BIOFORTIFIED VERMICOMPOST ENRICHED WITH ENDOPHYTIC BACTERIA ENHANCES GROWTH, NUTRIENT ACQUISITION, AND SOIL HEALTH IN OKRA (*ABELMOSCHUS ESCULENTUS* L

Akila Vaishnavi P[1], Thenmozhi M[2*]

[1]Research Scholar, Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu, India, akilaprabagar@gmail.com Orcid ID : 0000-0002-6675-6674
[2]*Associate Professor, Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu, India. , drmthenmozhi@gmail.com, ORCID ID: 0000000279576648

ABSTRACT
Biofortification of organic amendments with beneficial microbes provides a potential solution for increasing both crop productivity and soil sustainability. The current investigation assessed the effectiveness of organic amendments-namely vermicompost (VC) and endophyte biofortified VC-in terms of growth parameters, nutrient accumulation, and soil health of okra (*Abelmoschus esculentus* L.). To achieve biofortified VC, prepared from goat manure and *Celosia argentea* biomass, with some selected plant growth promoting (PGP) endophytic bacterial strains; it was incorporated into pot culture. Treated plants (biofortified VC and conventional VC) significantly enhanced the plant growth, biomass yield and yield attributes as compared to control. Increased accumulation of important macro- and micronutrients in treated plants revealed better nutrient availability and assimilation. Analysis of soil samples showed an increased level of soil organic carbon, nutrient status, and microbial population in the treatments where biofortified VC was applied. Successful establishment and sustenance of applied endophytic microbes within rhizosphere was further confirmed by metagenomic analysis. Conclusively, biofortified VC acted as a stable platform of PGP endophytes, which provided excellent benefits to okra. This could be proposed as a nutrient amendment for sustainable and green agriculture.

KEYWORDS: Biofortified vermicompost; Endophytic bacteria; Okra; Nutrient uptake; Rhizosphere microbiome; Soil health; Sustainable nutrient management.

1. INTRODUCTION

Growing demand for food production with finite natural resources, variable climate and degradation of natural resources have further increased the importance for sustainable nutrient management in agriculture.[24] Chemical fertilizer use has greatly contributed to maintain crop productivity in intensive farming system especially in developing countries, and it has caused degradation of soil organic carbon, nutrient imbalance, reduced microbial diversity and pollution of soil and water by imbalanced and long-term application.[15, 12] Vegetable growing areas of India especially intensive cultivation areas are severely affected due to loss of soil fertility by decreasing nutrient use efficiency, stagnated yield and increasing input costs. Hence soil health restoration through integrated and bio-based nutrient management practice has become a crucial goal for sustainable agriculture.[17, 38]

Among the various organic amendments vermicompost gained significance as a beneficial and biologically active soil conditioner which amends the physical, chemical and biological properties of soil. It is an ecofriendly and a stabilized product formed by the bio-oxidative decomposition of organic matter by earthworm and associated microflora and it is rich in plant available nutrients, humic compounds, enzyme and diverse beneficial microflora.[19, 8] Previous reports have shown that vermicompost application enhances the soil aggregation, pore-space, water retention capacity, cation exchange capacity and nutrient mineralization thus improving nutrient and root growth, efficiency and crop yield compared to common compost. Vermicompost contains higher quantity of plant available nutrients and beneficial microbial activity compared to common compost and it is an ideal organic component for nutrient management strategies.[13, 14]

Latest advancements in the field of sustainable agriculture showed that functional efficiency of vermicompost could be improved by microbial bio-fortification, by introducing plant growth promoting endophytic bacteria to organic manure; thereby enhancing organic nutrient supply with biological functions viz; biological nitrogen fixation, phosphate solubilization, production of phytohormones, production of siderophore mediated micronutrient availability and suppress plant diseases.[26, 7] So, biofortified vermicompost serves as both nutrient provider and efficient carrier of beneficial bacteria that ensure its survival, colonization and sustenance in the soil-plant ecosystem. Okra (*Abelmoschus esculentus* L. Moench) is a predominant vegetable crop of tropics and is responsive to organic and bio-inputs thus being an ideal model for assessment of such integrated approaches for nutrient management. The present study was undertaken to evaluate the effects of vermicompost and biofortified vermicompost on growth and nutrient uptake of okra and soil properties, coupled with the validation of microbial colonization and activity by molecular and metagenomic approach.[2, 36, 32]

2. MATERIALS AND METHODS

2.1 Preparation of Vermicompost

Standard methods were followed for the vermicompost preparation. Locally available goat dung and Celosia argentea weed biomass were used at a 1:1 (w/w) ratio under aerobic conditions for preparation of vermicompost. The material was chopped and mixed well, pre-decomposed for 15 days to minimize its phytotoxicity and promote microbial activity. Pre-decomposed material was loaded onto vermicomposting beds and inoculations with the epigeic earthworm, *Eisenia fetida*, were made uniformly at each bed.[27] Water was sprinkled as needed to maintain moisture level at around 60-70% throughout the composting period and bed was shielded against rain and sunlight. The process was carried out for 60 days until the vermicompost was dark in colour, granulated in nature and was odourless, signifying maturity.[40] The vermicompost obtained was collected, air-dried under shade, sieved using a 2-mm mesh, stored in clean containers and used for further studies

2.2 Biofortification of Vermicompost

To bio-Fortified, 10-day old Vermicompost was inoculated with individual selected, powerful endophytic bacterial strains viz *Azospirillum lipoferum* (AER2), *Bacillus thuringiensis* (AER6), *Bacillus cereus* (AEL6), *Bacillus subtilis* (AES1) and *Pseudomonas fluorescens* (AES3) having superior plant growth promoting and biocontrol characters. [21, 5] These strains were individually grown in nutrient broth under shaking conditions to exponential phase, cell density was standardized (approx 10 CFU ml) and were mixed together equally to make a multi-strain bacterial consortium to avoid any antagonistic interaction and provide complementarily in nitrogen fixation, phosphate solubilization, phytohormones production and plant pathogen inhibition traits and it was inoculated into the matured vermicompost at uniform rate and incubated for 10 days under shady condition with aeration in between at moisture level of 60-70% to provide sufficient time for microbes colonization into vermicompost. To facilitate proper uniform colonization into vermicompost the vermicompost was mixed up randomly for 10 days. The consortia biofortified vermicompost was used for pot culture experiments and soil biological analyses.[11]

