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ORIGINAL ARTICLE

Improvement of antioxidant and defense properties of Tomato (var. Pusa Rohini) by application of bioaugmented compost

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Abstract Nutrient management practices play a significant role in improving the nutritional quality of tomato. The present study deals with the evaluation of compost prepared using Effective Microorganisms (EM), on antioxidant and defense enzyme activities of Tomato (*Lycopersicon esculentum*). A field experiment with five treatments (control, chemical fertilizer and EM compost alone and in combination) was conducted in randomized block design. An increment of 31.83% in tomato yield was recorded with the combined use of EM compost and half recommended dose of chemical fertilizers ($N_{50}P_{30}K_{25}$ + EM compost at the rate of 5 t ha^{-1}). Similarly, fruit quality was improved in terms of lycopene content (35.52%), antioxidant activity (24–63%) and defense enzymes activity (11–54%), in tomatoes in this treatment as compared to the application of recommended dose of fertilizers. Soil microbiological parameters also exhibited an increase of 7–31% in the enzyme activities in this treatment. Significant correlation among fruit quality parameters with soil microbiological activities reveals the positive impact of EM compost which may be adopted as an eco-friendly strategy for production of high quality edible products.

1. Introduction

Organic farming is one of the fastest growing sectors of agriculture worldwide and its goal is to balance systems of soil organisms, plants, animals and humans (Karanatsidis and Berova, 2009). An ideal organic fertilizer should be capable of giving reasonable yields, increase soil fertility and quality and sustain productivity. The concept of organic

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fertilizers was popularized because the negative effect of the intensive use of chemical fertilizers resulted in soil degradation. Moreover, excessive fertilization has been reported to have an influence on the phyto-nutritional quality of crops and reduction in the antioxidant levels, besides causing pollution (Arancon et al., 2004; Toor et al., 2006).

Compost prepared using Effective Microorganism (EM) is a type of bio-organic fertilizer whose concept was developed in 1971 by Professor Teruo Higa, University of the Ryukyus, Okinawa, Japan (Higa and Wididana, 1991). Effective Microorganism (EM) is a combination of microbial inoculant which can stimulate plant growth and soil fertility in agriculture (Mayer et al., 2010). EM compost is a good source of nutrients for vegetable crops, which can provide favorable conditions for the growth of crops, promoting the mobilization of insoluble nutrients and activating the beneficial microorganisms in soil (Higa, 2000). Application of EM is known to enhance crop growth and yield in many vegetable crops (Sheng and Lian, 2002; Daiss et al., 2008). Although many reports are available on the beneficial effect of EM on agronomic aspects, the effect of compost prepared by EM on quality aspects of food has been less investigated.

Tomato (*Lycopersicon esculentum*) is one of the most popular and versatile vegetables in the world, because of its taste, color, high nutritive value and its diversified use. It is the world's largest vegetable crop after potato and sweet potato, and tops the list of canned vegetables. According to recent FAO (Food and Agricultural Organization) statistics, approximately 160 million tones tomatoes are produced annually on 4.7 million hectares of land (FAOSTAT, 2011). Tomato and its products are rich in antioxidants and considered to be a good source of vitamins C, E and carotenoids, particularly lycopene and β-carotene and other phenolic compounds (Ilahy et al., 2011; Pinela et al., 2012). Tomatoes have endogenous defense mechanisms which include oxidative enzymes Peroxidase (PO) and Polyphenol Oxidase (PPO) which are generally produced in response to pathogens (Bhonwong et al., 2009). These enzymes catalyze the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure (Avdiushko et al., 1993). On the other hand, Phenylalanine Ammonia Lyase (PAL) is a key enzyme in the biosynthetic pathway of phenylpropanoids (Berner et al., 2006). The reactive oxygen species (ROS) like superoxide and hydrogen peroxide (H_2O_2) can cause direct damage to membrane lipids, proteins and DNA leading to cell death (Mittler, 2002; Simova-Stoilova et al., 2008). The enzymes Superoxide dismutase (SOD), Catalase (CAT) Peroxidase (PO), Ascorbate Peroxidase (APO) and Glutathione Reductase (GR) are key antioxidants playing a central role in the defense against ROS (Noctor and Foyer, 1998; Simova-Stoilova et al., 2008). Due to the presence of antioxidants, the regular consumption of tomatoes and their products can reduce the risk of several types of cancer and cardiovascular diseases (Clinton, 1998).

The main aim of this study was to determine the effect of EM compost on the level of antioxidant compounds and fruit quality of tomato. These parameters were also correlated with soil microbiological parameters to understand the impact of EM compost on soil health.

