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Full Length Article

Vermicompost Augmented with Plant Growth Promoting Rhizobacteria Improved Soil Fertility and Growth of *Brassica rapa*

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Abstract

The excessive use of chemical fertilizers in agriculture has created several health and environmental issues which necessitates the finding of alternate means for sustainable crop production. This study was designed to compare the effects of plant growth promoting rhizobacteria (PGPR)-enriched, and plain vermicompost on growth and yield of turnip (*Brassica rapa* L.) and soil fertility. Microbial strains were isolated from the previously manufactured vermicompost and characterized *in vitro* for plant growth promoting traits (*e.g*., phosphate (P) solubilization, growth hormone and enzyme production). Based on exhibition of maximum traits of PGPR, 11 out of 22 strains were selected for inoculating the vermicompost before its application to the turnip plants in pots under natural conditions. Results revealed that 3 strains *i.e*., MV-9, MV-17 and MV-21 showed significantly higher potential of plant growth promoting traits (mentioned above). Similarly, vermicompost enriched with these PGPR strains significantly improved germination rate (43%) and turnip yield (30%) along with increased number of leaves, leaf length, diameter, fresh/dry weights of plant shoot/root compared with control. PGPR treated vermicompost unquestionably improved the macro and micronutrients in plant and soil, despite a notable variability was observed between treatments regarding contribution towards soil and plant nutrients. Inter strain comparison affirmed that, MV-17 treated vermicompost resulted in 63% higher soil NO_3-nitrogen compared with control, whereas in roots, same isolate increased phosphate (P) and potassium (K) contents, but not NO_3-N. In general comparison of PGPR strains, MV-9, MV-17 and MV-21 significantly increased turnip fresh weight, soil macro and micronutrient contents. Overall, it is concluded that vermicompost augmented with bacterial strains; MV-9, MV-17 and MV-21 imparted beneficial effects on growth, yield and nutritional value of turnip and increased soil fertility.

Keywords: Vermicompost; PGPR; Plant nutrition; Soil fertility; *Brassica rapa*

Introduction

After green revolution, the use of chemical fertilizers has increased manifolds making them a lifeline for conventional agricultural systems. Undoubtedly, excessive use of chemical fertilizers has significantly increased crop yields over the decades. However, imbalanced use of these chemicals has disintegrated soil physical health resulting in stagnant crop yields (Kumar *et al*., 2018). Along with other irregularities, superfluous use of chemical fertilizers has acutely regressed the soil fertility, microbial biodiversity, coupled with increasing ground water pollution and compromising human and environmental health (Bakar *et al*., 2015). High cost and associated environmental health risks of chemical fertilizers warrant investigating the alternate means of low cost and environment friendly sustainable crop production. Vermicomposting is well recognized and established technique for improving the crop growth and yields by transforming organic wastes into valuable nutrients for soil and plants (Ibrahim *et al*., 2008). It has several benefits over chemical fertilizers including: 30-40% higher crops yield, instant bio accessibility of nutrients to the plants, and reducing up to 40% water requirement for irrigation compared with chemical fertilizer application (Ganeshnauth *et al*., 2018).

Vermicomposting, a biochemical method consisting of consortia of earthworms and microorganisms for degradation/breakdown of organic materials is recognized as a plausible solution to improve the crop yield, maintaining/improving the soil fertility and that too with no environmental hazards. Earthworms, known as the nature's plowman, are excellent indicators of soil fertility which simultaneously improve physical, chemical, and biological composition of host soil. Most of the soil digested by earthworms is discharged into soil environment in the form of fine mucus covered granular aggregates, which are rich

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source of NPK, micronutrients and beneficial microbes. The excreta of earthworms called 'vermicasting' are very useful in combating the soil salinity and being antipathogenic reduces the use of pesticide on crops (Sinha *et al*., 2010, 2014). Earthworms serve as critical drivers of vermicomposting thereby increasing aerobic microbial activity area, accelerating enzyme activities and breaking up the complex organic compounds into simpler ones to be further degraded by microorganisms (Fu *et al*., 2014). Although bacterial counts vary in the compost but usually the numbers of *Rhizobium*, *Azotobacter*, *Actinomycetes*, Phosphate solubilizing and *nitrobacter* may range from 10^2–10^6 in one gram of vermicompost (Sinha *et al*., 2010). Earthworms ingest plant growth promoting rhizosphere bacteria, where the engulfed PGPR start multiplying because of favorable conditions in the gut of earthworm. This particular group of microorganisms stimulates growth of plants by several direct and indirect mechanisms including growth hormone production, phosphate solublization, fighting against pathogens and nitrogen fixation amongst others (Sinha *et al*., 2010; Pathma and Sakthivel, 2012; Asad *et al*., 2018).

