

# Enhancement of Antioxidants and Nutritional Quality of Tomato Inoculated with Agriculturally Importance Microorganisms (AIMs) Fortified Vermicompost

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## ABSTRACT

The effect of biofortified vermicompost was studied on growth parameter and nutritional quality of tomato. Microorganisms used for fortification were *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis*. Vermicompost was prepared by using temple waste and cow dung and earthworm *Eisenia fetida* was used in vermicomposting. The experimental findings showed significant variation in growth parameter and amount of antioxidants in treatments. The growth parameter such as root length, shoot length and dry weight was recorded after treatments. Maximum growth of plants was found in Vermicompost + *T. harzianum* treatment. Biochemical constituents of leaves such as total soluble protein, phenol, ascorbic acid and carotenoids were also recorded enhanced in treatments. Lycopene content in fruit was also increased in biofortified vermicomposts. Nutritional and antioxidants were recorded highest in vermicompost + *P. fluorescens* treatment.

## Highlights

- Biofortified vermicompost would be environmentally safe and contribute to sustainable agriculture.
- Maximum antioxidant content were recorded in biofortified vermicompost.

**Keywords:** Biofortified vermicompost, antioxidant, *Trichoderma*

Tomato (*Solanum lycopersicum*) member of family solanaceae, is one of the most widely grown vegetable crop and stand second to the potato in terms of production globally. The worldwide production of tomatoes in year 2018 was estimated around 170.8 million tons. China, the leading producer of tomatoes, accounted for 31% of the total production. India and United States followed with the second and third highest production of tomato in the world. In India, tomato with overall production of 19541 (000' tons) stands third in production share of total vegetable production after potato and onion (National Horticulture Database 2018). Tomato is a treasure of riches when it comes to antioxidants and contributes largely to the daily consumption of a major amount of these molecules. Tomato is not only traded in the fresh market but

is also used in the processing industry in soups, as paste, concentrate, juice, and ketchup (Lenucci *et al.* 2006). It is an incredible source of important nutrients such as lycopene,  $\beta$ -carotene and vitamin C, which all have positive impacts on human health. Recent studies show the wide consumption of chemical fertilizers and pesticides in agricultural practices which cause direct or indirect effect on the environment and human health. Excessive use of these chemicals induces the genetic resistance in pathogens. Keeping these negative impacts of synthetic chemicals in mind, scientists are trying to find out new technique with minimum effect on the environment and human health. Vermicomposting is a non-thermophilic biological oxidation process in which organic material are



converted into vermicompost (Pathma and Sakthivel 2012). Vermicomposting is a combined symbiotic action of earthworms and microorganisms on organic materials. Vermicompost (VC) is fine peat-like substance with good aeration, high porosity and water holding capacity (Arancon *et al.* 2004; Atiyeh *et al.* 2002). Owing to its environmental friendliness, nutrients richness, easy and low cost production procedure, VC as a quality biofertilizer has attracted worldwide farmer's community and agricultural industries. Along with solid vermicompost, aqueous extract of vermicompost is also an important organic input for sustainable agriculture (Singh *et al.* 2013). Vermicomposting is the method that provides biodegradable farming and tends to increase soil stability and productivity. During the last 40 years, vermicomposting is extensively used in both developing and non-developing countries. As compared to thermophilic aerobic compost vermicompost are more efficient as organic fertilizers. Various greenhouse and field studies have examined the effects of a variety of vermicompost on a wide range of crops including vegetables, ornamental and flowering plants and field crops. The main aim of this research was to determine the effects of vermicompost alone and in combination with agriculturally important microorganisms on the growth, yield and fruit quality of tomato under field conditions.

## MATERIALS AND METHODS

### Preparation of vermicompost

Vermicompost was prepared by the methods validated by Singh *et al.* (2013) using floral offering from temple. The temple wastes mainly consisted of *Aegle marmelos* leaves, *Datura stramonium*, *Tagetes erecta* and *Hibiscus rosa sinensis* flowers. The offerings were collected from different temples in the city but the bulk from two temples namely "Vishwanath temple" and "Sankatmochan temple" which receive most of the devotees. Nine rectangular plastic boxes with dimensions measuring 320 cm × 150 cm × 50 cm, were used for vermicomposting. Forty five holes (0.65 cm diameter) were drilled at the base of the container for allowing proper exchange of gases. Mature cow dung was added at a ratio of about 1:7 to provide an instant source of food to the earthworms. Finally the boxes were covered with a

layer of soil for decomposition. Adult worms, *Eisenia fetida*, ranging in length from 4 to 8 cm were added at the rate of 1.5 kg/m<sup>2</sup> through the developed cracks after 15 days of partial decomposition of waste to prevent worms from the thermophilic reaction occurring during composting. The moisture content of the feedstock was adjusted to 70 ± 10% at the start of vermicomposting and maintained throughout the period by sprinkling of water. Watering was stopped when the VC was ready as indicated by uniform dark brown to black coloured granular structure. Three days later the compost along with worms was harvested and the worms were removed by sieving (<2 mm).