2. Methods and material

2.1. Site, experimental design and field layout

A field experiment was carried out with Tomato var. Pusa Rohini (*L. esculentum*), during winter (Rabi) season, with harvesting upon reaching maturity in March 2013 at the Indian Agricultural Research Institute, New Delhi (India) farm. The research farm is located at latitude of 28°40′N 77°12′E and 228.6 m above the mean sea level of Arabian Sea. The physico-chemical characteristics of the soil in the experimental field were – pH 8.3, EC, 0.1 mS cm^{-1}, organic carbon 0.56% (Walkley and Black, 1934), available N – 223.24 kg ha^{-1} (Subbiah and Asija, 1956), available P - 37.36 kg ha^{-1} (Olsen et al., 1954) and available K – 662.96 kg ha^{-1} (Hanway and Heidel, 1952).

2.2. Preparation of EM (Effective Microorganism) compost

EM compost was prepared by the method of Sharma et al. (2014); in brief paddy straw was used as raw material for preparation of EM compost. Paddy straw (40 kg) was amended with poultry droppings (5 kg) and rock phosphate (1% w/w) to provide nitrogen and phosphorous in composting pits. The EM consortium consisting of *Candida tropicalis* (Y6), *Phanerochaete chrysosporium* (VV18), *Streptomyces globisporus* (C3), *Lactobacillus* sp. and photosynthetic bacteria, was added 1% (v/w). All the substrates were mixed and water was sprinkled at regular intervals to maintain 60% moisture level throughout composting. The physicochemical characteristics of the mature EM compost used were: pH, 7.8; EC, 3.8 mS cm^{-1}; humus, 7.55%; C/N ratio, 15.66; available P, 0.31%; C, 26%; N, 1.66%.

2.3. Agronomic practices, management and treatment details

One month old tomato seedlings were transplanted in plots (10 m^2), with the spacing of 30–40 cm, in the month of December, 2012. The experiment was conducted with the five treatments having selected combinations of chemical fertilizers and EM compost, alone or in different ratios. Earlier experiments undertaken with EM compost had shown that it can provide 50 kg N ha^{-1} (Pers. Comm.). The five treatments were: Absolute control (T1); recommended full dose of chemical fertilizers (RDF) $N_{100}P_{60}K_{50}$ (T2); half of the recommended dose of chemical fertilizers $N_{50}P_{30}K_{25}$ (T3); $N_{50}P_{30}K_{25}$ + EM compost 5 t ha^{-1} (T4); EM compost 10 t ha^{-1} (T5). The experiment was conducted in four replicates, as a randomized block design (RBD). The recommended chemical fertilizers (T2) for tomato included 100 kg ha^{-1} nitrogen which was applied as prilled urea, whereas 60 kg ha^{-1} phosphorous and 50 kg ha^{-1} potassium were applied in the form of single super phosphate (SSP) and muriate of potash (MOP), respectively. Additionally, treatment-T4 receiving EM at the rate of 5 t ha^{-1} compost was supplemented with only half of the recommended dose of N, P and K fertilizers. The recommended package of practices was followed for raising the tomato crop.

2.4. Soil microbiological parameters

Soil samples were taken from plots using auger from each plot at harvest stage. A set of three to five soil cores (5 cm diameter,

0–15 cm depth) were taken from each plot and pooled together. The soil samples were placed in polyethylene bags and transported to the laboratory. The soil samples were thoroughly mixed and sieved (2 mm mesh) and visible plant material was removed manually, if any. The samples were stored at 4 °C, until microbiological analyses was undertaken.

Acid (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) activity was assayed by the method of Tabatabai and Bremner (1969) using *p*-nitrophenol phosphate as a substrate, at pH 6.0 and 11.0, respectively. The absorbance was measured at 440 nm and the enzymatic activities were expressed as μg of *p*-nitrophenol released g^{-1} soil h^{-1}. Dehydrogenase activity was assayed using the method of Casida et al. (1964). The values were expressed as μg of triphenyl formazan (TPF) released g^{-1} soil d^{-1}. Microbial biomass C (MBC) was estimated by the method of Nunan et al. (1998), using aliquots of K_2SO_4 extracts through dichromate digestion. Microbial biomass carbon was calculated after back titration with ferrous ammonium sulfate using the equation: Biomass C = 2.64 × CE, where CE = (organic C from fumigated soil) – (organic C from unfumigated soil) and expressed as μg C g^{-1} soil. Fluorescein di acetate (FDA) hydrolysis was estimated by the method of Green et al. (2006) using FDA as a substrate and expressed as μg of fluorescein released g^{-1} of soil h^{-1}.

2.5. Estimation of lycopene and total phenol content

The total phenol in tomato fruit was estimated by the method proposed by Mallick and Singh (1980) and total phenol content was expressed as mg tannic acid100 g^{-1} fresh wt of tomato. The total lycopene content in tomato fruit was estimated by the method of Thimmaiah (1999) and expressed as mg of lycopene 100 g^{-1} fresh weight of tomato.

