	6.71 c (± 0.060)	2.43 e (± 0.051)	1.92 g (± 0.034)
Compost	52.3 a (± 0.368)	7.33 a-c (± 0.222)	3.11 a-c (± 0.025)	2.55 a-c (± 0.042)
F & V wastes	52.8 a (± 0.307)	6.86 bc (± 0.019)	3.17 ab (± 0.017)	2.84 a (± 0.019)
Press Mud	52.5 a (± 0.103)	7.23 a-c (± 0.028)	3.05 a-d (± 0.019)	2.01 fg (± 0.000)
Cow Dung	53.0 a (± 0.115)	7.52 ab (± 0.067)	2.93 b-d (± 0.043)	2.72 ab (± 0.026)
PGPR	52.2 a (± 0.024)	7.12 a-c (± 0.037)	2.77 c-e (± 0.057)	2.49 b-d (± 0.009)
PGPR + Compost	51.6 a (± 0.191)	7.67 a (± 0.019)	3.35 a (± 0.051)	2.11 ef (± 0.015)
PGPR + F & V wastes	52.6 a (± 0.014)	7.63 a (± 0.039)	2.72 de (± 0.035)	2.32 c-e (± 0.035)
PGPR + Press Mud	52.6 a (± 0.024)	7.44 ab (± 0.030)	3.39 a (± 0.056)	2.40 c-e (± 0.024)
PGPR + Cow Dung	51.9 a (± 0.302)	7.72 a (± 0.041)	3.33 a (± 0.032)	2.20 d-f (± 0.033)
HSD (p≤ 0.05)	1.7158	0.6943	0.3563	0.3057
CV	1.13	3.28	4.07	4.51

The data are presented as mean values of three replications; Means followed by same letter(s) within the column are statistically non-significant according to Tukey's HSD test at *p≤0.05*. Data in parenthesis represent standard error. F & V waste, fruit and vegetable waste; POD, peroxidase dismutase activity; CAT, catalase activity; APX, ascorbate peroxidase activity; POX, peroxidase activity; FW, fresh weight

Table 4. Comparative effect of PGPR and different organic carrier materials on growth parameters of wheat crop in pot trial.

Treatments	SL	LA	SFB	SDB	NT
	cm	cm^2	g pot^{-1}		pot^{-1}
Control	55.4 g (± 0.319)	2.51 j (± 0.005)	31.4 f (± 0.440)	7.5 g (± 0.003)	25 h (± 0.333)
Compost	55.4 g (± 0.316)	3.43 f (± 0.003)	35.1 de (± 0.332)	9.2 f (± 0.003)	28 e-g (± 0.333)
F & V wastes	62.1 bc (± 0.141	3.81 d (± 0.003)	36.0 c-e (± 0.342)	11.4 b (± 0.003)	32 bc (± 0.333)
Press Mud	56.2 fg (± 0.289	2.84 I (± 0.003)	34.2 ef (± 0.367)	9.4 e (± 0.005)	26 gh (± 0.333)
Cow Dung	56.3 fg (± 0.300	4.03 c (± 0.003)	38.3 bc (± 0.318)	9.3 ef (± 0.030)	27 f-g (± 0.333)
PGPR	59.2 de (± 0.255	3.01 h (± 0.003)	41.4 a (± 0.340)	11.5 b (± 0.028)	36 a (± 0.333)
PGPR + Compost	61.0 cd (± 0.333	4.24 b (± 0.005)	35.8 c-e (± 0.315)	12.6 a (± 0.034)	34 ab (± 0.333)
PGPR + F & V wastes	58.3 ef (± 0.346	4.44 a (± 0.007)	42.1 a (± 0.330)	11.5 b (± 0.003)	30 c-e (± 0.333)
PGPR + Press Mud	63.7 ab (± 0.097	3.23 g (± 0.005)	37.6 b-d (± 0.373)	10.6 c (± 0.003)	31 cd (± 0.333)
PGPR + Cow Dung	65.3 a (± 0.387)	3.64 e (± 0.004)	39.4 ab (± 0.305)	10.3 d (± 0.005)	29 d-f (± 0.333)
HSD (p≤0.05)	2.5329	0.0381	3.0186	0.1966	2.8909
CV	1.48	0.37	2.81	0.66	3.36