2.3 Pot Experiment

A pot culture experiment was conducted with polythene pot bags in controlled conditions to assess the independent and interaction effects of vermicompost and inoculation of endophytic bacteria on okra (*Abelmoschus esculentus* L.). The experiment was conducted in a completely randomized design (CRD) with three replications. The treatments are T1- Soil (Control), T2-Vermicompost, T3-Vermicompost + *Azospirillum lipoferum* (AER2), T4-Vermicompost + *Bacillus thuringiensis* (AER6), T5-Vermicompost + *Bacillus cereus* (AEL6), T6-Vermicompost + *Pseudomonas fluorescens* (AES3), and T7-Vermicompost + consortium bacteria comprising *Azospirillum lipoferum* (AER2), *Bacillus thuringiensis* (AER6), *Bacillus cereus* (AEL6), *Bacillus subtilis* (AES1), and *Pseudomonas fluorescens* (AES3). All the

amendments were applied as per recommended doses. The okra seeds were sown with uniform population in all the polythene bags, and standard agronomic practices were followed throughout the experimental period. All the growth parameter like plant height, number of leaves, biomass yield and yield related characters were measured at scheduled intervals and at the harvest.

2.4 Soil and Plant Analysis

2.4.1 Soil and Plant Nutrient Analysis

The soils were sampled from each treatment after harvesting the crop, and air-dried at room temperature, grounded carefully and passed through a 2 mm mesh size before its analysis. Soil Physicochemical characters and availability of nutrients status was determined according to the standard analytical procedures. Soil pH and electrical conductivity were measured using soil-water suspension (1:2.5 w/v) and total organic carbon was estimated by Walkley Black wet oxidation method. Available N determined by alkaline permanganate method, available P by Olsen's method,[22] and available K by flame photometer following neutral ammonium acetate extraction method. Plant parts were harvested and dried in oven at 65 C till constant weight then finely grounded and digested through acid digestion. Macronutrients (N, P, and K) and micronutrients (Fe, Zn, Cu and Mn) were determined from the digests using standard analytical methods.[28, 30, 1]

2.4.2 Soil Metagenomic Analysis

To verify the introduction and maintenance of inoculated endophytic bacteria in treatments selected, soil samples from the selected treatments were harvested, and its molecular identification.[3] Soil total DNA was extracted using a soil DNA isolation kit commercial supplied and following instructions to get isolated DNA which was examined for quality and quantity before running further steps. In order to estimate microbial community structure and to detect functional groups of microbes responsible of nutrient cycling and plant growth promoting action, metagenomic analysis were carried out.[37] Sequence analysis was performed using bioinformatic common tool and further phylogenetic interpretation were conducted with the software MEGA [Molecular Evolutionary Genetics Analysis].[39, 34]

2.5 Statistical Analysis

The data from the experiment were statistically analysed using an ANOVA for a Completely Randomized Design (CRD) for comparing treatment differences. Treatment means that were significantly different were tested by Duncan's Multiple Range Test (DMRT) at p 0.05.

3. RESULTS

3.1 Physicochemical Properties of Vermicompost and Biofortified Vermicompost

The vermicompost made from the goat dung and *C. Argentea* reached mature in 60 days that shown by its dark, granulated and non-smell. That showed effectively stabilized organic matter. Biofortification by endophytic bacterial inoculums, the vermicompost is significantly improve by their physicochemical quality (Table 1) when compared to vermicompost solely (T2). Organic carbon increased from 1.27% of vermicompost to 1.56% of vermicompost amended by consortium inoculums (T7). Meanwhile, available nitrogen was increased from 106.0 kg ha (T2) to 138.8 kg ha (T7), the available phosphorus from 16.8 to 26.9 kg ha and available potassium from 137.3 to 189.6 kg ha. Individually inoculums showed *Azospirillum lipoferum* (T3) resulted in higher nutrient enhancement compared to other single inoculums. Besides, the C:N ratio in the biofortified vermicompost treatments reduced significantly and it showed 0.006 for T7 which means compost has advanced maturity and higher efficiency in nutrient mineralization.

3.2 Effect of Vermicompost and Biofortified Vermicompost on Okra Growth Parameters

Vermicompost and endophytic bacterial inoculation affected growth characteristics in Okra ($p<0.05$; Table 2). Shoot length (47.9±1.4 cm), root length (23.2±0.9 cm), number of leaves (13.1±0.6) and biomass production (13.54±0.38g plant -1) were maximal under treatment with the consortium (T7). Comparing to the control (T1) the treatment T7 enhanced shoot length by about 67% and biomass production by about 111%. Vermicompost significantly affected growth over control (T2) and all biofortified treatments T3-T6 further enhance growth significantly over vermicompost treatment. Among the single strain treatments *Azospirillum lipoferum* (T3) shows better result than other *Bacillus spp* and *Pseudomonas fluorescens* but their values were less than consortium T7.

3.3 Biomass Accumulation and Yield-Related Attributes

The overall biomass production shows a distinct treatment related trend (Table 2). The maximum biomass production was under the treatments T7 (13.54 0.38 g plant) followed by T3 (12.48 0.35 g plant) and T4 (11.92 0.33 g plant). Biomass accumulation under vermicompost only (T2) and control was 8.94 0.28 g plant and 6.42 0.21 g plant respectively. The higher biomass production under consortium treatments indicates increased effects of consortium on nutrient supply, root elongation and assimilate partitioning compared to individual treatments.

3.4 Nutrient Uptake by Okra Plants

Both macro and micro-nutrients uptake were highly influenced by the treatments (Table 3 and Table 4). Nitrogen uptake was raised from 42.6 1.8 mg plant (T1) up to 92.8 3.1 mg plant (T7) while phosphorus uptake was raised from 6.8 0.3 up to 21.6 0.8 mg plant. Potassium uptake was nearly triple between T1 (38.4 1.6 mg plant) and T7 (112.7 3.8 mg plant).

Micro-nutrients uptake was also boosted, iron and zinc uptake were highest for T7 reaching 1184 36 g plant and 346 15 g plant, respectively. For the individual inoculations, uptake of micro-nutrients was varied between the bacterial strains and *Azospirillum lipoferum* consistently presented higher values than the rest of the bacterial inoculations.