### Fortification of Vermicompost

The biological control agents used in this study *viz.* *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from the culture repository of Plant Health Clinic Laboratory of the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. All these selected BCAs were used to fortify the vermicompost individually. Five days old *T. harzianum* culture grown in 1L PDB with CFU count approximately 4×10<sup>7</sup> was used to fortify 25 kg vermicompost tray. Likewise, two days old bacterial cultures grown in 1L NB with CFU count approximately 2×10<sup>8</sup> was thoroughly mixed with 25 kg of freshly prepared vermicompost in separate trays.

### Pot Experiments

Pot experiments were carried out with tomato variety Kashi Amrit sown in plastic pots of 15 cm × 10 cm. Soil was autoclaved for 30 min. at 15 psi for three consecutive days and pots were filled with soil mixture containing sterile soil and microbially fortified vermicompost in ratio of 1:1 (w/w). In first three treatments, vermicompost was individually fortified with *T. harzianum*, *B. subtilis* and *P. fluorescens*. Fourth treatment was only vermicompost and fifth treatment was only soil. Plant samples were uprooted carefully and following growth parameters were measured and recorded for all the treatments.

1. Root Length (cm)
2. Shoot Length (cm)
3. Dry Weigh

## Biochemical analysis

### Determination of ascorbic acid content

Plant materials were homogenized in ice cold mortar pestle with 10 ml of 6% T.C.A. The filtrate was then extracted at 0°C. 2 ml of DNPH (2%) and 1 drop of thiourea (10%) were then added to 4 ml of extracts. Mixture kept in boiling water bath for 15 min and cooled down by keeping in ice at 0 °C and 5 ml of 80% H<sub>2</sub>SO<sub>4</sub> added to it at 0 °C. Quantitative estimation of ascorbic acid was carried out following the method as described earlier by Lichtenthaler (1987), and using a standard curve of ascorbic acid.

### Extraction and estimation of carotenoid

Carotenoid were extracted and estimated by method given by Lichtenthaler (1987). 1 g of plant material was homogenized in methanol. The homogenate was then filtered through Whatman no. 1 filter and the volume was made up accordingly. Absorbance of the filtrates was determined at 480 nm, 645 nm, and 663 nm, in UV-VIS spectrophotometer and the carotenoid content was calculated.

### Estimation of lycopene content in tomato fruits

Lycopene from tomato fruit was extracted by making it pulp in acetone with the help of mortar and pestle. Ten g of the pulp extracts was then transferred to a separating funnel containing 20 ml of petroleum ether and mixed gently. 20 ml of sodium sulphate (5%) solution was added and shaken gently. The two phases were separated and the lower aqueous phase was re-extracted until the aqueous phase was colourless. The petroleum ether extracts were pooled out and washed with distilled water. The washed petroleum ether extract containing carotenoid was poured into a brown bottle and kept aside for 30 min. Then the petroleum ether extract was decanted into a 100 ml volumetric flask through a funnel containing cotton wool. The sodium sulphate slurry was washed with petroleum ether until it was colourless and washings were also transferred to the volumetric flask. The volume was made up and the absorbance was measured in a spectrophotometer at 503 nm using petroleum ether as blank. Quantification was done on the basis of absorbance using the following formula:

$$\text{Absorbance (1 unit)} = 3.1206 \mu\text{g lycopene/ml.}$$

### Determination of total soluble protein

Protein was extracted from the tomato leaf using phosphate buffer (pH7.2) and protein content was determined following the method as described by Lowry *et al.* (1951) using BSA as standard.