The data are presented as mean values of three replications; Means followed by same letter(s) within the column are statistically non-significant according to Tukey's HSD test at *p≤0.05*. Data in parenthesis represent standard error. F & V waste, fruit and vegetable waste; SL, shoot length; LA, leaf area; SFB, shoot fresh biomass; SDB, shoot dry biomass; NT, number of tillers

Table 5. Comparative effect of PGPR and different organic carrier materials on root growth of wheat crop in pot trial.

Treatments	TRL	RFB	RDB	RSA	RV	NRT
	cm	g pot^{-1}		cm^2	cm^3	plant^{-1}
Control	760 j (± 0.357)	22 g (± 0.369)	9.2 h (± 0.033)	294 i (±1.011)	8.5 g (± 0.039)	5474 j (± 0.509)
Compost	984 a (± 0.352)	28 c-e (± 0.333)	12.7 a (± 0.033	336 bc (± 0.315)	8.8 d-f (± 0.028)	5894 c (± 0.667)
F & V wastes	875 f (± 0.359)	26 ef (± 0.333)	12.2 b (± 0.033	343 a (± 0.803)	9.1 a-c (± 0.044)	6010 a (± 1.667)
Press Mud	785 i (± 0.358)	29 cd (± 0.333)	9.7 g (± 0.033)	306 h (± 0.255)	8.6 fg (± 0.033)	5537 i (± 0.667)
Cow Dung	815 h (± 0.308)	32 ab (± 0.333)	11.2 d (± 0.033	331 de (± 0.627)	9.2 ab (± 0.039)	5954 b (± 0.839)
PGPR	937 c (± 0.362)	25 fg (± 0.333)	12.7 a (± 0.033	340 ab (± 0.509)	9.3 a (± 0.035)	5817 d (± 0.667)
PGPR + Compost	851 g (± 0.364)	33 a (± 0.333)	10.1 f (± 0.033	313 g (± 0.302)	8.7 e-g (± 0.036)	5586 h (± 0.333)
PGPR + F & V wastes	961 b (± 0.349)	30 bc (± 0.333)	12.2 b (± 0.033	323 f (± 0.342)	9.4 a (± 0.037)	5777 e (± 0.333)
PGPR + Press Mud	892 e (± 0.310)	26 ef (± 0.333)	10.6 e (± 0.057	329 e (± 0.313)	8.9 c-e (± 0.015)	5695 f (± 0.667)
PGPR + Cow Dung	912 d (± 0.320)	27 d-f (± 0.333)	11.7 c (± 0.035	334 cd (± 0.350)	9.0 b-d (± 0.001)	5650 g (± 0.667)
HSD (p≤ 0.05)	2.9876	2.9230	0.3033	4.6790	0.2856	6.8206
CV	0.12	3.64	0.93	0.50	1.10	0.04

The data are presented as mean values of three replications; Means followed by same letter(s) within the column are statistically non-significant according to Tukey's HSD test *p≤0.05*. Data in parenthesis represent standard error. F & V waste, fruit and vegetable waste; TRL, total root length; RFB, root fresh biomass; RDB, root dry biomass; RSA, root surface area; RV, root volume; NRT, number of root tips