Diverse microbial groups accelerate the vermicomposting which slows down over time. The slowing down process of vermicomposting is provoked while mixing with nitrogen fixers and phosphate solubilizers including: *Azospirillum brasilense*, *Azotobacter chroococcum* and *Pseudomonas maltophila* (Kaushik *et al*., 2008). Gopinathan and Prakash (2014) observed significant difference in the growth and yield of tomato when treated with bacterial enriched vermicompost as compared to plain compost *i.e*., which was not supplemented with additional bacterial cultures. Similarly, actinomyces isolated from the vermicompost improved the agronomic and economic yield of rice (Gopalakrishnan *et al*., 2014). Vermicompost augmented with phosphate solubilizing bacteria (*Bacillus* spp.) positively influenced the growth of cowpea (Manivannan *et al*., 2012). The role of PGPRs in improving the plant growth and development is extensively studied over the last many decades and has remarkable role in crop productivity (Asad *et al*., 2004; Nadeem *et al*., 2010; Hussain *et al*., 2016; Asad *et al*., 2018, 2019). These microbes live in the close proximity of roots and impart several direct and indirect benefits by establishing symbiotic relationship with plant roots and along with other provisions also help plants to uptake the nutrients and cope with toxic environments (Belimov *et al*., 2005; Asad *et al*., 2018, 2019).

Although a significant amount of work has been published highlighting the beneficial role of vermicompost and PGPR on the growth and yield of several crops including: legumes, banana, cassava, okra but those investigations involved either of the vermicompost or PGPR but not both in any single study. Moreover, most of the research was conducted under controlled conditions which necessarily do not mimic the natural environment. Current research focus on the value addition of prepared vermicompost by supplementing it with additional PGPR under natural conditions. Experimental work under this research was aimed to investigate the joint effects of vermicompost and PGPR on turnip. Augmentation of the vermicompost with PGPR will not only enhance its efficiency, but will also reduce its demand up to 50%, which may significantly curtail the cost of production of turnip. Parallel with these advantages, application of PGPR supplemented vermicompost to the crop would improve the nutritional value of turnip and soil fertility.

Materials and Methods

Vermicompost-sampling and Analysis

Freshly prepared vermicompost was obtained from the vermiculture and vermicomposting research unit of National Agricultural Research Centre (N.A.R.C.), Islamabad. Collected samples were sealed in sterilized zipper bags and stored at 4°C before being processed to isolate bacteria. For this purpose, serial dilutions were prepared as per the methods devised by Somasegaran and Hoben (1994). Briefly, one drop of each serial dilution was poured on the solidified agar media plates and streaked over the entire surface to observe bacterial growth. Plates were incubated at 37ºC for 24 h. Afterwards, the full-grown bacterial colonies were purified by transferring them to freshly prepared agar media plates. The process was repeated until pure bacterial colonies were obtained.

Microbial Characterization

Bacterial cultures isolated from the vermicompost were morphologically and biochemically characterized. The morphological observations were carried out on the full grown isolated bacterial colonies and studied for color, shape, and size. Gram staining (Vincent, 1970) was performed to further classify the bacterial isolates into gram positive and gram negative. Biochemical characterization of bacterial cultures was performed as below.

Indole Acetic Acid (IAA) Production

Indole acetic acid (IAA) production capability of bacterial isolates was determined as per Okon *et al*. (1977). For this purpose, isolated bacterial colonies were inoculated in L-tryptophan (precursor of IAA) containing LB liquid broth. Inoculated culture media was incubated at 28 ± 2°C for 7 days followed by addition of few drops of kovacs reagent. Development of cherry red color on top of medium was indication of IAA production by isolates. To quantify the IAA concentration, 50 mL of broth culture was centrifuged at 10,000 rpm for 10 min. After centrifugation, pellet was discarded and Salkowiski

reagent was mixed with supernatant in the ratio of 1:4 (Loper and Schroth, 1986). After 30 min of reaction, absorbance was recorded with spectrophotometer at 535nm wavelength to quantify IAA.

Phosphate Solubilization

For this essay, bacterial isolates were placed on Pikovskaya agar plates and incubated at 28°C for 7 days (Pikovskaya, 1948). Formation of a clear zone around colonies indicated the phosphate solubilization by bacterial isolates; the important characteristic of plant growth promoting rhizobacteria (PGPRs). Qualitative estimation of phosphate solubilized by bacteria was determined by calculating the phosphate solubilization index (PSI) and phosphate solubilization efficiency (PSE%) while taking in to account the colony and halo zone diameter as of equation 1 and 2 respectively.

$$PSI = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$ Eqn.1 (Qureshi *et al*., 2012)

$$PSE(\%) = \frac{\text{Diameter of clearance zone}}{\text{Diameter of growth zone}} X100$$ Eqn. 2 (Srividya *et al*., 2009)

For quantifying phosphate solubilized by bacteria, a bacterial colony was added in to 100 mL of sterilized liquid Pikovaskaya media and incubated at 28°C for 7 days. Afterwards, the bacterial cultures were centrifuged for 10 min at 10,000 rpm. Then 0.1 mL of the supernatant was diluted by adding distilled water and making the total volume of 10 mL. After dilution, 2.5 mL of color developing reagent was added to the samples and vortexed. After 30 min of reaction, absorbance was recorded on spectrophotometer at 880nm wavelength (Soltanpour and Workman, 1979). Solubilized phosphate was calculated according to equation 3.

$$P(ppm) = P\ (ppm)\ \text{from calibration curve X Dilution factor}$$ (Eqn. 3)

Ammonia (NH_3) Production

Isolates were tested for their capability to produce ammonia (NH_3). For this purpose, isolates were inoculated in peptone water for 48 h at 30°C (Dye, 1962). After incubation, few drops of Nessler's reagent were poured in each sample. Appearance of brownish to deep yellow color indicated that strain was strongly capable to produce NH_3, while light yellow color of the sample showed weak ability of bacteria to produce ammonia (Cappucino and Sherman, 1992).