3.5 Metagenomic Confirmation of Establishment and Functional Potential of Inoculated Endophytes

Soil metagenomics demonstrated successful establishment and maintenance of inoculated endophytic bacteria in biofortified vermicompost-amended soils. Treated soil with the consortium (T7) revealed greatest relative abundance and highest enrichment of inoculated taxa along with significant enrichments in functional genes related to nitrogen fixation (nifH), phosphorus solubilization (phoA, phoD), siderophore biosynthesis, carbon and nitrogen and phosphorus cycling pathways. Moreover, both inoculants had a stable colonization in single inoculant soils, *Azospirillum lipoferum* (T3) was highly enriched compared to other inoculants. These results indicated that biofortified vermicompost can be used as an efficient delivery vehicle and support persistence and functional activity of microbes to contribute the soil quality and plants performance.

Table 1. Physicochemical properties of vermicompost and biofortified vermicompost

Treatment	Organic carbon (%)	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})	C:N ratio
T1 – Soil (Control)	0.78	82.4	12.6	104.8	0.021
T2 – Vermicompost	1.27	106.0	16.8	137.3	0.012
T3 – VC + *Azospirillum lipoferum*	1.52	132.4	25.8	176.8	0.007
T4 – VC + *Bacillus thuringiensis*	1.46	128.2	24.3	170.6	0.008
T5 – VC + *Bacillus cereus*	1.42	124.6	22.4	162.5	0.008
T6 – VC + *Pseudomonas fluorescens*	1.39	121.3	21.6	158.4	0.009
T7 – VC + Consortium	1.56	138.8	26.9	189.6	0.006

Figure 1A. Organic carbon (%) in vermicompost and biofortified vermicompost treatments

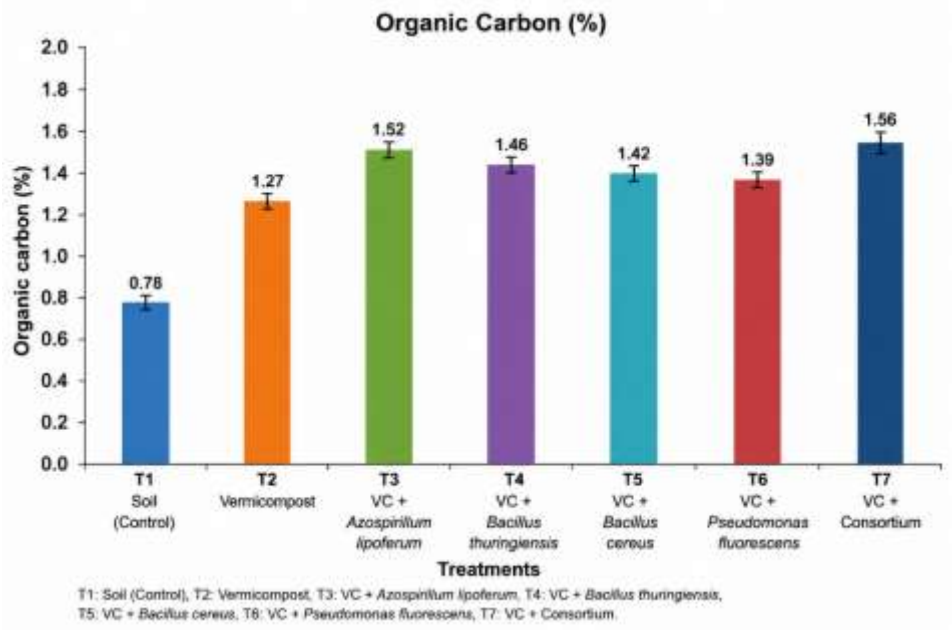


Figure 1B. Effect of Vermicompost and Biofortified Vermicompost on Available Nitrogen Content in Soil

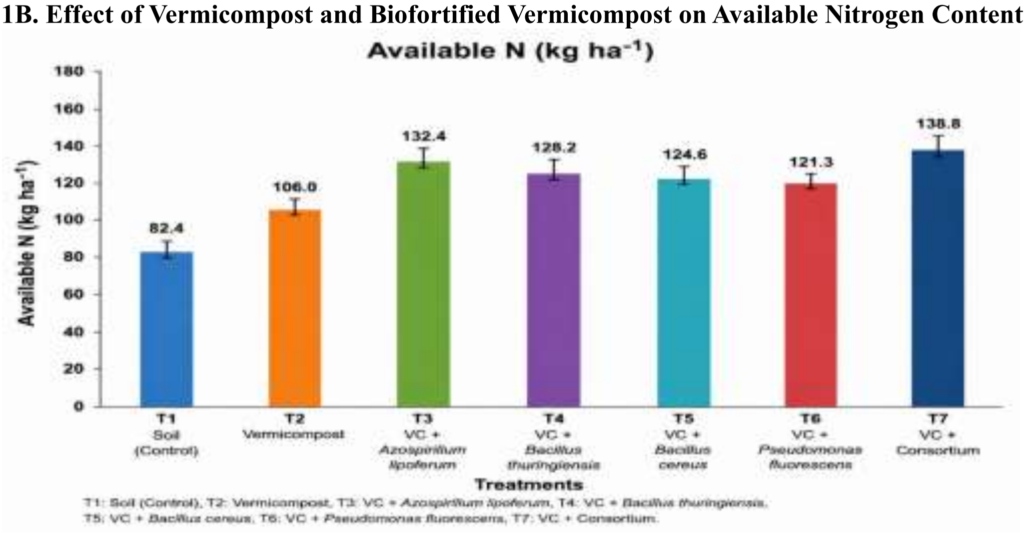


Figure 1C. Effect of Vermicompost and Biofortified Vermicompost on Available Phosphorus Content (kg ha^{-1})