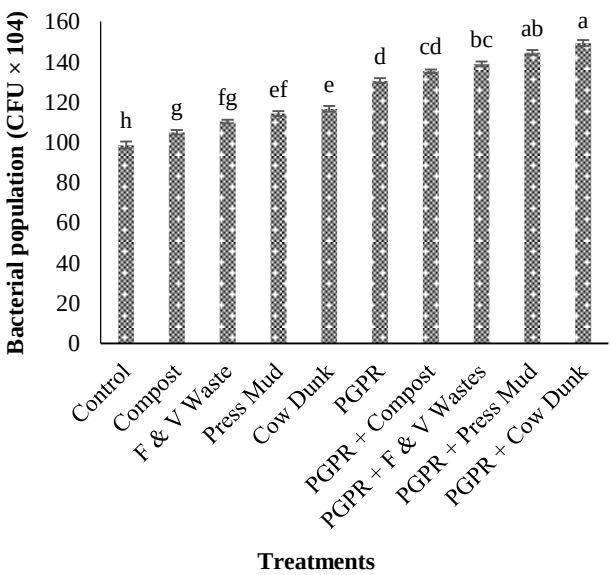

Fig. 1. Comparative effect of PGPR and different organic carrier material on microbial population in soil after harvesting of wheat crop in pot trial; The data are presented as mean values of three replications; Bars followed by same letter(s) are statistically non-significant according to Tukey's HSD test at *p≤0.05*; Standard Error for comparison is 1.78.

Sole and combined inoculation of PGPR and different organic carrier materials improved microbial population in the rhizosphere: Results in Fig. 1, indicates comparative effectiveness of separate as well as the combined application of different carrier organic materials and plant growth promoting rhizobacteria (PGPR) to improve soil biological properties. Among separate applications, PGPR made an increase in bacterial population to the highest extent of 32% followed by cow dung which showed 18 % increase in the bacterial population. However, the combined inoculation of PGPR + cow dung observed with maximum bacterial population (149×10^4) which was 51% more than control. Combined inoculation of PGPR + press mud was at second showing a rise of 47% in the bacterial population if compared with control.

Discussion

The soil is a dynamic matrix that supports plant production. However, in soil-plant growth is hampered by several environmental stresses including biotic and abiotic, for instance, plant pathogens, weeds, salinity, drought, heavy metals, temperature and flooding conditions (Glick *et al*., 2007; Nadeem *et al*., 2014; Ali *et al*., 2017; Mustafa *et al*., 2019a). The excessive utilization of agrochemicals to combat such stresses and recompense the crop production losses, on the other hand, threatens environmental quality (Mustafa *et al*., 2019a). Significant and advance studies have been required to find the effective role of PGPR to improve the growth, production and quality of different crops on a sustainable basis.

Bio-fertilizers containing PGPR nourishes soil by different ways like boost up biological nitrogen fixation, prompt solubilization of insoluble nutrients and assist different enzymatic activities in soil and make the plant to grow well even in the presence of stressed conditions (Ali *et al*., 2017; Ahmad *et al*., 2019). In our study, Plant growth, physiological and biochemical parameters were increased by sole as well as the combined use of multi-trait plant growth-promoting rhizobacteria with different organic carrier materials. Our results agreed with the flindings of Mumtaz *et al*., (2017) who stated that the inoculation of PGPR improved antioxidant enzyme activities. Hussain *et al*., (2019) specified that combined inoculation of PGPR and different organic fertilizers improved growth, yield and quality of grain along with other growth parameters in maize.

In the present study, growth parameters include plant height and shoot fresh and dry biomass, number of fruits per plant, weight of fruits, number of tillers and number of leaves were recorded high by the mixed use of PGPR with different organic carrier materials (Fig. 2) (Picture). Similar results were also noted by Fatima *et al*., (2018) who stated that the inoculation of PGPR promoted plant growth and yield. Such indigenous soil microbs lead to a remarkable gain in shoot height and weight (Majeed *et al*., 2015). Priyanka & Koshy (2016) reported that organic wastes showed potential rise in

plant growth parameters like plant length, plant fresh and dry biomass, germination percentage and length of fruit emerged. This was due to the incorporation of manure to the soil. Addition of organic materials to soil makes biomass of plant higher including growth characteristics (Dotaniya *et al*., 2016). Effect of rhizobacteria on plant growth includes thick vegetative growth, increased plant height and weight by their growth promoting characteristics. Remarkable changes were noted in plant height, shoot fresh and dry biomass, root fresh and dry biomass due to inoculation of PGPR and different organic materials (Mumtaz *et al*., 2017; Hussain *et al*., 2015).