Enzymes Production by Bacteria

Bacteria isolated from the vermicompost were tested for their ability to produce enzymes: catalase, amylase, protease and pectinase. Essays were performed as detailed below.

Catalase: enzyme production was determined by pouring a drop of hydrogen peroxide (H_2O_2) on the bacterial colony already placed on sterilized slide. Appearance of air bubbles within 10 sec of additions marked the strain as catalase positive and vice versa (MacFaddin, 2000).

Amylase: For this essay, isolated bacteria were streaked over the amylase medium. Bacterial cultures were incubated at 35°C for 48 h followed by addition of few drops of iodine. Clear zone formation around the bacterial colonies was the indication of amylase production by bacteria (Ashwini *et al*., 2011).

Pectinase: Production of pectinase by bacterial isolates was determined by inoculating the bacterial strains on pectinase medium and incubating at 35°C for 48 h. Colonies forming clear zone around them were capable to produce this enzyme (Namasivavam *et al*., 2011).

Protease: Protease activity of bacterial isolates was determined by growing the colonies on skimmed milk agar medium (SKM) as described by Kazempour (2004). After incubation time of 48 h at 35°C, halo zone formation around the bacterial isolates was an indication of the protease production trait and vice versa.

Microbial Augmentation of Vermicompost

Based on microbial potential to produce IAA, enzymes and phosphate solubilization under controlled conditions, 11 out of 22 strains were selected for mass multiplication in LB broth. Strains were allowed to multiply for three days and poured in to already prepared vermicompost and incubated at 35ºC for four days before application to the experimental plants.

Evaluation of Enriched Vermicompost on *Brassica rapa*

Vermicompost augmented with beneficial bacterial strains was evaluated on *Brassica rapa* L. plants at the National Agricultural Research Centre (N.A.R.C.). The experimental pots were not placed in any environmental control, but placed in protection from the animals etc. Hence, it simulated with natural experimental conditions. Experimental design, plant growth parameters studied, plant and soil analysis are detailed below.

Experiment Description

For evaluating the effects of PGPR augmented vermicompost on turnip, thirty-six (36) pots were filled with 10 kg of soil consisting of sand, silt and clay in the ratio of 3:1:1 respectively. There were three replicates for each of the twelve treatments, consisting of a control (receiving plain vermicompost) and 11 treatments of bacterial augmented vermicompost, each inoculated with 1 out of 11 bacterial isolates. Soil in each pot was spiked with 10 g of vermicompost. At this rate of soil and vermicompost application per pot, it would make the utility of vermicompost at 2 tons ha^{-1} on weight basis. Twelve seeds of experimental plant were sown in each pot.

Germination (%)

Germination percentage (%) of turnip seeds was calculated according to Mukhtar (2008) as in equation 4.

$$\text{Germination}(\%) = \frac{\text{No. of germinated seeds}}{\text{Total number of seeds}} \text{X100} \quad \text{(Eqn. 4)}$$

Growth Parameters

Plants were destructively harvested at maturity stage. Harvested plant biomass was washed with tap water and dried. After drying, agronomic traits of plants including number of leaves plant^{-1}, leaf length, width, fresh and dry weights of shoots and roots of each plant were determined. For dry weights determinations, shoots and roots were oven dried at 65°C until constant weight was obtained.

Plant Nutrient Analysis

Harvested plant material was ground by seed mill grinder homogenously for nutrients analysis. Nitrogen contents in plants samples were determined by Kjeldahl's method while remaining nutrients were analyzed through acid digestion protocol, thereby carrying the digestion on hot plate in fume hood. After the disappearance of green color, aliquots of this solution were analyzed for micro and macro nutrients determinations (Ryan *et al*., 2007). Phosphorus (P) was measured by spectrophotometer at 410 nm wavelength, while potassium (K), was analyzed on flame photometer. Calcium, magnesium, iron, zinc, copper and manganese determined by using atomic absorption spectrophotometer.

Soil Analysis

Chemical properties of soil were determined before sowing and after harvesting the plants. For these analyses, soil samples were air-dried at room temperature, mixed homogenously and sieved through a 2 mm mesh size. A homogenous soil slurry was prepared by mixing 15 g of air-dried soil in 15 mL distilled water for measuring the pH and EC. Macro and micro nutrients in the soil samples were worked out by using ammonium bicarbonate-diethylene triamine pentaacetic acid (AB-DTPA) method formulated by Soltanpour and Workman (1979). Quantification of nitrogen and phosphorus were done by recording the absorbance at wavelengths of 54 nm and 880 nm respectively. Potassium analysis was conducted on flame photometer, while calcium, magnesium and micronutrients were computed by running the samples on atomic absorption spectrophotometry against respective standards.