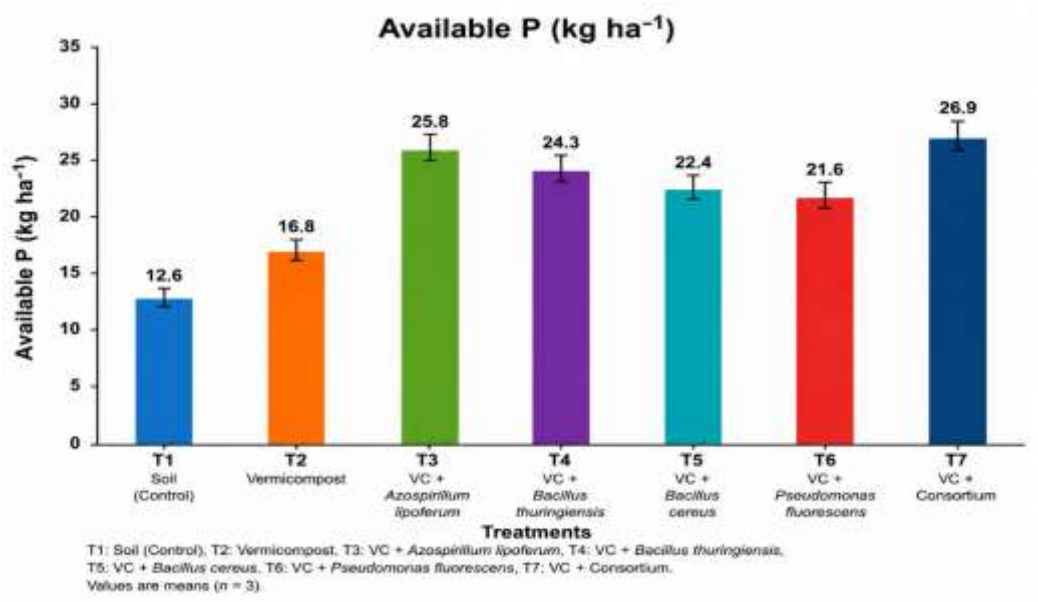


Figure 1D: Effect of Vermicompost and Biofortified Vermicompost on Available Potassium Content (kg ha⁻¹)

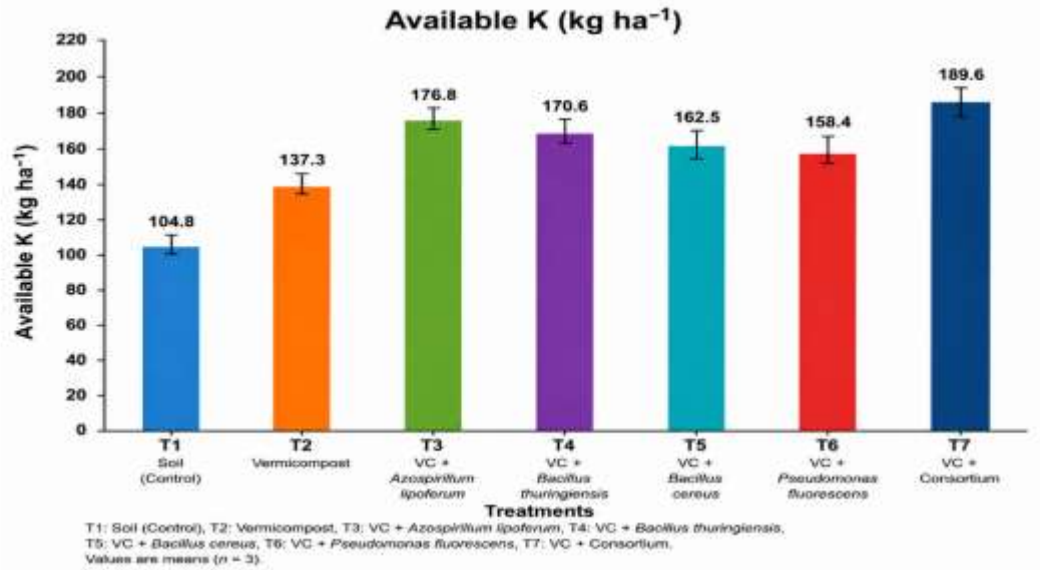


Figure 1E: Effect of Vermicompost and Biofortified Vermicompost on Soil C:N Ratio

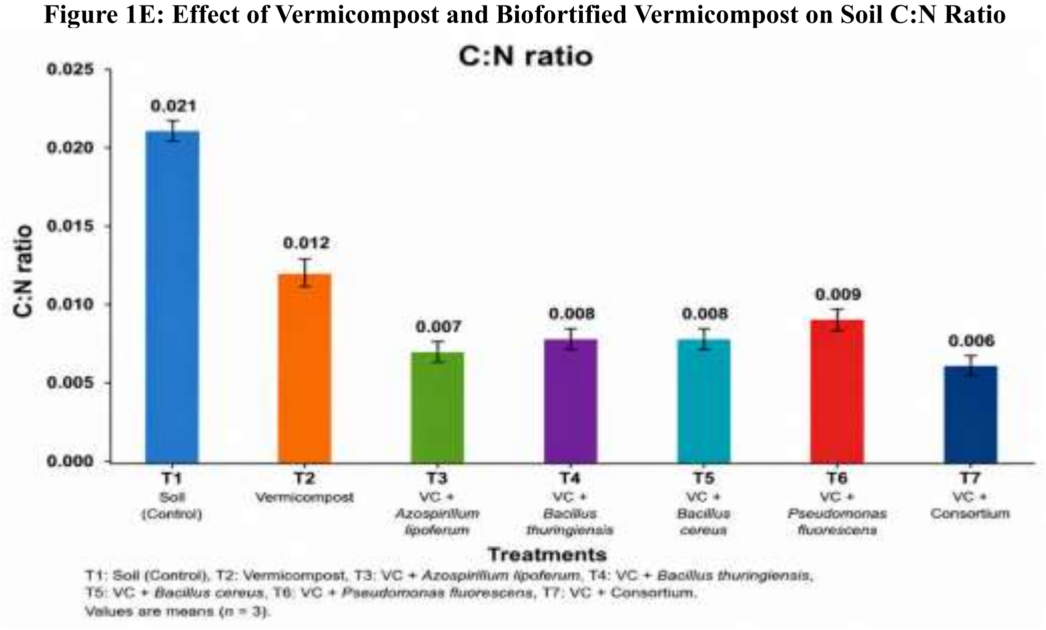


Table 2. Effect of vermicompost and biofortified vermicompost on okra growth parameters

Treatment	Shoot length (cm)	Root length (cm)	Number of leaves	Total biomass (g plant^{-1})
T1 – Soil (Control)	28.6 ± 0.8	11.2 ± 0.5	7.1 ± 0.3	6.42 ± 0.21
T2 – Vermicompost	36.4 ± 1.1	15.8 ± 0.6	9.3 ± 0.4	8.94 ± 0.28
T3 – VC + *A. lipoferum*	45.7 ± 1.3	22.4 ± 0.8	12.3 ± 0.6	12.48 ± 0.35
T4 – VC + *B. thuringiensis*	43.9 ± 1.2	21.6 ± 0.7	11.8 ± 0.5	11.92 ± 0.33
T5 – VC + *B. cereus*	41.8 ± 1.1	20.4 ± 0.6	11.2 ± 0.4	11.36 ± 0.31
T6 – VC + *P. fluorescens*	40.9 ± 1.0	19.8 ± 0.6	10.9 ± 0.4	10.94 ± 0.30
T7 – VC + Consortium	47.9 ± 1.4	23.2 ± 0.9	13.1 ± 0.6	13.54 ± 0.38