### Total phenolic content (TPC)

TPC was determined using the method of Zheng and Shetty (2000). Five ml of ethanol (95%) was added to 0.1 g leaf tissues and mixture was kept at 4 °C for 48 h. The mixture was centrifuged at 13000 x g for 10 min. one ml of ethanol (95%), 5 ml of distilled water and 0.5 ml of Folin-ciocalteau reagent (50%) were added to 1 ml of supernatant and mixed thoroughly. One ml of sodium carbonate (5%) was added after 5 min. and the reaction mixture was kept at room temperature for 1h and absorbance of the colour developed was recorded at 725 nm against a reagent blank. Standard curves were prepared for each assay using various concentrations of gallic acid (GA; Sigma, USA) in 95% ethanol. Absorbance values were converted to mg GA equivalents (GAE) g<sup>-1</sup> fresh weight (FW).

## RESULTS AND DISCUSSION

The influence of different microbes used for fortification of vermicompost on the growth characters was clearly observed after 15 days of transplanting. All treated plants showed significant improvement in root length in comparison to the control. Tomato plants treated with vermicompost fortified with *Trichoderma* showed maximum root length (14.95 cm) after 15 days of sowing followed by T-2 (11.25 cm) and T-3 (9.85 cm). Similar trends were observed after 45, 60 and 90 days after sowing (Figure 1). In the present study it was observed that the application of vermicompost in addition with other bioinoculants in tomato promoted growth and yield of plant. Gandhi and Sundari (2011) also reported the similar results and concluded that the availability of nutrients in vermicompost fortified with PGPRs enhanced plant growth and yield in brinjal (*Solanum melongena* L.).

Shoot length was recorded at 15, 45, 60 and 90 days after sowing. Tomato plants treated with vermicompost fortified with *Trichoderma* showed maximum shoot length at every interval. Maximum shoot length 57.5 cm was observed in T-1 followed

by T-2 (45.13 cm) and T-3 (42.25 cm) after 15 DAS. Similar pattern was observed after 45, 60 and 90 days after sowing. After 90 DAS maximum shoot length was observed in T-1 (142.25 cm). After 90 DAS significant difference was observed in treated and control plants.

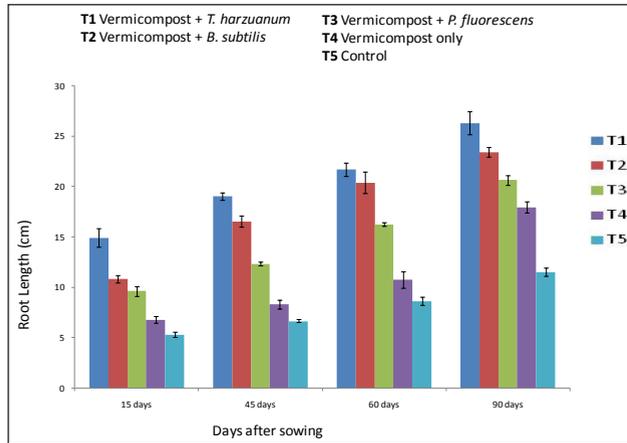


Fig. 1: Effect of different treatments on root length of tomato

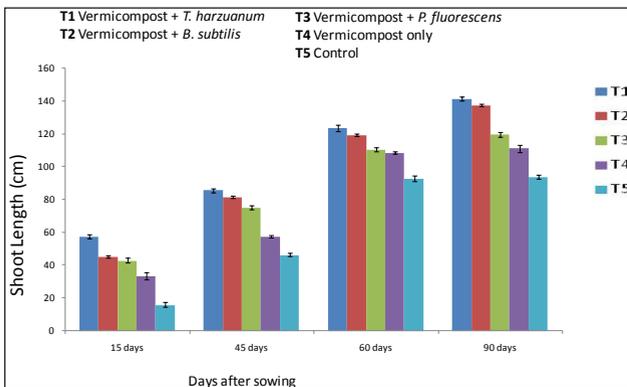


Fig. 2: Effect of different treatments on shoot length of tomato

Dry weight was recorded at 15, 45, 60 and 90 days after sowing. Maximum dry weight was observed in plants treated with vermicompost fortified with *Trichoderma*. After 15 DAS, 3.15 g dry weight was in T-1 followed by T-2 and T3. Bachman and Metzger (2008) reported that the application of vermicompost enhanced the dry weight of tomato plant.

The results presented in this study showed that there was a clear difference in growth promotion in tomato plants grown in microbial fortified vermicompost as well as in vermicompost alone. Highly significant variations were observed in root length, shoot length and dry weight among the treatments. Results of the current study are in accordance with the report of Wang *et al.* (2017)

who reported that the application of vermicompost in addition with other bioinoculants in tomato promoted growth.