2.6. Plant defense enzymes

The activity of plant defense enzymes – Peroxidase (PO), Polyphenol Oxidase (PPO) and Phenylalanine Ammonia Lyase (PAL), was measured both in leaves and tomato fruits. The defense enzymes in leaves were measured at mid stage, while activity of these enzymes in fruits was measured in the ripened tomatoes. Five tomatoes/few leaves from each plot were taken and immediately kept in ice box to prevent degradation of enzymes. The samples from each plot were chopped and mixed together. One gram of sample was weighed and homogenized in a pre-chilled mortar and pestle by adding 5 ml of pre-chilled 50 mM phosphate buffer, pH 7.0. The homogenate was centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant so obtained was stored at 4 °C and used as enzyme extract for the estimation of following defense enzymes except for PAL.

2.7. Peroxidase (PO; EC 1.11.1.7) activity

PO activity was determined by the spectrophotometric method of Jennings et al. (1969). The reaction mixture consists of 0.05 M Tris–HCl buffer, 1% (v/v) guaiacol and enzyme extract. The reaction was started by the addition of 1% (v/v) H_2O_2. Changes in the optical density (at 470 nm) of the reaction mixture were taken at 30 s intervals up to 3 min. One unit of Peroxidase activity is defined as the change in absorbance of 0.001 min^{-1} g^{-1} fresh weight of leaves/fruit.

2.8. Polyphenol Oxidase (PPO; EC 1.10.3.1) activity

PPO activity was also measured by the method of Jennings et al. (1969) with slight modifications. The mixture contained 20 mM citrate phosphate buffer (pH 6.0), proline (5 mg ml^{-1}) and enzyme extract. Catechol (2 mg ml^{-1}) was added to initiate the reaction and the absorbance was read at 546 nm. One unit of Polyphenol Oxidase activity is defined as the change in absorbance of 0.001 min^{-1} g^{-1} fresh weight of leaves/fruit.

2.9. Phenylalanine Ammonia Lyase (PAL; EC 4.3.1.24) activity

PAL activity was determined spectrophotometrically by the method of Gonzalez-Aguilar et al. (2004) with some modification. Tomato fruit and leaves (0.2 g) were extracted in 1.4 ml ice-cold sodium borate buffer (2.5 mM 2-mercaptoethanol, 20 mM boric acid, 50 mM borax, pH 8.8) at 4 °C. The mixtures were centrifuged for 10 min at 4000×*g*, and the resulting supernatants were used for PAL activity analysis. The reaction mixture, in a final volume of 3.3 ml, consisted of enzyme extract, 20 mM L-phenylalanine and deionized water. The enzyme reaction was started by the addition of enzyme extract and stopped by the addition of 5 M HCl. The reaction mixture was incubated for 60 min at 28 °C, after which the absorbance at 290 nm was measured. The enzyme activity was expressed as nmoles of cinnamic acid min^{-1} g^{-1} fresh weight of leaves/fruit.

2.10. Determination of antioxidant defense enzyme specific activities

The activity of antioxidant enzymes – Tyrosine Ammonia Lyase (TAL), Ascorbate Peroxidase (APO) and Glutathione Reductase (GR) was measured in tomato fruit at harvest stage. The enzyme extract was obtained by the same method as described in Section 2.6.

2.11. Tyrosine Ammonia Lyase (TAL; EC 4.3.1.5) assay

TAL assay was performed by the same method used to determine PAL activity with few changes. The reaction mixture contained 20 mM L-tyrosine instead of L-phenylalanine and the amount of *p*-coumaric acid released from L-tyrosine was measured spectrophotometrically at 335 nm. The enzyme activity was expressed as nmoles of *p*-coumaric acid min^{-1} g^{-1} fresh weight of tomato.

2.12. Ascorbate Peroxidase (APO; EC 1.11.1.11) assay

APO activity was estimated according to the method of Nakano and Asada (1981). Enzyme activity was determined by the decrease in absorbance of ascorbate at 290 nm. The reaction mixture consisted of enzymatic extract, 50 mM potassium phosphate buffer (cold), pH 7, 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 0.1 mM EDTA (Ethylene Diamine Tetraacetic Acid), in a 3 ml final volume. The reaction started with the addition of hydrogen peroxide. The molar extinction coefficient 2.8 mM^{-1} cm^{-1} was used to calculate ascorbate Peroxidase activity and expressed as μmole H_2O_2 reduced min^{-1} g^{-1} fresh

wt of tomato. One unit of ascorbate was required for the reduction of 1 mol of H_2O_2 reduced per minute at 25 °C.

2.13. Glutathione Reductase (GR; EC 1.6.4.1) assay

GR activity was determined spectrophotometrically according to the method of Carlberg and Mannervik (1985). The assay system contained 0.2 M phosphate buffer pH 7.5, including 1 mM EDTA, 3.0 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB), 0.2 mM NADPH, 20 mM oxidized glutathione (glutathione disulfide-GSSG) and enzyme extract. The increase in absorbance at 412 nm was recorded spectrophotometrically and expressed as μmol NADPH oxidized min^{-1} g^{-1} fresh wt of tomato. One enzyme unit is defined as the oxidation of 1 μmol NADPH per minute under the assay condition.

