

Effect of vermicompost on fruit yield and quality of bell pepper

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Abstract

In this study effect of application of food waste vermicomposts to soil on antioxidant compounds, fruit yield and quality of sweet pepper (*Capsicum annum* L.) were investigated in field condition. Four vermicompost levels (0, 5, 10 and 15 t/ha) were applied to soil based on a randomized complete block design with three replications. The results showed that the highest (21.87 kg m²) and lowest (14.69 kg m²) fruit yield were achieved in plants treated with 5 t/ha vermicompost and control, respectively. Vermicompost treatments positively influenced fruit antioxidant compounds (antioxidant activity, total phenolic, carbohydrate content and total flavonoid). The highest antioxidant activity (81%) and carbohydrate content were obtained in plants treated with 10 t/ha vermicompost, while their lowest values were recorded in the control plants. Fruit quality indices (pH, titratable acidity, ascorbic acid and fruit firmness) were significantly influenced by vermicompost treatments. However, no significant difference was found for total soluble solids between treatments. There were 40, 61 and 56% increase in the amount of Titratable acidity, ascorbic acid content and fruit firmness following application of vermicompost (15 t/ha) when compared to their values in control, respectively. In conclusion, soil application of vermicompost can positively influence antioxidant compounds, fruit yield and quality of pepper.

Keywords: ascorbic acid, carbohydrate content, capsicum annum, fruit firmness, vegetable crop.

Introduction

There has been a growing awareness to reduce application of inorganic fertilizers to soils at global level in order to avoid their adverse effects. Vermicomposts are stabilized and non thermophilic products, which are produced by interactions of earthworms and microorganisms and have a great potential as soil amendments (Arancon *et al.*, 2005). Vermicomposts are finely divided peat-like materials with high porosity, aeration, drainage, water-holding capacity and microbial activities, which make them excellent soil amendments or conditioners (Atiyeh *et al.*, 2000).

Pepper (*Capsicum annum* L.) belongs to Solanaceae family, which are known for their versatility as a vegetable crops. The species of this family are consumed as fresh vegetables or dried spices (Bosland and Vostava, 2000). Pepper fruit is a rich source of ascorbic acid, carotenoids and phenolic compounds and is considered as an excellent source of antioxidant in human diets (Marin *et al.*, 2004). Fruit antioxidant contents can be influenced by exposure to different environmental cues (e.g. light intensity, temperature and drought) and internal factors (e.g. cultivar, fruit load and position) (Slimestad and Verheul, 2005). Furthermore, plant growth

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media and fertilizer regime can also influence fruit antioxidant contents (Ahn *et al.*, 2005). Asami *et al.* (2003) reported that total phenolic compounds in Marionberries grown under organic agricultural methods were significantly higher than conventional method. Olsson *et al.* (2006) demonstrated that organic growing of strawberries would result in production of fruits with higher levels of all antioxidants including total phenolic, ellagic acid and flavonols, than conventionally grown strawberries. It has been reported that, cabbages from organic cultivation methods have higher phenolics content than cabbages produced by the conventional methods (Sousa *et al.*, 2005). Karakurt *et al.* (2009) reported that humic acid application can significantly influence total carbohydrate content and total yield of pepper. Although positive influences of vermicompost on plant growth and development have been well established, their effects on fruit antioxidant activity and quality have not received much attention. Therefore, in this study we investigated the influence of vermicompost application in growth medium on fruit antioxidant activity and quality of pepper under field conditions.

Materials and Methods

Plant preparations

The experiment was conducted during the 2015 growing season at the experimental field of the Agricultural Faculty, Ferdowsi University of Mashhad, Iran (latitude 36° 17' N, longitude 59° 35' E and 985 m elevation). Soil sample (0-30 cm depth) was collected after the site had been prepared for

cultivation. The sample was analysed for physical and chemical properties using standard laboratory procedures described by Mylavarapu and Kennelley (2002) and the corresponding data are shown in Table 1. The experimental field was cleared, ploughed, harrowed and divided into plots. Sweet pepper seeds (*Capsicum annuum* L. var. 'California Wonder', M&B Seed, USA) were sown in a greenhouse in large trays containing 1:1 mixture of sand and peat (1:1 v/v). After sowing, trays were watered when necessary. Seven-week-old pepper seedlings with 12 true leaves were hand-transplanted into well-prepared beds in the field. Between and within rows spaces were 50 and 35 cm, respectively. All necessary cultural practices and plant protection measures were uniformly followed for all the plots during the entire period of experimentation.