Statistical Analysis

Data collected were analysed through analyzing the variance (ANOVA). Means were compared by least significant difference (LSD) at 5% level of significance by using statistical software, statistix, version 8.1, Tallahassee, F.L.

Results

Chemical Properties of Soil

Before planting, a detailed investigation was carried out to determine the fertility status of soil used for experimentation. For this purpose, both macro and micronutrients along with pH and EC was assessed in the soil samples. Chemical characteristics of the soil are provided in Table 1.

Morphological and Biochemical Characteristics of Isolates

Morphological characteristics of bacterial isolates used to augment the vermicompost are provided in Table 2. Data indicated that majority of the isolates were gram positive, coccus shaped with non-transparent colonies having flat elevation and entire margins. About 40% of the bacterial isolates were gram negative. Only one out of eleven isolates exhibited translucent opacity. Biochemical characteristics as depicted in Table 3, which revealed that all bacterial strains used in the current study produced growth hormone, IAA and solubilized phosphate and made it available for plant uptake. Results affirmed that highest IAA production potential was observed in the isolate, MV-9 which produced 48.21 μL mL^{-1} of this plant hormone followed by that of produced by MV-17 (36.88 μL mL^{-1}). Minimum concentration of IAA was observed in the culture solution of microbial isolate MV-19. All of the bacterial isolates were reported to be phosphate solubilizers but with varying potential, as realized from the quantity of phosphate solubilized by respective strains (Table 3). Maximum amount of phosphate solubilized was observed in MV-21 followed by MV-17 (158.1 and 120.7 μg mL^{-1} respectively). Microbial isolate, MV-8 was least efficient to solubilize phosphate. Surprisingly all the bacterial isolates were catalase positive but lacked the ability to produce pectinase enzyme. However, only one bacterial isolate (MV-20) was unable to produce ammonia (NH_3). No trend was observed regarding amylase and protease production ability of bacterial isolates (Table 3).

Effect of PGPR Enriched Vermicompost on Plant Growth

Effect of vermicompost augmented with PGPR was observed on various growth parameters of study plant. Results shown in Table 4, revealed that germination percentage of plants were significantly higher (LSD=9.065) in PGPR enriched vermicompost as compared to those in plain vermicompost *i.e*., without PGPR addition. Sprouting was highest in the seeds sown in vermicompost inoculated with strains, MV-9 and MV-17 where about 95% of seeds were germinated in each of these two treatments.

Table 1: Chemical analysis of soil before sowing

Nutrients/Parameters analysed	Concentration (mg kg^{-1})
Phosphorus	04.62
Nitrate-Nitrogen	27.95
Potassium	60.00
Calcium	20.00
Magnesium	23.28
Copper	05.20
Iron	30.34
Manganese	18.23
Zinc	04.00
Electrical Conductivity (dS/m)	00.29
pH	8.31

Values are means of three replicates

Table 2: Morphological characteristics of isolated bacterial strains

Isolate	Margin	Elevation	Form	Opacity	Shape	Gram staining
MV-1	Lobate	Flat	Circular	Opaque	Cocci	+ve
MV-6	Lobate	Pulvinate	Irregular	Opaque	Cocci	-ve
MV-8	Entire	Flat	Circular	Opaque	Cocci	-ve
MV-9	Entire	Flat	Punctiform	Opaque	Bacillus	+ve
MV-13	Entire	Raised	Irregular	Opaque	Bacillus	-ve
MV-14	Erose	Flat	Spindle	Translucent	Bacillus	-ve
MV-15	Entire	Convex	Punctiform	Opaque	Cocci	+ve
MV-17	Undulate	Umbonate	Filamentous	Opaque	Bacillus	+ve
MV-19	Undulate	Raised	Circular	Opaque	Cocci	+ve
MV-20	Entire	Flat	Punctiform	Opaque	Cocci	-ve
MV-21	Undulate	Flat	Circular	Opaque	Cocci	+ve

Table 3: Biochemical characteristics of bacterial isolates

Isolate	IAA μg mL^{-1}	PSI	PSE (%)	pH	P availability μg mL^{-1}	Ammonia	Catalase	Amylase	Protease	Pectinase
MV-1	33.23	2.08	107.7	5.30	61.72	++	+	+	+	-
MV-6	23.77	2.08	108.3	6.7	41.62	+	+	+	+	-
MV-8	16.32	2.09	109.1	6.56	38.22	+	++	-	+	-
MV-9	48.21	2.39	128.6	5.15	78.04	++	+	+	+	-
MV-13	12.32	2.21	121.4	5.95	58.67	+	+	+	+	-
MV-14	28.81	2.16	125	7.38	58.49	++	+	+	+	-
MV-15	22.74	2.29	128.6	5.6	38.75	++	+	-	+	-
MV-17	36.88	2.18	150	5.1	120.7	+	+	+	+	-
MV-19	9.48	2.00	100	5.51	76.61	++	+	-	++	-
MV-20	32.92	2.06	116.7	6.45	33.73	-	+	-	-	-
MV-21	33.65	2.33	400	4.78	158.1	++	+	-	+	-