2.14. Morphometric and biometrical parameters

The plant parameters – plant height, number of branches per plant and fruit yield were recorded at the time of harvesting.

2.15. Statistical analysis

The datasets of the various parameters was analyzed in triplicates and subjected to two-way ANOVA (Analysis of variance) in accordance with the experimental design (RBD) using SPSS-16 statistical package to quantify and evaluate the source of variation and interactions among the cultivars. The treatment means were compared at a significance level of 0.05 and the ranking of treatments denoted by alphabets. The data denoted by different letters in each column of the tables or in figures represent significantly different values among the treatments. Further, the correlation among the different parameters was measured using Microsoft Excel program.

3. Results

3.1. Effect of EM compost on tomato yield and other plant biometric parameters

Plant morphological parameters (plant height, number of branches and fruits per plant) were measured at harvest stage of the crop. Treatment T4 receiving EM compost at the rate of 5 t ha^{-1} along with half dose of recommended fertilizer ($N_{50}P_{30}K_{25}$) showed significantly higher values of plant morphological parameters as compared with chemical fertilizer control ($N_{100}P_{60}K_{50}$ – T2). The values and percent increase over T2 (RDF) for plant height (70.80 cm; 4.66%), number of branches per plant (6.00; 10%) and number of fruits per plant (47.13; 30.20%) illustrated the superiority of treatment T4 (Table 1; $P < 0.05$). Similarly, a significantly higher yield (31.83%) of tomato was recorded with the integrated application of EM compost and chemical fertilizer ($N_{50}P_{30}K_{25}$ + EM compost at the rate of 5 t ha^{-1} – T4), over the fertilizer control ($N_{100}P_{60}K_{50}$ – T2). A significant correlation was also recorded among the plant morphological parameters and tomato yield ($r = 0.683 - 0.966$, Supplementary Table 1; $P < 0.05$).

3.2. Total phenol and lycopene content

Total phenol and lycopene content in tomato fruit were measured at maturity. A significantly higher amount of lycopene (8.81 mg 100 g^{-1} fresh wt) and phenols (118.49 mg tannic acid 100 g^{-1} fresh wt) was recorded in the fruits harvested from treatment T4 supplemented with $N_{50}P_{30}K_{25}$ + EM compost at the rate of 5 t ha^{-1} (Table 2; $P < 0.05$). In addition, lycopene and phenol content were enhanced by 35.52% and 2.06%, respectively by treatment T4, over the fertilizer control T2 ($N_{100}P_{60}K_{50}$). Furthermore, lycopene and phenol content were found significantly correlated with each other ($r = 0.896$, Supplementary Table 2; $P < 0.05$).

3.3. Influence of EM compost on soil microbiological parameters

At harvest stage, a pronounced increase in the treatment T4 was recorded in all the soil microbiological parameters (Fig. 1A–E; $P < 0.05$). In addition, a significant increase in soil microbiological parameters, relative to fertilizer control ($N_{100}P_{60}K_{50}$ – T2) equivalent to 11.10%, 20.28%, 7.04%, and 31.33% for alkaline phosphatase, acid phosphatase, dehydrogenase activity and fluorescein diacetate hydrolase, respectively was observed. Although microbial biomass carbon was also higher (420.18 μg C g^{-1} soil; 31.33%) in treatment T4 over the fertilizer control (T2), it was statistically at par with treatment T5 (EM compost at the rate of 10 t ha^{-1}). All the soil parameters showed a significant correlation with each other ($r = 0.773 - 0.969$; $P < 0.05$), and tomato yield was found to be significantly correlated with soil microbiological parameters ($r = 0.860 - 0.946$, Supplementary Table 2; $P < 0.05$).

3.4. Effect of EM compost on antioxidant enzymes

The activity of antioxidant enzymes – Tyrosine Ammonia Lyase (TAL), Ascorbate Peroxidase (APO) and Glutathione

Table 1 Effect of EM compost on yield parameters of Tomato.

Treatment details	Plant height (cm)	Number of branches per plant	Number of fruits per plant	Yield per plant (kg)
T1 Absolute control	55.00 ± [#]1.45^{c}	3.00 ± 0.27^{b}	24.93 ± 3.44^{c}	1.28 ± 0.14^{d}
T2 $N_{100}P_{60}K_{50}$	67.50 ± 0.60^{b}	5.40 ± 0.63^{a}	32.90 ± 2.19^{b}	2.02 ± 0.21^{c}
T3 $N_{50}P_{30}K_{25}$	66.20 ± 4.92^{b}	4.00 ± 0.41^{ab}	28.60 ± 1.56^{bc}	1.85 ± 0.18^{c}
T4 $N_{50}P_{30}K_{25}$ + EM compost 5 t ha^{-1}	70.80 ± 4.24^{a}	6.00 ± 0.47^{a}	47.13 ± 3.80^{a}	2.96 ± 0.19^{a}
T5 EM compost 10 t ha^{-1}	67.20 ± 1.19^{ab}	5.50 ± 0.78^{a}	42.63 ± 2.39^{a}	2.54 ± 0.13^{b}
SEM	1.84	0.4	1.97	0.09
LSD ($P < 0.05$)	5.18	1.13	5.55	0.26

[#] Mean ± standard deviation, $n = 3$; superscripts indicate significant differences based on LSD at 0.05 levels.