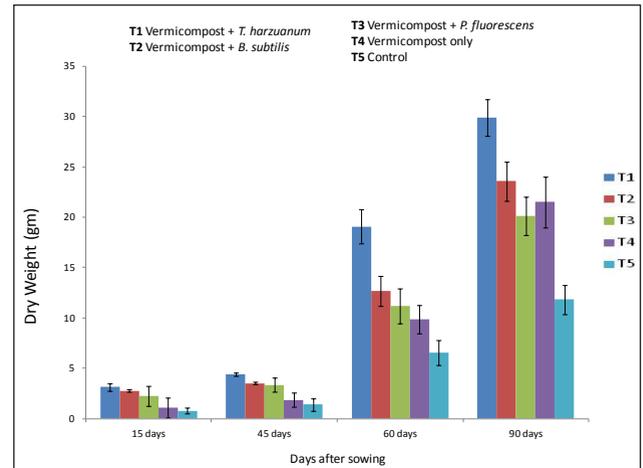


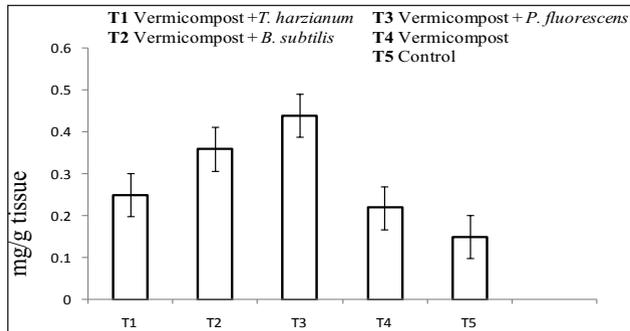
Fig. 3: Effect of different treatments on dry weight of tomato

In their study using different treatments, it was found that maximum growth in tomato plants was obtained where a combination of different treatments i.e. vermicompost, *Bacillus pumilus*, *Trichoderma* and a mycorrhizal fungus *Glomus mosseae* were used. Similar results were also reported by Bachman and Metzger (2008) who stated positive effect on productivity enhancement and nematode management through vermicompost and bio-pesticides in brinjal. The findings of this study are also in agreement with experimental findings which showed that vermicompost or its combination with *Pseudomonas fluorescens* based biopesticide have played a vital role in promoting growth in tomato (Bora and Deka, 2007).

### Ascorbic acid

Maximum ascorbic acid content in tomato leaves were found in plant treated with vermicompost + *P. fluorescens* which was 0.44 mg g<sup>-1</sup>. All the plants treated with biofortified vermicompost showed higher ascorbic acid content in leaves in comparison to control. 0.25 mg g<sup>-1</sup> and 0.36 mg g<sup>-1</sup> ascorbic acid in leaves was recorded in Vermicompost + *T. harzianum* and Vermicompost + *B. subtilis* treated plants respectively (Fig. 4). T3 (vermicompost + *P. fluorescens*) showed 2.4 fold increase in ascorbic acid content in leaves in comparison to control. Same result was found in case of chlorophyll content. It was previously reported that vermicompost may affect different aspects of plant biochemical

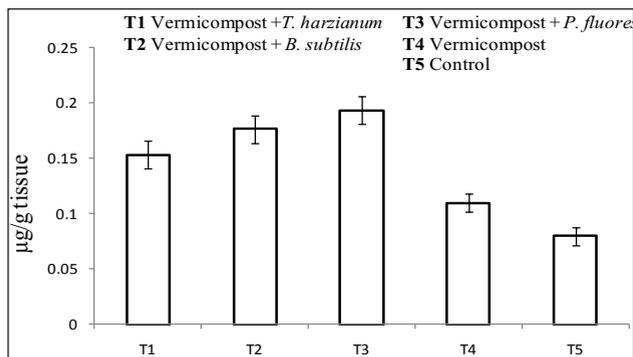
processes (Ladan *et al.*, 2012; Adewole and Ilesanmi, 2011). They significantly promoted contents of vitamin C, phenols and flavonoids in the vermicompost treated plants has been recorded (Sahni *et al.*, 2008).



