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"IMPROVING ANTIOXIDANT LEVELS AND NUTRITIONAL VALUE OF CARROTS THROUGH BIOFORTIFIED VERMICOMPOST INOCULATION

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Abstract

The effect of biofortified vermicompost was studied on growth parameter and nutritional quality of carrot. Microorganisms used for fortification were Trichoderma harzianum, Pseudomonas fluorescens and Bacillus subtilis. Vermicompost was prepared by using farm and kitchen waste from Rama University, Kanpur. 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. Carotenoid content was also increased in biofortified vermicomposts. Nutritional and antioxidants were recorded highest in vermicompost + P. fluorescens treatment.

Keywords: Biofortified vermicompost, antioxidant, Trichoderma,

Introduction

The significance of human nutrition is profound as it underpins energy production, growth, tissue repair, immune function, and various metabolic processes within the body. Essential nutrients include proteins, fats and oils, carbohydrates, fiber, vitamins, minerals, and water. Additionally, certain plant-derived compounds known as antioxidants play a crucial role in mitigating oxidative stress and safeguarding the body from cellular damage. Recently, antioxidants have been increasingly utilized as food additives to enhance the shelf life of products, stabilize fats and lipid-rich foods, and help retain flavor and nutritional value.

Carrots are believed to have originated in the regions of present-day Afghanistan and Persia around 1100 years ago. Today, they rank among the top ten vegetables cultivated worldwide. Approximately 1.2 million hectares are dedicated to carrot farming globally, with an estimated economic value of $14 billion. Although carrots can be grown in various climates, they thrive best in cooler seasons and are predominantly cultivated in Europe and Asia. High temperatures can be challenging for carrot growth, making tropical regions less suitable for their cultivation.

Carrots are well-regarded for their benefits to vision, largely due to their rich content of bioactive compounds such as carotenoids, which are vital for the production of vitamin A. Carotenoids, along with anthocyanins, serve as important non-enzymatic antioxidants. These antioxidants contribute to the vibrant colors of carrots—alpha- and beta-carotene provide the orange hue, lycopene adds red tones, and lutein gives a yellowish tint.

Recent research highlights the widespread use of chemical fertilizers and pesticides in agriculture, which has raised concerns about their direct and indirect impacts on both the environment and human health. Overuse of these chemicals can lead to the development of genetic resistance in pathogens. In response to these issues, scientists are exploring alternative methods that minimize environmental and health risks. One such method is vermicomposting, a biological process where organic materials are transformed into vermicompost through the action of earthworms and microorganisms (Pathma and Sakthivel, 2012).

Vermicomposting is characterized by its non-thermophilic nature, meaning it does not require high temperatures, and results in a fine, peat-like substance known as vermicompost (VC) (Arancon et al., 2004; Atiyeh et al., 2002). This product boasts excellent aeration, high porosity, and a substantial capacity for water retention. Due to its environmental benefits, nutrient richness, and cost-effective production, vermicompost has gained popularity among farmers and agricultural industries worldwide. In addition to solid vermicompost, its aqueous extract is also valued as a sustainable agricultural input (Singh et al., 2013).

Over the past four decades, vermicomposting has been adopted extensively across both developed and developing countries. Compared to thermophilic aerobic composting, vermicompost has been shown to be a more effective organic fertilizer. Various greenhouse and field studies have assessed the impact of different types of vermicompost on a range of crops, including vegetables, ornamental plants, flowering plants, and field crops. This research specifically aims to evaluate the effects of vermicompost, both alone and in combination with beneficial microorganisms, on carrot growth, yield, and fruit quality under field conditions.

Materials and Method

Preparation of vermicompost

Vermicompost was prepared by the methods validated by Singh et al (2013) using kitchen waste and farm waste from Rama University campus. 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^2 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 Vermicomost

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 4×10^7 was used to fortify 25 kg vermicompost tray. Likewise, two days old bacterial cultures grown in 1L NB with CFU count approximately 2×10^8 was thoroughly mixed with 25 kg of freshly prepared vermicompost in separate trays.