Table 2 Effect of EM compost on lycopene and phenol content of Tomato fruit.

Treatment details	Lycopene (mg lycopene 100 g^{-1} fresh wt)	Total phenol (mg tannic acid 100 g^{-1} fresh wt)
T1 Absolute control	2.72 ± [#]0.06[c]	112.59 ± 0.17[e]
T2 $N_{100}P_{60}K_{50}$	5.68 ± 0.08[b]	116.05 ± 0.04[c]
T3 $N_{50}P_{30}K_{25}$	5.25 ± 0.76[b]	114.07 ± 0.08[d]
T4 $N_{50}P_{30}K_{25}$ + EM compost 5 t ha^{-1}	8.81 ± 0.45[a]	118.49 ± 0.21[a]
T5 EM compost 10 t ha^{-1}	5.98 ± 0.22[b]	117.38 ± 0.30[b]
SEM	0.32	0.15
LSD ($P < 0.05$)	0.91	0.42

[#] Mean ± standard deviation, $n = 3$; superscripts indicate significant differences based on LSD at 0.05 level.

Reductase (GR) in tomatoes was measured at harvest stage. Higher values of TAL (1.69 nmol *p*-coumaric acid min^{-1} g^{-1} fresh wt), APO (2.10 µmol H_2O_2 reduced min^{-1} g^{-1} fresh wt) and GR (2.57 µmol NADPH oxidized min^{-1} g^{-1} fresh wt) were recorded in tomatoes harvested from T4 treatment plots with EM compost and chemical fertilizer ($N_{50}P_{30}K_{25}$ + EM compost at the rate of 5 t ha^{-1}). TAL and GR activity showed a significant increase of 49.57% and 24.12%, respectively in this treatment as compared with full dose of fertilizer control ($N_{100}P_{60}K_{50}$ – T2) (Table 3; $P < 0.05$). The activity of antioxidant enzymes showed a good correlation with one another [TAL – GR ($r = 0.867$; $P < 0.05$), TAL – APO ($r = 0.758$; $P < 0.05$) and APO – GR (0.870; $P < 0.05$)].

3.5. Defense enzyme activity exhibited by leaves and fruits of tomato

The activity of defense enzymes: Peroxidase (PO); Polyphenol Oxidase (PPO); Phenylalanine Ammonia Lyase (PAL) in tomato leaves was quantified at mid stage of the crop. Significantly higher values of PO (1.45 IU min^{-1} g^{-1} fresh wt), PPO (16.43 IU min^{-1} g^{-1} fresh wt) and PAL activity (165.10 IU h^{-1} g^{-1} fresh wt), were recorded in leaves harvested from T4 fertilized with half dose of the chemical fertilizers $N_{50}P_{30}K_{25}$ + EM compost at the rate of 5 t ha^{-1} – T4 (Fig. 2A; $P < 0.05$). An increase of 25%, 17.41% and 13.02% was recorded for PO, PPO and PAL, respectively as compared with fertilizer control ($N_{100}P_{60}K_{50}$ – T2). Defense enzymes (PO, PPO and PAL) in tomato fruit were measured after its ripening. The defense enzyme activities were also higher in the fruits harvested from treatment T4 ($N_{50}P_{30}K_{25}$ + EM compost at the rate of 5 t ha^{-1} – T4). The PO activity (41.03 IU min^{-1} g^{-1} fresh wt) was enhanced by 34.40% over the fertilizer control – T2, but it was statistically at par in fruits inoculated with EM compost alone – T5. Furthermore, significantly higher PPO (0.72 IU min^{-1} g^{-1} fresh wt; 54.17%) and PAL (48.72; 10.96%) activities were measured in $N_{50}P_{30}K_{25}$ + EM compost at the rate of 5 t ha^{-1} – T4 as compared with fertilizer control – T2 (Fig. 2B; $P < 0.05$). Significant correlations were observed among the defense enzymes measured in leaves ($r = 0.899 - 0.976$; $P < 0.05$) and fruit ($r = 0.795 - 0.897$; $P < 0.05$).

4. Discussion

During the last few decades the use of organic fertilizers is preferred over inorganic/chemical fertilizer, because the former option improves biological, chemical and physical properties of the soils in an eco-friendly manner (Palm et al., 1997). Few reports suggest that organic fertilizer especially EM compost improves the nutritional quality and antioxidant content in plants along with improving the soil health (Xu et al., 2000; Toor et al., 2006; Ncube et al., 2011).