± signs indicated the presence (+) or absence (-) of respective enzyme production ability of microbial isolate

Amongst PGPR enriched vermicompost treatments, the lowest number of turnip seeds germinated in MV-15 treated vermicompost (55.56%), but even this lowest germination ratio was 43% higher than that observed in the control treatment (38.89%). Similarly, number of leaves, leaf length, width, fresh and dry weights of above and below ground biomass were significantly different when grown on PGPR amplified vermicompost as compared to the counterparts grown in plain vermicompost (Table 4). Notably, the length and diameters of leaves in PGPR supplemented vermicompost were significantly higher compared with those grown in uninoculated vermicompost. Surprisingly, no significant variability was detected between different treatments of enriched vermicompost. Contrary to this observation, fresh and dry weights of plant biomass were statistically different among all treatments. In numerical terms, the highest fresh (17.19 g) and dry weights (2.28 g) of leaves were observed in plants growing on vermicompost supplemented with MV-17 against the respective fresh and dry weight of 1.76 g and 0.21 g in control treatment. Similar observations were recorded in the plant roots (Table 4).

Effect of PGPR Supplemented Vermicompost on Nutrients in Plant Tissues

Macronutrients including: NO_3-N, P, K, Ca and Mg and micronutrients; Cu, Mn, Zn and Fe were analyzed in the leaves of experimental plants grown in vermicompost with and without PGPR. As expected, both macro and micronutrients were statistically higher ($P < 0.001$) in the leaves grown in PGPR supplemented vermicompost as compared to those grown in plain vermicompost *i.e.*, control (Table 5). However, great variations were observed among treatments. Highest NO_3-N (% age) was observed in plants growing on vermicompost treated with bacterial isolate

Table 4: Effect of enriched vermicompost on growth of turnip

Treatments	Germination %	Leaf attributes			Leaves Biomass (g)		Root Biomass (g)	
		Nos.	Length (cm)	Diameter (cm)	f. wt.	d. wt.	f. wt.	d. wt.
Control	38.89 h	3.00 f	8.933 c	1.31 b	1.76 j	0.21 g	4.26 k	0.49 d
MV-1	86.11 abc	7.33 b	18.49 ab	4.52 a	4.16 g	0.96 c	10.73 e	1.48 bc
MV-6	72.22 def	7.00 bc	17.50 b	4.60 a	3.39 hi	0.47 f	7.51 j	0.70 d
MV-8	88.89 ab	5.67 d	18.07 ab	4.71 a	4.76 e	0.77 e	8.27 i	1.18 c
MV-9	94.44 a	8.67 a	23.74 a	5.11 a	8.39 b	1.07 b	14.80 b	1.83 a
MV-13	80.56 bcd	6.00 d	18.03 ab	4.31 a	3.10 i	0.50 f	8.46 h	1.19 c
MV-14	63.89 fg	5.67 d	17.95 ab	4.75 a	3.56 h	0.54 f	9.07 g	1.43 bc
MV-15	55.56 g	5.00 e	17.32 b	4.60 a	4.37 fg	0.87 d	10.80 e	1.49 bc
MV-17	94.44 a	6.00 d	20.81 ab	5.66 a	17.19 a	2.28 a	15.97 a	1.95 a
MV-19	77.78 cde	7.00 bc	18.74 ab	4.24 a	4.67 ef	0.79 de	10.39 f	1.45 bc
MV-20	69.44 ef	6.66 c	19.26 ab	4.33 a	6.14 d	0.76 e	12.78 d	1.67 ab
MV-21	94.44 a	9.00 a	19.27ab	5.17 a	6.57 c	1.02 bc	13.88 c	1.82 a
LSD	9.065	0.628	6.068	1.586	0.3295	0.0829	0.1277	0.3356

Data are means of three replicates and values with different letters indicate significant difference at $P \leq 0.05$
f. wt= fresh weight; d. wt= dry weight

Table 5: Effect of PGPR augmented and plain vermicompost on the concentration of nutrients in Turnip leaves. Macronutrients were calculated as percentage (%), while micronutrients were expressed in weight (mg kg^{-1})