Significant improvements in plant growth, yield and biochemical properties can be achieved with the introduction of organic manures like composts (Niamat *et al*., 2019). We found, increased root growth, fresh and dry biomass of root, surface area of root and number of root tips due to the inoculation of PGPR with different carrier organic materials. Kumar *et al*., (2017) described that the use of organic substances could cause a remarkable increase in root growth, root proliferation, increase number of root hairs which in results directly increase in yield of crop plant. In the conducted research, outcomes are also assisted by Fatima *et al*., (2018) who used native bacteria to improve root growth along with root length, root fresh and dry biomass. The latest study by Hossain *et al*., (2019) revealed a strong co-relation between plant growth, yield and nutrients homeostasis and use of composts. Bio inoculants can be used for enhancing biological yield in a number of ways like by improving root colonization (Owen *et al*., 2015). By applying microbes, increased N availability could play a major role in increasing root length and weight and root colonization potential (Majeed *et al*., 2015). Nutrient rich organic chemicals are released from roots from which microbes get the benefit and facilitate the growth of plant. High values of root length and root weight were reported by their introduction to soil (Mumtaz *et al*., 2017).

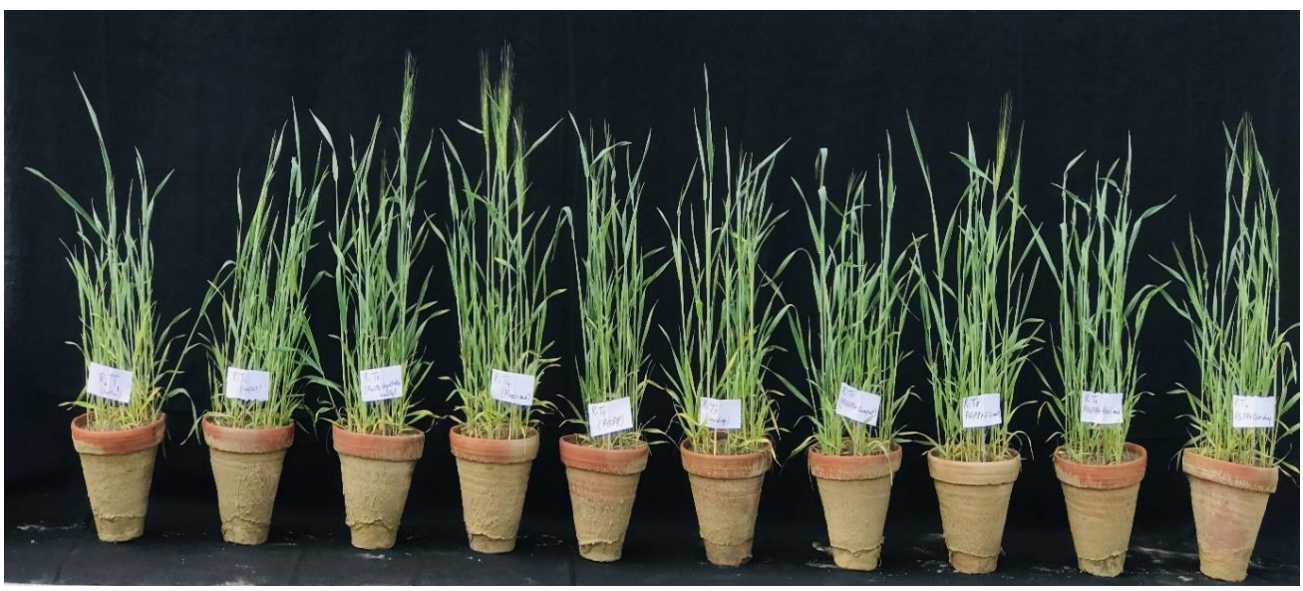

Fig. 2. Pictorial overview of plant growth (45 days after sowing) due to separate as well as combined inoculation of PGPR and different organic carrier materials. Pots are arranged from left to right as control, compost, fruit and vegetable waste, press mud, cow dung, PGPR, PGPR + compost, PGPR + fruit and vegetable waste, PGPR + press mud and PGPR + cow dung.

Plant physiological properties like chlorophyll contents of plants were also observed higher by use of these beneficial microbes. The research conducted reveals higher values of Chlorophyll contents like chlorophyll a and b. These results are also supported by Fatima *et al*., (2018) who reported a raise in chlorophyll by using microbes. Use of inborn rhizobacteria reduces the effect produced by the scarcity of nutrients as they have the potential to make them available to plant. As a result, the plant shows maximum growth characteristics and an increase in vegetative growth. A remarkable increase in plant physiological properties like chlorophyll contents in our study also match with the results of Mumtaz *et al*., (2018).