Compost, specifically prepared by EM has the ability to effectively mineralize soil organic matter and consequently improve nutrient availability, soil health and crop growth (Piyadasa et al., 1995). The bioaugmented compost is also associated with improved soil structure and enhanced soil fertility, increased soil microbial activity and improved moisture-holding capacity of the soil (Arancon et al., 2004). The EM compost applied in tomato field was endowed with high humus content (7.55%), which in general influences the microbial diversity in soil and helps the plant to improve their physiological processes for mineralization of nutrients in soil. Our results proved that the plots treated with half of the recommended dose of the chemical fertilizers; $N_{50}P_{30}K_{25}$ + EM

Table 3 Effect of EM compost on antioxidant enzymes of Tomato fruit.

Treatment details	Tyrosine Ammonia Lyase (nmoles *p*-coumaric acid min^{-1} g^{-1} fresh wt)	Ascorbate Peroxidase (µmoles H_2O_2 reduced min^{-1} g^{-1} fresh wt)	Glutathione Reductase (1 µmol NADPH oxidized min^{-1} g^{-1} fresh wt)
T1 Absolute control	0.67 ± [#] 0.03[c]	0.36 ± 0.02[c]	1.56 ± 0.12[c]
T2 $N_{100}P_{60}K_{50}$	0.85 ± 0.04[b]	0.77 ± 0.33[b]	1.95 ± 0.02[b]
T3 $N_{50}P_{30}K_{25}$	0.84 ± 0.11[b]	0.54 ± 0.08b[c]	1.50 ± 0.12[c]
T4 $N_{50}P_{30}K_{25}$ + EM compost 5 t ha^{-1}	1.69 ± 0.13[a]	2.10 ± 0.08[a]	2.57 ± 0.04[a]
T5 EM compost 10 t ha^{-1}	0.92 ± 0.04[b]	1.94 ± 0.05[a]	2.08 ± 0.09[b]
SEM	0.06	0.11	0.64
LSD ($P < 0.05$)	0.16	0.30	0.18

[#] Mean ± standard deviation, $n = 3$; superscripts indicate significant differences based on LSD at 0.05 levels.