Treatments	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Control	0.02h	3.10 g	2.35 e	0.12 d	0.08 f	1.07 e	155.8 f	11.13 e	11.4 f
MV-1	1.62 cde	8. 5.6 b	2.46 de	0.32 c	0.43 bc	5.60 ab	219.8 cd	42.20 ab	35.2 cd
MV-6	1.23 fg	6.40 de	2.73 cde	0.35c	0.21 e	3.00 d	251.07 bc	19.33 cde	34.87 cd
MV-8	1.50 def	7.88 bc	3.05 bc	0.32 c	0.37 cd	4.53 bc	238.6 bcd	13.73 cd	17.47 e
MV-9	1.93 ab	9.70 a	3.22 abc	0.48 ab	0.49 ab	5.80a	322.27 a	47.00 a	42.13 a
MV-13	1.35 ef	7.33 cd	2.38 de	0.29 c	0.44 ab	4.93 abc	197.8 def	30.33 bc	38.60 abc
MV-14	1.03 g	7.29 cd	2.9 bcd	0.29 c	0.35 cd	3.73 cd	166.6 ef	25.20 cd	18.07 e
MV-15	1.43 ef	4.63 f	2.84 bcde	0.31 c	0.31 d	3.80 cd	217.07 cd	16.67 de	22.53 e
MV-17	1.99 ab	8.09 bc	3.33 ab	0.40 bc	0.49 ab	4.13 cd	367.2 a	45.00 a	40.33 ab
MV-19	1.85 abc	8.40 b	3.21 abc	0.36 bc	0.45 ab	4.00 cd	240.27bcd	20.20 cde	31.47 d
MV-20	1.77 bcd	6.11 e	2.78 cde	0.35 c	0.48 ab	3.73 cd	208.2 cde	18.13 de	30.93 d
MV-21	2.12 a	10.39 a	3.65 a	0.55 a	0.50 a	6.13 a	272.4 b	46.07 a	35.93 bcd
LSD	0.2941	1.0282	0.5284	0.1249	0.0741	1.2548	47.620	11.881	5.0956

Data presented are means of three replicates and values significantly different at $P \leq 0.05$ are indicated with different letters
Here N = Nitrogen; P = Phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; Cu = Copper; Mn = Manganese; Zn= Zinc; Fe = Iron

MV-21 while the lowest in control plants. Moreover, other four macronutrients are also found to be maximum in the same treatment of vermicompost and were significantly different from the plant leaves harvested from control treatment. Micronutrients in the plant leaves varied from one treatment to another. These trace elements: Cu, Fe, Mn and Zn were many folds higher in leaves as is evident from respective LSDs; 1.25, 47.62, 11.88 and 5.09. Similar to the observations in brassica plant growth parameters in previous section, a non-symmetric variability was recognized among the PGPR augmented vermicompost treatments.

Effect of PGPR Supplemented Vermicompost on Nutrients in Roots

Influence of PGPR containing vermicompost was determined on the macro and trace elements in the turnip roots. Roots harvested from plants exposed to plain vermicompost contained undoubtedly lower concentration of all tested elements (Table 6). The data explained that plants growing on MV-9 treated vermicompost resulted in highest accumulation (%) of NO_3-N, Ca and Mg in the roots which were statistically higher than those growing on unamended vermicompost. However, MV-17 proved to be better, resulting in higher accumulation of P and K in the roots. Micronutrients were also higher in roots of plants growing in PGPR treated vermicompost. Cu, Fe and Zn was found to be maximum in roots grown in the vermicompost treated with microbial isolate MV-21 while, Mn concentration was higher in roots growing on vermicompost amended with microbial isolate MV-17. Concentration variation between treatments was significant but a great variability among treatments was observed (Table 6).

Effect of PGPR Enriched Vermicompost on Soil Fertility

After harvesting the plant biomass, soil analyses were performed to assess the nutrient status of soil in response to PGPR application. The concentration of all tested nutrients was significantly higher in PGPR amended vermicompost treatments (Table 7). Data demonstrated that MV-17 treated vermicompost resulted in highest addition of NO_3-N into the soil which was about 63% higher than control treatment. This trend was followed by MV-9 and MV-21 for phosphorus, potassium and micronutrients content.

Table 6: Effect of PGPR enriched and plain vermicompost on the macronutrients (%) and micronutrients (mg kg^{-1}) in the turnip root (fruit; fleshy tape root)

Treatments	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Control	0.59 f	6.14 f	1.9 g	0.31 e	0.18 f	2.2 e	177 f	21.73 g	1.27 e
MV1	1.19 cd	11.91 bc	3.21 cde	0.65 b	0.26 d	9.4 b	210.1 de	38.87 ef	7.87 cd
MV6	0.87 e	11.97 bc	2.85 def	0.53 c	0.23 de	6.3 cd	209.9 de	44.07 de	4.87 de
MV8	1.24 cd	11.59 c	2.96 def	0.46 cd	0.25 d	6 cd	209.1 de	43.73 de	4.80 de
MV9	2.08 a	13.21 ab	3.46 abc	0.75 a	0.43 a	11.5 a	246 a	50.27 cd	35.87 a
MV13	0.88 e	9.68 de	2.62 f	0.46 cd	0.25 d	5 d	207.5 de	30.80 f	4.67 de
MV14	1.20 cd	8.95 e	2.59 f	0.42 d	0.21 ef	6.6 c	211.8 de	30.67f	6.93 d
MV15	1.15 cd	10.70 cd	2.83 ef	0.45 d	0.27 d	7.1 c	216.8 cd	31.07 f	29.93 b
MV17	1.27 cd	14.18 a	3.85 a	0.64 b	0.35 b	9 b	223.7 bc	64.13 a	31.00 b
MV19	1.54 b	11.68 bc	3.67 ab	0.63 b	0.25 d	10.3 ab	205.4 e	51.93 bcd	11.53 c
MV20	1.09 d	10.90 cd	2.94 def	0.45 d	0.30 c	6.3 c	209.6 de	52.60 bc	32.80 ab
MV21	1.32 c	13.21 ab	3.26 bcd	0.74 a	0.35 b	11 a	232.8 b	58.93 ab	31.53 b
LSD	0.2032	0.8901	0.4154	0.0698	0.4154	1.5888	10.392	8.4120	4.1358