Figure 2A: Effect of Vermicompost and Biofortified Vermicompost on Shoot Length of Okra

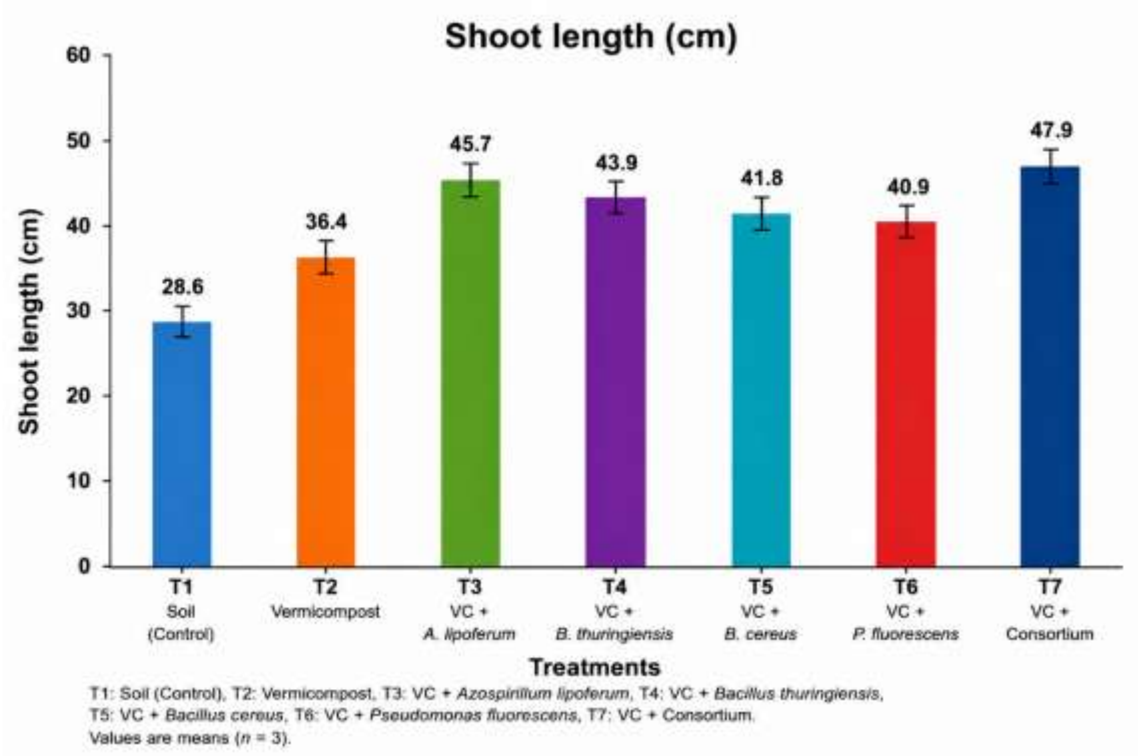


Figure 2B: Effect of Vermicompost and Biofortified Vermicompost on Root Length of Okra

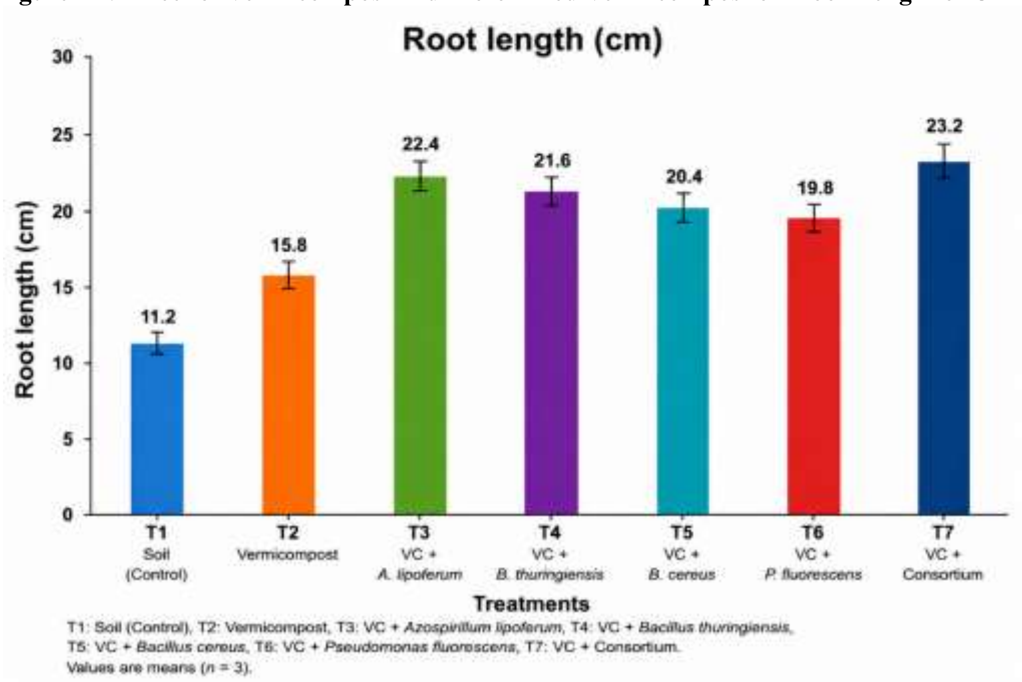


Figure 2C: Effect of Vermicompost and Biofortified Vermicompost on Number of Leaves per Plant in Okra