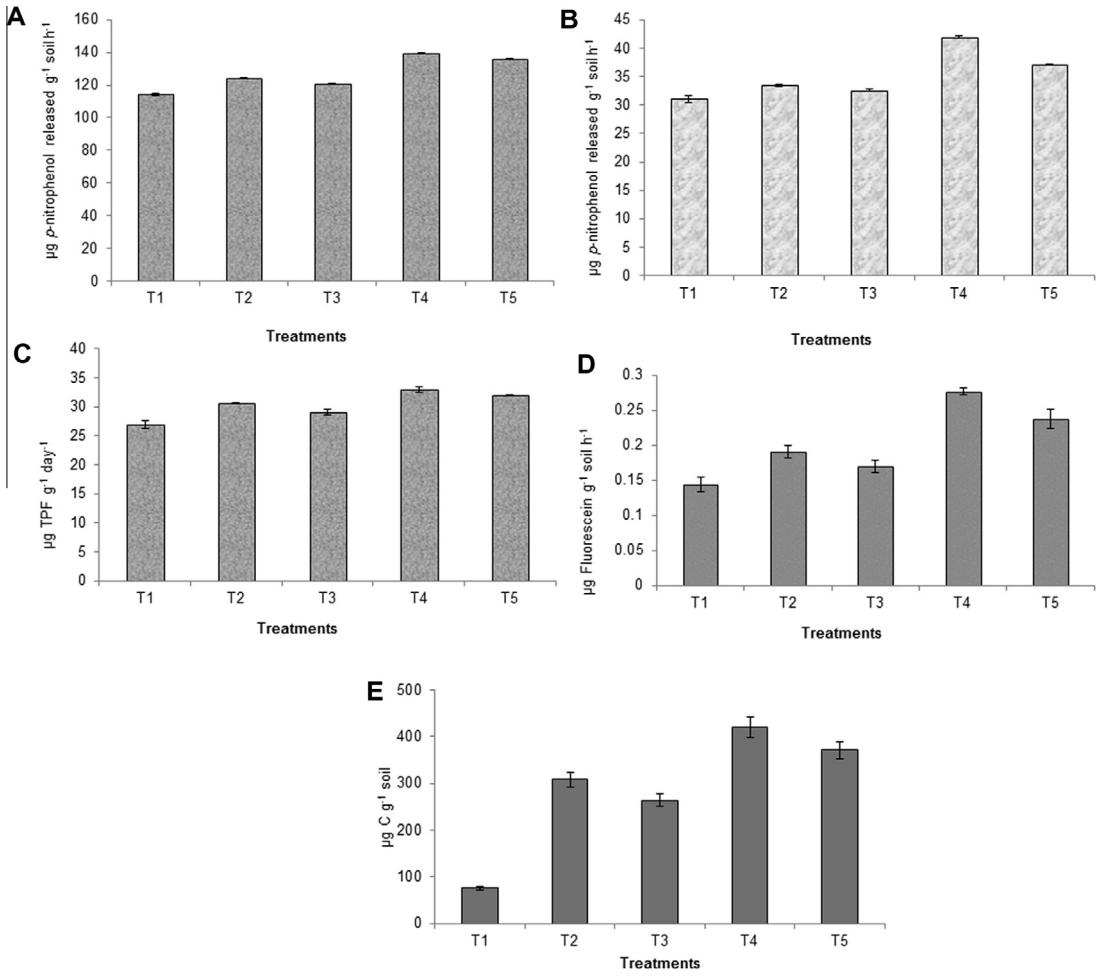

Figure 1 Influence of EM compost on soil microbiological parameters, at harvest-stage on Tomato. (A) Alkaline phosphatase activity (µg *p*-NP g^{-1} h^{-1}). (B) Acid phosphatase activity (µg *p*-NP g^{-1} h^{-1}). (C) Dehydrogenase activity (µg TPF g^{-1} day^{-1}). (D) Microbial biomass carbon (µg C g^{-1} soil). (E) Fluorescein diacetate hydrolase activity. Error bars represent standard deviation. T1, Absolute control; T2, $N_{100}P_{60}K_{50}$; T3, $N_{50}P_{30}K_{25}$; T4, $N_{50}P_{30}K_{25}$ + EM compost 5 t ha^{-1}; T5, EM compost 10 t ha^{-1}.

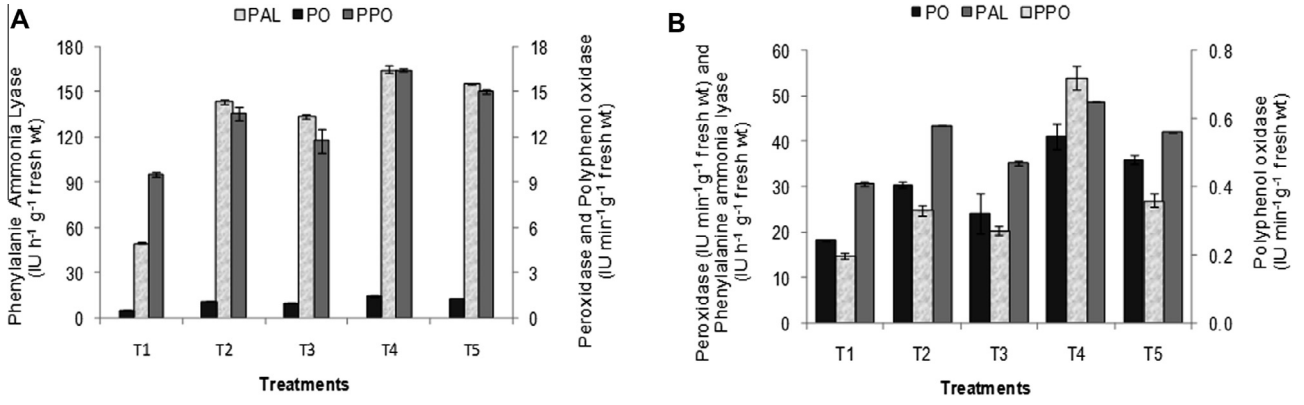

Figure 2 Influence of EM compost on defense enzyme activity of Tomato. (A) Leaves at mid stage. (B) Fruit at harvest stage. Error bars represent standard deviation. T1, Absolute control; T2, $N_{100}P_{60}K_{50}$; T3, $N_{50}P_{30}K_{25}$; T4, $N_{50}P_{30}K_{25}$ + EM compost 5 t ha^{-1}; T5, EM compost 10 t ha^{-1}.

compost at the rate of 5 t ha^{-1}, showed a pronounced increase in microbial activity. Higher values of dehydrogenase (32.96 µg TPF g^{-1} day^{-1}), fluorescein diacetate hydrolase (0.28 µg fluorescein g^{-1} soil h^{-1}) and microbial biomass carbon (420.18 µg C g^{-1} soil) in this treatment reflect a significant increase in microbial population in soil.

In the present study, tomato yield and other plant biometric parameters were positively influenced by mature compost

prepared with EM, which may be attributed to the pronounced increase in microbial population in the soil. An integrated use of EM compost and chemical fertilizer ($N_{50}P_{30}K_{25}$ + EM compost at the rate of 5 t ha^{-1} – T4), significantly increased tomato yield by 31.83% over the fertilizer control. Khaliq et al. (2006) also advocated the integrated use of organic and inorganic nutrient sources with Effective Microorganisms (EM) for improving crop yield. Similar results were obtained by Xu et al. (2000) where application of EM inoculated organic fertilizer integrated with chemical fertilizer, increased fruit yield and plant growth of tomato crop. Application of EM to peas, sweet potato and onions increased yield by 31%, 23% and 29%, respectively (Daly and Stewart, 1999). Riahi et al. (2009) also reported the influence of different organic fertilizers on the yield of tomato. According to their report, yield of tomato and quality increased by using organic compost in comparison to usual yield found for conventionally grown tomatoes.