Values are means of three replicates and those differing significantly at $P \leq 0.05$ are indicated with different letters
Here N = Nitrogen; P = Phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; Cu = Copper; Mn = Manganese; Zn= Zinc; Fe = Iron

Table 7: Effect of PGPR enriched and plain vermicompost on soil nutrient concentrations (mg kg^{-1})

Treatments	pH	EC (dS m^{-1})	NO_3-N	P	K	Ca	Mg	Cu	Mn	Zn	Fe
Control	8.58	0.25	33.44 f	5.80 f	70 e	28.71 h	25.24 j	5.59 c	16.99 e	4.63 f	31.40 j
MV1	8.52	0.24	36.65 f	11.37 b	94.6 cd	29.46 g	26.54 i	6.00 abc	26.47 d	5.06 def	39.67 f
MV6	8.50	0.26	43.65 cde	9.56 c	88 d	32.37 d	26.3 i	5.88 bc	26.75 d	6.02 bc	41.38 e
MV8	8.45	0.22	35.05 f	6.66 e	74 e	31.36 e	29.39 h	5.60 c	19.47 e	4.69 ef	36.25 h
MV9	8.49	0.22	46.49 bc	12.69 a	121 a	37.42 a	36.55 a	6.74 a	36.26 b	6.45 ab	48.38 b
MV13	8.47	0.31	44.31 cd	9.43 c	86 d	30.31 f	31.98 d	5.78 bc	24.98 d	5.47 cde	37.56 g
MV14	8.43	0.29	36.84 ef	8.11 d	98.67 c	35.36 c	30.71 fg	5.61 c	31.75 c	4.91 ef	36.3 h
MV15	8.45	0.23	39.87 cdef	8.06 d	85.33 d	29.69 g	31.38 e	6.16 abc	32.55 bc	6.01 bc	33.50 i
MV17	8.43	0.25	54.53 a	12.63 a	108.67 b	36.35 b	33.70 c	6.45 ab	43.37 a	7.15 a	49.50 a
MV19	8.45	0.24	44.41 cd	9.76 c	98 c	36.31 b	30.88 f	6.06 abc	17.45 e	5.81 bcd	36.52 h
MV20	8.42	0.31	39.11 def	8.15 d	94.67 cd	36.21 b	30.36 g	5.87 bc	18.58 e	6.02 bc	42.68 d
MV21	8.46	0.28	52.45 ab	9.77 c	116.67 ab	36.60 b	35.39 b	6.08 abc	40.38 a	6.08 bc	46.31 c
LSD			6.8931	0.7370	9.3489	0.4238	0.4311	0.7856	3.8688	0.8095	0.4374

Data are means of three replicates and values significantly different at $P \leq 0.05$ are indicated with different letters
Here EC = Electrical conductivity; N = Nitrogen; P = Phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; Cu = Copper; Mn = Manganese; Zn= Zinc; Fe = Iron

However, the concentration of nitrate nitrogen in the compost treated with isolates; MV-1, MV-8 was statistically similar with that observed in control. The amount of P, Mn, Zn and Fe retained in soil was also maximally contributed by MV-17 treated vermicompost whereas, the manganese concentration contributed to the soil was statistically similar in MV-8, MV-19, MV-20 which was at par with control. Elements including: K, Ca, Mg and Cu were maximally contributed to the soil by the vermicompost augmented with microbial isolate MV-9. Significant variability between and among treatments was observed regarding soil nutrients. Soil pH and EC were non-significantly affected by treatments. However, these values were higher than pre-sowing conditions (Table 1 and 7).

Discussion

A clear difference was observed in soil chemistry in response to inoculation with PGPR enriched vermicompost compared with pre-sowing characteristics, where post treatment pH of soil was higher about pre-sowing. This increased pH may be attributed to increased amount of nutrients in vermicompost and also because of excessive liberation of nutrients by PGPR (Cox *et al*., 2001). PGPR are well researched to liberate nutrients which otherwise would not be available. These bacteria enhance plant growth and assist the natural acquisition of sparingly available nutrients (Richardson and Simpson, 2011). EC of the vermicompost amended soil was lower, although not significantly different from that of unamended soil. Possible mechanisms behind these changes depend on factors including type of nutrients involved, climatic conditions, and competency of microbial species (Ortiz-Castro *et al*., 2009; Dutta and Podile, 2010). In current study, the microbial isolates exhibited the characteristics of phosphate solubilization, antipathogenecity, production of enzymes and growth hormone (Table 3) which are well recognized traits of PGPR, hence in view of these observations, it can be ascertained that increase in pH of PGPR amended vermicompost was because of PGPR inoculation which increased the nutrients availability thereafter increasing soil pH. Post treatment rise in pH in PGPR augmented vermicompost might also be because of increased root growth (Weber *et al*., 2018), which is reflected by an increased fresh and dry weights (g) of root in this investigation (Table 7). Vermicompost enrichment had a significant effect on the plants (cowpea, cassava, and banana) and soil nutrients including: N, P, K, Mg and Mn

(Padmavathiamma *et al*., 2008). These researchers further noted that P-contents in soil and plants were significantly higher when vermicompost was inoculated with P-solubilizing organisms compared with uninoculated vermicompost. This observation highlights the need of using nutrient specific microbial strains to cope with the deficiency of any specific nutrient in the soil.