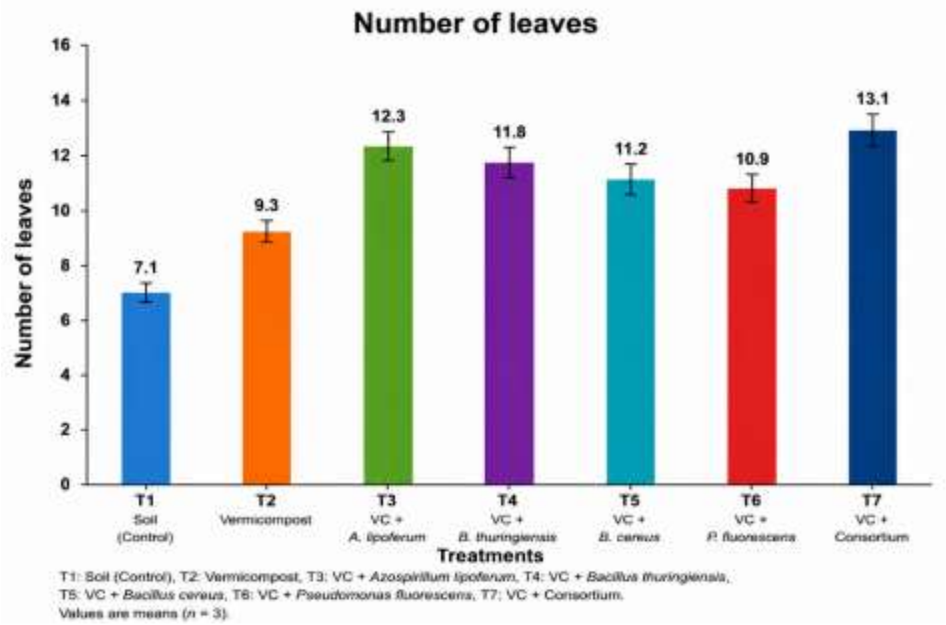


Figure 2D: Effect of Vermicompost and Biofortified Vermicompost on Total Biomass Production of Okra

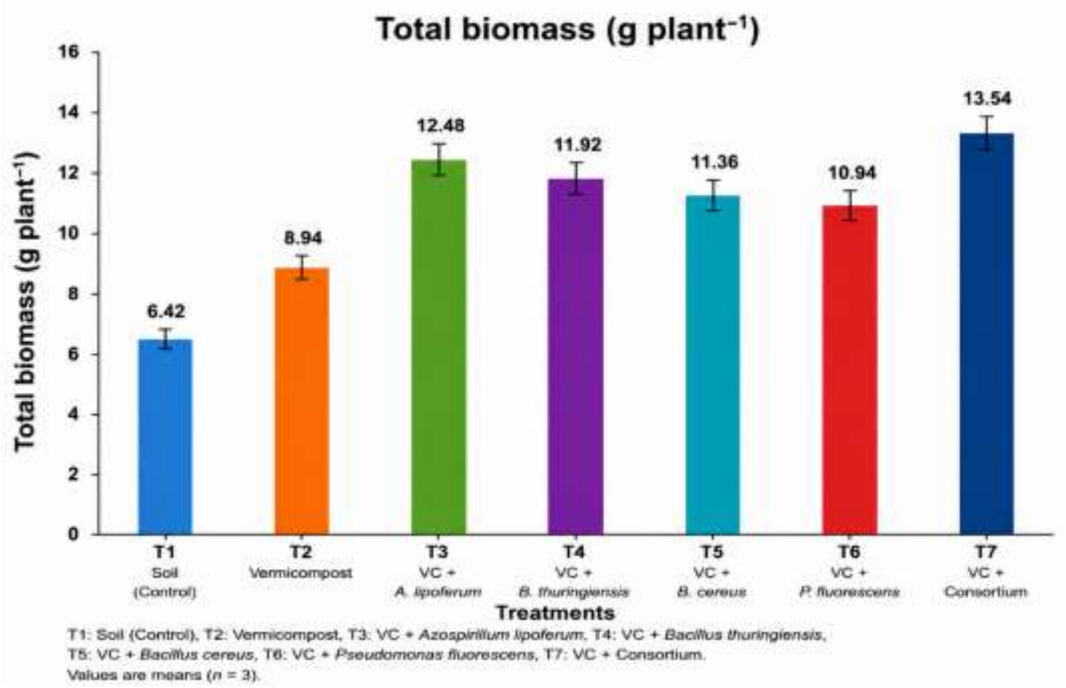
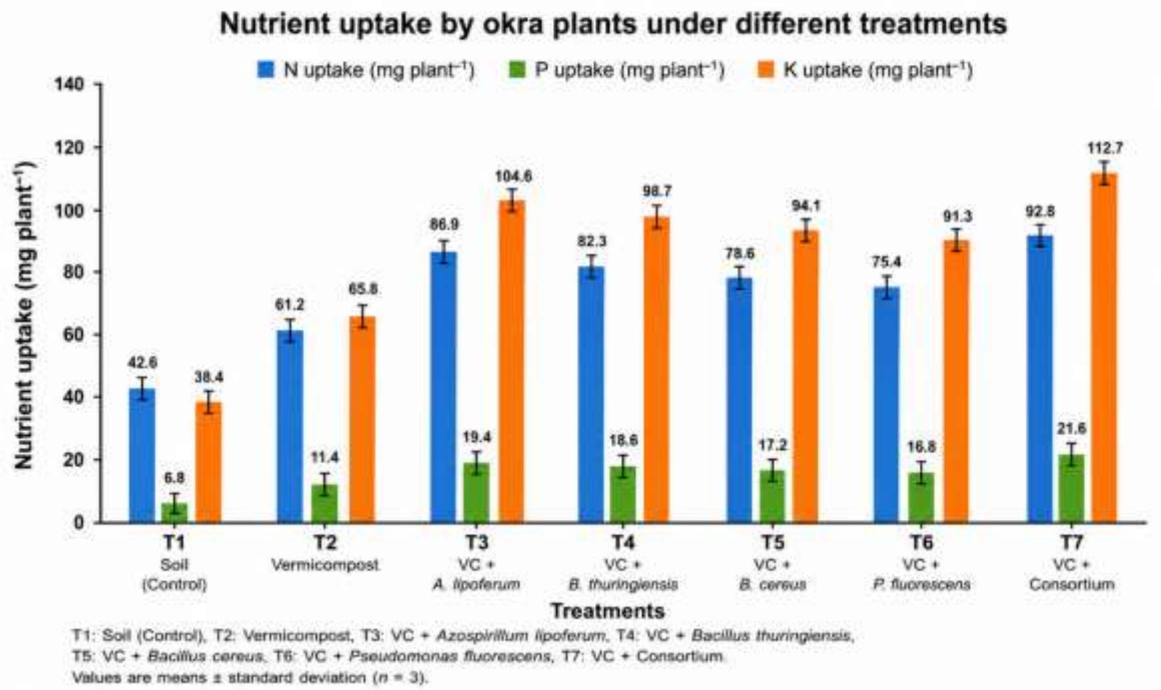