**Fig. 4:** Effect of biofortified vermicompost on ascorbic acid content in tomato leaves

### Carotenoid

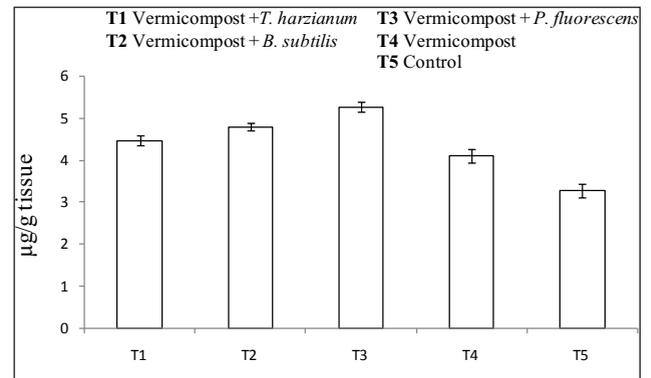
Maximum carotenoid content in tomato leaves were recorded in leaves from plant treated with vermicompost + *P. fluorescens* (T3) followed by T2 (Vermicompost + *B. subtilis*) and T1 (Vermicompost + *T. harzianum*). 0.19  $\mu\text{g g}^{-1}$  plant tissues carotenoid was estimated in tomato leaves treated with vermicompost + *P. fluorescens* which is 2.37 fold higher than the control. All the plants treated with biofortified vermicompost showed higher carotenoid content in leaves in comparison to control (Fig. 5). The results of our study agreed with the previous findings where similar results on the effects of soil amendments on the nutritional quality of okra (*Abelmoschus esculentus* [L.] Moench) were obtained (Osonubi *et al.* 1991). Bhattacharjee *et al.* (2015) reported that the application of vermicompost along with PGPRs in tomato increased the amount of carotenoid in tomato leaves.



**Fig. 5:** Effect of biofortified vermicompost on carotenoid content in tomato leaves

### Lycopene

Tomato fruit harvested from the plants treated with vermicompost + *P. fluorescens* (T3) showed maximum lycopene content which was 5.26  $\mu\text{g g}^{-1}$ . 4.8 and 4.6  $\mu\text{g g}^{-1}$  lycopene content was recorded in fruits from treatment T2 (Vermicompost + *B. subtilis*) and T1 (Vermicompost + *T. harzianum*). Minimum lycopene content (3.26  $\mu\text{g g}^{-1}$ ) was estimated in control (Fig. 6).



**Fig. 6:** Effect of biofortified vermicompost on lycopene content in tomato fruit

### Total phenol content (TPC)

TPC in all treatment was ranges from 4.28 to 11.32  $\text{mg g}^{-1}$ . Maximum TPC was recorded in T3 followed by T2 (Vermicompost + *B. subtilis*), T1 (Vermicompost + *T. harzianum*) and T4 (only vermicompost). In control it was recorded 4.28  $\text{mg g}^{-1}$ . T3 showed 2.6 fold more accumulation of TPC in plant tissues (Table 1).

**Table 1:** Effect of biofortified vermicompost on total phenol and protein content in tomato

Treatments	Total phenol (mg/g tissue)	Total protein (mg/g tissue)
T1 Vermicompost + <i>T. harzianum</i>	7.55±1.21 <sup>b</sup>	3.56±0.24 <sup>b</sup>
T2 Vermicompost + <i>B. subtilis</i>	8.6±1.09 <sup>b</sup>	4.74±0.36 <sup>c</sup>
T3 Vermicompost + <i>P. fluorescens</i>	11.32±0.69 <sup>c</sup>	5.1±0.2 <sup>c</sup>
T4 Vermicompost	6.44±0.84 <sup>b</sup>	3.1±0.11 <sup>b</sup>
T5 Control	4.28±0.70 <sup>a</sup>	1.8±0.29 <sup>a</sup>

### Total soluble protein

Like TPC, protein content was recorded highest in T3 (vermicompost + *P. fluorescens*) which was 5.1  $\text{mg g}^{-1}$  followed by T2 (4.74  $\text{mg g}^{-1}$ ), T1 (3.56  $\text{mg g}^{-1}$ )



and T4 (3.1 mg g<sup>-1</sup>). T3 showed 2.8 fold increase in protein content in comparison to control (Table 1). Minimum 1.8 mg g<sup>-1</sup> protein content was recorded in T5 (control).

## CONCLUSION

Vermicompost is one of the most widely used organic fertilizer which increase crop yield in sustainable manner. The soil amended with vermicompost offers supplementary substances that are absent in chemical fertilizers (Arancon *et al.* 2008). It is thus apparent from our study that application of vermicompost alone or with other agriculturally important microorganisms would be environmentally safe and contribute to long term sustainable agriculture. In conclusion it can be said that application of vermicompost in addition with other microorganisms such as *Trichoderma*, *Pseudomonas* and *Bacillus* maximize plant growth, total yield and fruit quality and nutritional parameters of tomato plants.

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