Tomatoes are a very good source of antioxidants, vitamins C carotenoids (lycopene and β-carotene) and phenolic compounds (Ilahy et al., 2011; Pinela et al., 2012). Organically grown fruits and vegetables have high levels of vitamin C, iron, magnesium, phosphorus and antioxidant activity (SOD, GR, APO, PO, phenols) and less lipid peroxidation level than conventional grown products (Worthington, 2001; Barron, 2010; Montalba et al., 2010). The present study also validates positive influence of EM compost on tomato yield and fruit quality in terms of antioxidant and defense properties of tomato. Rein et al. (2006) reported that daily consumption of 15 mg of lycopene may reduce C-reactive proteins, a predictor of cardiovascular diseases and it acts as an *in vivo* antioxidant, and thus plays an important role in the prevention of cancer (Rao and Agarwal, 1998). Smita et al. (2013) reported 23 μg lycopene g^{-1} fresh wt in Pusa Rohini variety of tomato while in our experiment a significant increase (35.5%) in lycopene content (88.1 μg lycopene g^{-1} fresh wt) was observed under integrated nutrient management practices involving EM compost along with half dose of recommended chemical fertilizers. Similarly, Riahi and Hdider (2013) also reported that compost as organic fertilizers influences the lycopene content and antioxidant properties in different cultivars of tomato. In their experiment lycopene content varied ranging 78.0–117.8 μg g^{-1} fresh wt in different cultivars by application of different organic fertilizers.

The major phenolics in tomato exhibit a wide range of physiological properties, such as anti-inflammatory, antimicrobial, cardio protective, hepatoprotective, hypoglycemic and antiviral effects (Navarro-González et al., 2011). Phenolic compounds are of great importance in terms of the nutritional and commercial properties of agricultural products through their contribution to sensory properties such as color and flavor (Perez-Lopez et al., 2007). In this study, it was observed that EM compost alone at the rate of 10 t ha^{-1} (T5) and in combination with chemical fertilizers ($N_{50}P_{30}K_{25}$ + EM compost at the rate of 5 t ha^{-1} – T4) increased the phenol content in tomatoes. Similarly, Toor et al. (2006) also reported a higher level of total phenolics in organically fertilized tomatoes than in those that received chemical fertilizers.

The functional quality and antioxidant constituents of tomato are strongly influenced by environmental factors, mineral nutrition and genetics (Kaur et al., 2013). Awareness has increased about role of antioxidants in human health, as the levels of free radicals and other 'reactive oxygen species' (ROS) are controlled by a complex web of antioxidant defenses, which minimize oxidative damage to biomolecules. Any imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage, cancer, aging, atherosclerosis, inflammation, and neurodegenerative disorders such as Parkinson's and Alzheimer's diseases (Getoff, 2007; Kaur and Kapoor, 2002). Our results in tomato reveal that the values of antioxidant enzyme activity of GR was significantly higher (24.12%) in the fruits receiving $N_{50}P_{30}K_{25}$ + EM compost at the rate of 5 t ha^{-1} – T4, which are involved in detoxification of xenobiotics or serve in detoxification metabolism, of carcinogens, toxins and drugs causing hemolytic anemia, diabetes and neurologic disorders (Knapen et al., 1999; Gul et al., 2000; Hayes et al., 2005). The high levels of APO rose by 63.33% in the treatment T4 ($N_{50}P_{30}K_{25}$ + EM compost at the rate of 5 t ha^{-1}), making the tomato plant resistant against any stress. In addition, Simova-Stoilova et al. (2008) and Noctor and Foyer (1998) stated that PO, APO and GR are the key antioxidants playing a central role in the defense against ROS. Furthermore, Abdel-Fattah and Al-Amri (2012) reported that compost significantly increased the activity of TAL, PO, PPO, alkaline phosphatase and acid phosphatase as defense enzymes in tomato. Similar results were obtained in the present investigation where the defense enzymes PO, PPO and PAL were increased in leaves ranging from 13% to 25%. The activity of these defense enzymes in fruits increased in the range of 11–55% over the full dose of fertilizer control (T2) by combined inoculation of chemical fertilizers and EM compost (T4).

5. Conclusion

The present study revealed the promise of EM compost as soil supplements, for deriving multiple benefits to tomato crop, not only in terms of plant biometric parameters (plant height, fruit yield, etc.), but also improving the fruit quality, thereby emphasizing the need to reduce the use of chemical fertilizers in agriculture, particularly for edible food crops.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.sjbs.2014.11.003.

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