In the present study, plant antioxidant enzymes activities like POD, CAT, POX and APX were improved as organic additives used separately and in combination with PGPR even in stress conditions. Similar results were reported by Saeed *et al*., (2019) who observed antioxidant enzymes inaction under Cd stress condition along with improved brassica growth and physiological properties. Similarly, Altaey *et al*., (2018) also noted activeness of peroxidase and catalase as an organic natured substance when added to salt affected soil. Meeting the salt stress problems, PGPR are believed to be an important element of the scavenging process by increasing anti-oxidant enzyme production like CAT and POD. In the presence of these species, plant shows optimum growth in stress condition (Babaei *et al*., 2017). Israr *et al*., (2016) also reported that local microbes also influence the activity of antioxidant enzymes as un-inoculated seedlings show variations in outcomes.

In our study, separate as well as the combined inoculation of PGPR and different organic carrier materials seemed to enhance the bacterial population. However, the

outcome was distinct in case of combined inoculation of PGPR + different organic carrier materials than a separate application. The microbial population play a key role in enzymatic activities, mineralization of soil organic carbon, availability of vitamins and following the development of bacterial community (Hartmann *et al*., 2015). There is a robust correlation between soil fertility and SOC content in the response of greater microbial diversity (Chakraborty *et al*., 2011). Earlier researches report that use of natural organic amendments develops soil biological properties such as microbial population, MBC and enzymatic activities, and water holding ability (Ros *et al*., 2006; Tejada *et al*., 2009; Schulz *et al*., 2014; Chen *et al*., 2018). Similar results were also observed by Hussain *et al*., (2019), they noted the enhancement in biological properties of soil by the combined use of native microbes and organic material optimized the biological yield of maize.

Conclusion

This study concluded that the inoculation of PGPR along with different organic carrier materials like compost, press mud, fruits & vegetable wastes and cow dung increased growth and productivity of wheat crop. Combined inoculation of PGPR and different organic carrier materials also improved the bacterial population in the rhizosphere of the wheat crop that resulted in increased uptake of nutrients by the plant. Separate inoculation of PGPR and different organic carrier materials increased plant growth parameters including shoot fresh biomass, root fresh biomass. Plant height, root length, root volume, leaves surface area etc. Plant physiological and bio-chemical properties were also enhanced due to applied amendments. Physiological attributes including relative water contents, membrane stability index, chlorophyll a, b and carotenoid contents were also increased due to the inoculation of PGPR and different carrier organic materials. The combined inoculation of PGPR with different organic carrier materials had more influence rather than a separate inoculation. However, cow dung as a carrier material performed significantly well as compared to other organic carrier materials.

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