Pot Experiments

Pot experiments were carried out with carrot seedsown 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.

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 0^0C. 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 0^0C and 5 ml of 80% H_2SO_4 added to it at 0^0C. 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 Litchtenthaler (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 Anthocyanins content in carrot fruits

Anthocyanins from carrot 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:

Absorbance (1 unit) = 3.1206 µg lycopene/ml.

Determination of total soluble protein

Protein was extracted from the carrot 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 4^0C 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^{-1} fresh weight (FW).

Results and discussion

Ascorbic acid

Maximum ascorbic acid content in carrot leaves were found in plant treated with vermicompost + P. fluorescens which was 0.44 mg g^{-1}. All the plants treated with biofortified vermicompost showed higher ascorbic acid content in leaves in comparison to control. 0.25 mg g^{-1} and 0.36 mg g^{-1} ascorbic acid in leaves was recorded in Vermicompost + T. harzianum and Vermicompost + B. subtilis treated plants respectively (Figure 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).

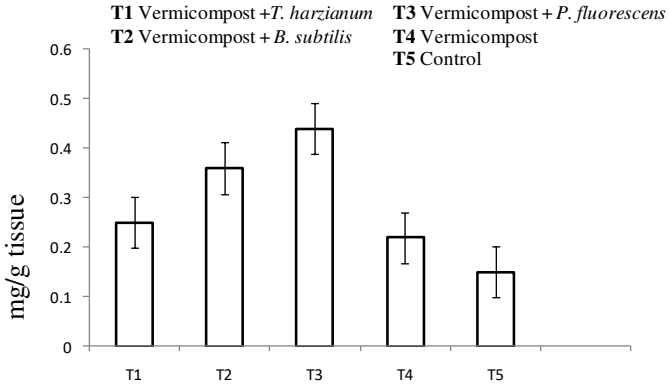

Figure 1 Effect of biofortified vermicompost on ascorbic acid content in carrot leaves

Carotenoid

Maximum carotenoid content in carrot 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 µg g^{-1} plant tissues carotenoid was estimated in carrot 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 (Figure 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 carrot increased the amount of carotenoid in carrot leaves.

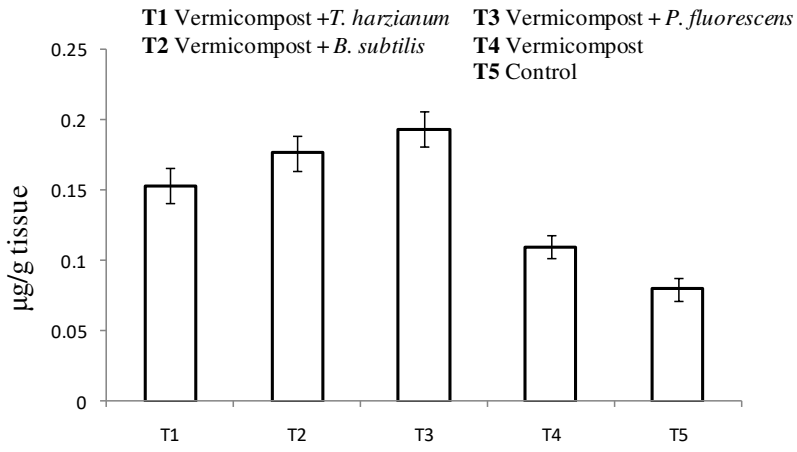

Figure 2 Effect of biofortified vermicompost on carotenoid content in carrot leaves