Significantly higher rate of seed germination was noticed when seeds were sown in vermicompost inoculated with PGPR compared with uninoculated ones. Several metabolites are released by PGPR during germination and this increased germination may be attributed to the increased secretion of metabolites. Vermicompost augmented with additional PGPR improved the germination rate of lettuce seeds, perhaps because of unique metabolic characteristics to produce plant growth regulators (Mangmang *et al*., 2014; Khosravi *et al*., 2018). This increased germination was the starting point for robust growth which was evidenced from increased fresh and dry biomass of plants. Phytohormone production increased seed germination, root elongation and stimulation of leaf expansion in cowpeas (*Vigna unguiculata* (L.) Walp as reported by Sivasankari *et al*. (2014). All bacterial isolates produced IAA, but some strains were more efficient than others regarding hormone production ability (Table 3). This is not surprising, as bacterial strains vary in their capacity for producing plant hormones (Asad *et al*., 2004). *Pseudomonas*, *Azospirillum*, *Azotobacter* and *Bacillus* spp. all exhibited varying abilities to produce IAA (Agrawal and Agrawal, 2013; Kundan *et al*., 2015). The auxins produced by these bacteria accordingly have varying effects on plant growth and fitness related traits. PGPR strains producing low levels of IAA exhibit significantly greater positive effect on plant biomass and other agronomic and physiological traits and seed nutrients (Sivasankari *et al*., 2014; Pérez-Fernández and Alexander, 2017). Our results negate the findings of these researchers, because in our study two isolates; MV-13 and MV-19 produced lowest concentration of IAA in whole slot of microbes, while their effects on plant growth parameters including: biomass, soil and plant nutrients were comparable with those of high IAA producing isolates. This disagreement might be due to synergistic effects of vermicompost, which could have increased the ability of bacteria many folds to influence growth parameters. This puts credence on our argument of synergistic effect between compost and PGPR, because in current study vermicompost was not sterilized, so possibly augmenting the PGPR capacity with those already present in vermicompost.

All PGPR isolates used to augment the vermicompost were phosphate solubilizers (Table 3) and their influence on phosphate solubilization is reflected in the high Phosphorous (P) concentration in turnip leaves (Table 5), whereby phosphorous contents in the leaves and roots of treated plants was significantly higher (LSD= 1.028 (leaves), 0.890 (roots)) than control treatment. The exceeded amount of P in response to vermicompost+ PGPR treatment may be attributed to the activity of these microorganisms, as phosphate solubilizers excessively solubilize the non-available phosphate and increase uptake by plants (Belimov *et al*., 1995; Kudoyarova *et al*., 2017). These microbes effectively solubilize phosphates by excreting phosphatases and organic acids (Richardson *et al*., 2009). Other nutrients also increased in the leaves and roots of treated plants as compared to control. This reflects the overall influence of rhizosphere microbes to liberate the nutrients from soil and rendering them available for plants. Moreover, vermicompost is a rich source of nutrients, available for plant uptake resulting in accelerated and robust plant growth. Vermicompost effects on growth are increasing biomass many folds when augmented with PGPR, as these microbes release these nutrients for plant uptake which would otherwise be non-available. Significant increases in fresh and dry biomass of lettuce (*Lactuca sativa*) were observed when treated with PGPR augmented vermicompost as compared to uninoculated vermicompost (Kohler *et al*., 2007; Bustamante *et al*., 2008). Nutrient availability status of soil was greatly enhanced by vermicompost application as an organic source. This evident from the post-harvest analysis of soil compared with pre-harvest and control treatments. Macronutrients and micronutrients after harvest enhanced the availability status of nutrients which could also be helpful for next crop. Results agree with those of previous researches (Padmavathiamma *et al*., 2008; Ansari and Sukhraj, 2010). This great influence of PGPR augmented vermicompost on plant health, nutrients and soil fertility may help to reduce the use of chemical fertilizers or even replace the chemical fertilizers. Moreover, this technology can also be used in other crops if crop specific PGPR are added to the vermicompost.

Conclusion

Quality of the vermicompost was improved by inoculating with additional inoculum of PGPR, which elevated the nutritional value of *Brassica rapa* and boosted soil fertility. Significant enhancement in crop yield (20–30%) after inoculation with PGPR augmented vermicompost suggested that PGPR supplemented vermi-technology could be a starting point for transition from chemical to bio-nutrition without compromising the yield and ecological processes. The use of PGPR enriched vermicompost is recommended for other brassica crops for improved and sustainable productivity.

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