Table 3. Nutrient uptake by okra plants under different treatments

Treatment	N uptake (mg plant^{-1})	P uptake (mg plant^{-1})	K uptake (mg plant^{-1})
T1 – Soil (Control)	42.6 ± 1.8	6.8 ± 0.3	38.4 ± 1.6
T2 – Vermicompost	61.2 ± 2.3	11.4 ± 0.5	65.8 ± 2.4
T3 – VC + *A. lipoferum*	86.9 ± 2.9	19.4 ± 0.7	104.6 ± 3.5
T4 – VC + *B. thuringiensis*	82.3 ± 2.7	18.6 ± 0.6	98.7 ± 3.3
T5 – VC + *B. cereus*	78.6 ± 2.6	17.2 ± 0.6	94.1 ± 3.1
T6 – VC + *P. fluorescens*	75.4 ± 2.5	16.8 ± 0.5	91.3 ± 3.0
T7 – VC + Consortium	92.8 ± 3.1	21.6 ± 0.8	112.7 ± 3.8

Figure 3. Effect of Vermicompost and Biofortified Vermicompost on Nutrient Uptake by Okra Plants

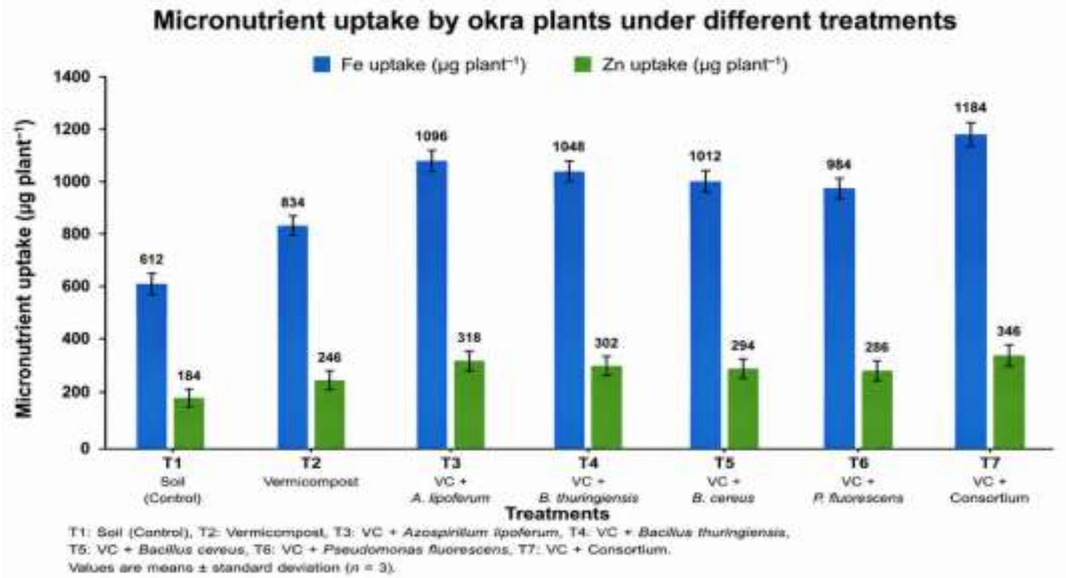


Note: Nitrogen (N), phosphorus (P), and potassium (K) uptake by okra (*Abelmoschus esculentus* L.) plants under different vermicompost and biofortified vermicompost treatments.

Table 4. Micronutrient uptake by okra plants

Treatment	**Fe (µg plant⁻¹)**	**Zn (µg plant⁻¹)**
T1 – Soil (Control)	612 ± 24	184 ± 8
T2 – Vermicompost	834 ± 31	246 ± 11
T3 – VC + *A. lipoferum*	1096 ± 35	318 ± 14
T4 – VC + *B. thuringiensis*	1048 ± 34	302 ± 13
T5 – VC + *B. cereus*	1012 ± 32	294 ± 12
T6 – VC + *P. fluorescens*	984 ± 31	286 ± 12
T7 – VC + Consortium	1184 ± 36	346 ± 15

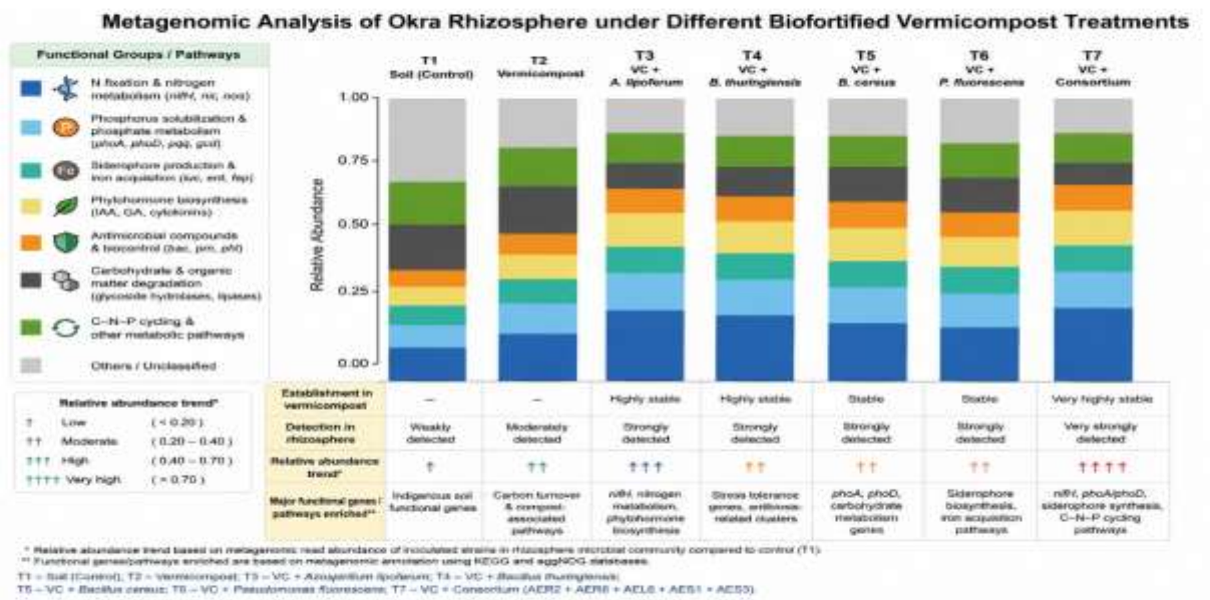
Figure 4. Effect of Vermicompost and Biofortified Vermicompost on Micronutrient Uptake by Okra Plants



Note: Values are expressed as mean ± standard deviation (n = 3). Error bars represent standard deviation.