Anthocyanins

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

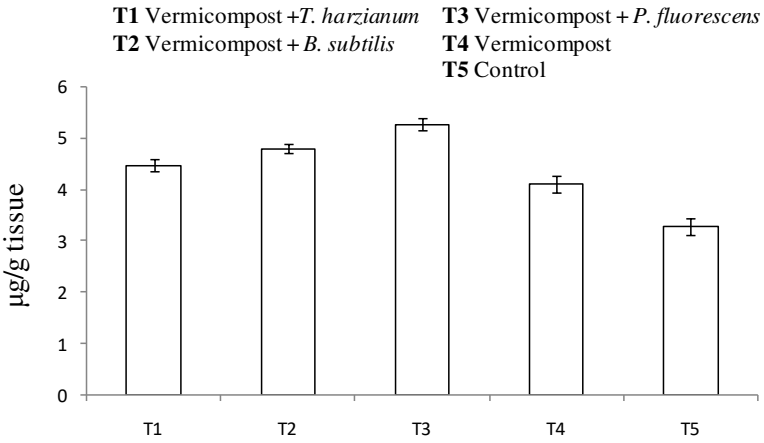

Figure 3 Effect of biofortified vermicompost on Anthocyanins content in carrot fruit

Total phenol content (TPC)

TPC in all treatment was ranges from 4.28 to 11.32 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 mg g^{-1}. T3 showed 2.6 fold more accumulation of TPC in plant tissues.

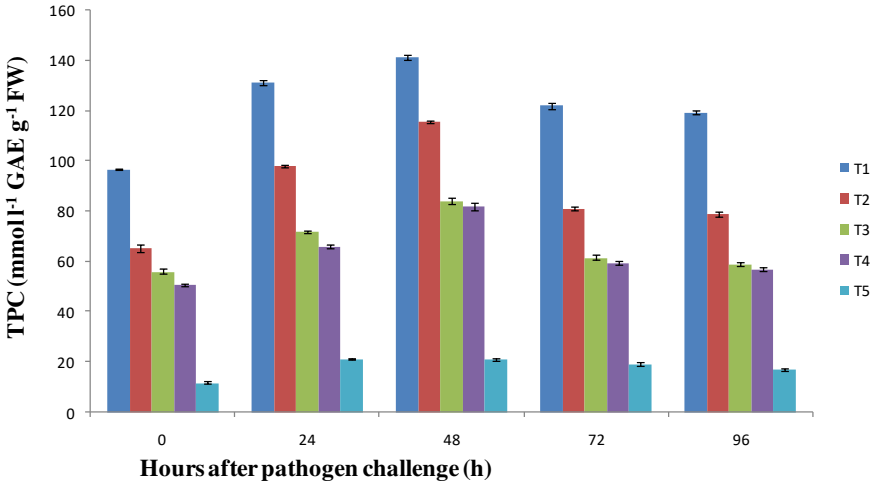

Figure 4 Figure TPC activity at different time intervals in tomato in different treatment. Results are expressed as means of three replicates and vertical bars indicate standard deviation of the mean.

Total soluble protein

Like TPC, protein content was recorded highest in T3 (vermicompost + P. fluorescens) which was 5.1 mg g^{-1} followed by T2 (4.74 mg g^{-1}), T1 (3.56 mg g^{-1}) and T4 (3.1 mg g^{-1}). T3 showed 2.8 fold increase in protein content in comparison to control (Table 1). Minimum 1.8 mg g^{-1} protein content was recorded in T5 (control).

Table 1 Effect of biofortified vermicompost on total phenol and protein content in carrot

Treatments	Total phenol (mg/g tissue)	Total protein (mg/g tissue)
T1 Vermicompost + T. harzianum	7.54 ± 1.11^{b}	3.46 ± 0.24^{b}
T2 Vermicompost + B. subtilis	8.7 ± 1.02^{b}	4.54 ± 0.34^{c}
T3 Vermicompost + P. fluorescens	10.31 ± 0.79^{c}	5.0 ± 0.3^{c}
T4 Vermicompost	6.41 ± 0.74^{b}	3.3 ± 0.21^{b}
T5 Control	4.24 ± 0.80^{a}	1.7 ± 0.29^{a}

Conclusions

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, Pseudomons and Bacillus maximize plant growth, total yield and fruit quality and nutritional parameters of carrot plants.

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