Table 5. Metagenomic confirmation of establishment and functional potential of inoculated endophytic bacteria in biofortified vermicompost–amended soil

Treatment	Isolate code / Consortium	Source tissue	Identified species	Major functional traits (in vitro)	Establishment in vermicompost	Metagenomic detection in rhizosphere	Relative abundance trend*	Major functional genes/pathways enriched**
T3	AER2	Root	*Azospirillum lipoferum*	Biological N fixation, IAA & GA production	Highly stable	Strongly detected	↑↑↑	*nifH*, nitrogen metabolism, phytohormone biosynthesis
T4	AER6	Root	*Bacillus thuringiensis*	Antagonistic activity, enzyme production, GA synthesis	Highly stable	Strongly detected	↑↑	Stress tolerance genes, antibiosis-related clusters
T5	AEL6	Leaf	*Bacillus cereus*	High IAA & GA production, phosphate solubilization, hydrolytic enzymes	Stable	Strongly detected	↑↑	*phoA*, *phoD*, carbohydrate metabolism genes
T6	AES3	Stem	*Pseudomonas fluorescens*	Siderophore production, phosphate solubilization, biocontrol	Stable	Strongly detected	↑↑	Siderophore biosynthesis, iron acquisition pathways
T7	Consortium (AER2 + AER6 + AEL6 + AES1 + AES3)	Root + Stem + Leaf	Mixed consortium	N fixation, P solubilization, siderophore production, phytohormones, enzymes	Very highly stable	Very strongly detected	↑↑↑↑	*nifH*, *phoA/phoD*, siderophore synthesis, C–N–P cycling pathways
T1	—	—	Native soil microbiota	Baseline microbial activity	—	Weakly detected	↑	Indigenous soil functional genes
T2	—	—	Vermicompost-associated microbiota	Organic matter decomposition	—	Moderately detected	↑↑	Carbon turnover and compost-associated pathways



4. DISCUSSION

Clearly, biofortification of vermicompost with endophytic bacteria can significantly increase agronomic efficacy over vermicompost alone. Although vermicompost alone improved soil organic carbon, nutrient availability and plant growth attributes, application of the selected endophytic bacterial strains individually or in combination demonstrated much better performance.[10] The reasons for higher agronomic efficiency of biofortified vermicompost are due to cooperative mechanisms between the organic matter and microbes, including stimulation of nutrient mineralization, nitrogen fixation, phosphate solubilization and production of phytohormones.[23] Similarly, beneficial effects of biofortified organic matter applications, where microbial inoculation helped in enhancing the efficiency of nutrient use and biomass accumulation over alone applied organic manure, were observed in vegetables.[21, 33, 16] Enhanced response under consortium treatment is in line with functional complementarities of individual microbes that their pooled trait can enhance plant growth more than single inoculation.

Significant increase in uptake of nutrients (N, P, K, Fe, Zn) under biofortified treatments indicated the efficiency of endophytic bacteria in the mobilization of nutrients from the soil to the roots.[31, 18] Nitrogen fixation (nifH), phosphorus solubilization (phoA, phoD), synthesis of phytohormones (auxins, gibberellic acid) and chelating of micronutrients via production of siderophore by common endophytic bacteria (*Azospirillum, Bacillus* and *Pseudomonas*) have already been reported.[25, 29] In our case, the shoot and root growth, plant biomass, nutrient uptake shown by okra are in good correlation with these attributes. Increased soil organic carbon content and reduced C:N ratio in biofortified vermicompost also indicates improved microbial activity, organic matter mineralization and nutrient cycling that are very essential for soil fertility and sustainability in the long term.[20, 9]

Molecular and soil metagenomic analyses showed proper establishment, colonization and functional activity of the introduced endophytic bacteria in the rhizosphere and therefore, a link was confirmed between inoculation and agronomic performance.[4] Functional genes such as nifH, phoA, phoD and genes involved in siderophore synthesis and carbon-nutrient cycle have been significantly enriched under biofortified vermicompost and demonstrated functional persistence and enhancement in soil biological activities over the alone applied vermicompost and compared to control. Metagenomic data supporting functional persistence of inoculated microbes has been recently demonstrated for biofortified composts and plants-microbiome systems.[35, 6] The present results validated biofortified vermicompost as an effective carrier of endophytic bacteria and science-based alternative, eco-friendly approach over chemical fertilizer based nutrient management that can be well suited in tropical agro-ecosystems like Tamil Nadu.

5. SUMMARY

The present study showed that the integration of organic matter recycling with microbial biofortification of vermicompost synthesized from goat dung and *C. Argentea* and supplemented with selected PGP endophytes significantly improved the soil fertility, nutrients availability, and okra growth. Organic matter recycling coupled with microbial biofortification remarkably increased soil OC content, macro-and micronutrient availability, accumulation of plant biomass, and uptake of nutrients. The mixed inoculation of endophytic bacterial consortium significantly stimulated soil nutrients availability and growth of okra than individual inoculated ones through effective nutrient mobilization, and hormonal-mediated growth stimulation, and better root development. The established roots system with inoculated endophytes was supported by molecular and soil metagenomic analyses which confirmed the survival and functioning of inoculants in the rhizosphere. Thus, the results show the application of biofortified vermicompost through organic matter recycling combined with beneficial endophytes can be considered as an ecofriendly, biologically oriented and sustainable nutrient management approach to boost the vegetable growth.

6. CONCLUSION

In the current work it has been concluded that biofortified vermicompost is a sustainable, environmentally friendly and biological tool for nutrients management for cultivation of okra crop. Enriched vermicompost with specified strains of endophytes not only results in the improvement of soil physicochemical characters, increase nutrient availability and uptake but also in the enhanced plant growth and biomass production as compared with vermicompost only. Confirmation of introduction of specified endophytes in soil and their functional integrity at molecular level and metagenomic analysis further increases the reliability of results. Overall, it can be concluded that such kind of organic-biological management can lead to reduced application of synthetic fertilizers for vegetables.

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