Experimental design and treatments

The experiment was arranged in a randomized complete block design (CRBD) with four treatments and three replications. Treatments consisted of four levels of vermicompost (0, 5, 10 and 15 ton/ha). Chemical analysis of vermicompost is shown in Table 2. Vermicomposts were applied before transplanting and supplemented with enough amounts of inorganic fertilizer correspond with the recommended full rate of 120-150-100 kg NPK/ha that was applied to the inorganic fertilizer-treated plots. Vermicompost (as per treatment) and the inorganic fertilizers were applied and incorporated to the top 15 cm layer of the soil in the experimental beds.

Table 1. Soil chemical characteristics of experimental field

N (%)	P (ppm)	K (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)	pH	EC (ds/m)
0.101	15.7	184	4.42	1.06	17.0	1.02	7.68	2.04

Table 2. Vermicompost characteristics of experimental field

N (%)	P (%)	K (%)	Fe (%)	Cu (ppm)	Mn (ppm)	Zn (ppm)	OC (%)	pH	Ec (ds/m)
1.45	1.75	1.11	1.76	92	660	350	19.6	8.25	5.05

Measurements

Pepper fruits were harvested at the green mature stage after 75 days of transplanting. There were three plots per treatment, and three replicates per plot were collected. Each replicate comprised of twenty peppers, which were harvested from ten different randomly selected plants. Fruits were weighted and fruit yield per square was calculated. Part of the samples were immediately used for some analyses (titratable acidity, pH, total soluble solids, ascorbic acid and fruit firmness) and the other parts were freeze-dried and ground then were stored at -18 °C for analysis of antioxidants.

Antioxidant activities and total phenolic

Methanol extracts of freeze-dried fruits were prepared for the determination of antioxidant activity and total phenolic content. Weighed pepper fruit samples (5 g) were placed in a glass beaker and homogenised with 50 ml of methanol at 24 °C overnight. The homogenate was filtered and then centrifuged at 6000 rpm for 15 min. Free radical scavenging activity of the samples was determined using 2,2-diphenyl-2-picryl-hydrazyl (DPPH) method (Turkmen *et al.*, 2005). An aliquot of 2 ml of 0.15 mM DPPH radical in methanol was added to a test tube with 1 ml of the sample extract. The reaction mixture was mixed using vortex for 30 s and left to stand at room temperature in the dark for 20 min. The absorbance was measured at 517 nm, using a spectrophotometer (Bio Quest, CE 2502, UK). The antioxidant activity was calculated using the following equation:

$$\text{Antioxidant activity (\%)} = 1 - \frac{\text{A Sample (517 nm)}}{\text{A Control (517 nm)}} \times 100.$$

The total phenolic content in methanol extracts was determined using Folin–Ciocalteu's reagent (Singleton and Rossi, 1965). Each methanol extract solution (0.5 ml) was mixed with 6 ml distilled water and 0.5 ml Folin–Ciocalteu's phenol reagent. After 5 min, 2 ml of 20 g L⁻¹

sodium carbonate solution was added and the mixture was vigorously vortexed. The same procedure was also applied to standard solutions of gallic acid. After incubation at room temperature for 2 h, the absorbance of each mixture at 750 nm was measured using spectrophotometer. Results were expressed as mg of gallic acid equivalents (GAE)/100g dry weight.

Total flavonoid content

The flavonoids content was spectrophotometrically determined based on formation of a flavonoid–aluminium complex (Yoo *et al.*, 2008). Each sample (2 g) was extracted with 10 ml methanol for 24 h. One ml of the extracts was added to a 10 ml volumetric flask. Distilled water was added to make a volume of 5 ml. At zero time, 0.3 ml of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.6 ml of 10% (w/v) AlCl₃ was added and then at 6 min 2 ml of 1 M NaOH were also added to the mixture, followed by the addition of 2.1 ml distilled water. Absorbance at 510 nm was immediately read. Quercetin was chosen as a standard and the levels of total flavonoid content were determined in triplicate and expressed as quercetin equivalents in mg/100g dry weight.

Carbohydrate content

Carbohydrate content was measured according to Yemm and Willis (1954) method using anthrone reagent. Sugars were extracted with 80% ethanol at 45 °C, followed by centrifugation at 5000 rpm for 10 min. The reaction mixture consisted of 0.5 ml extract and 5 ml of anthrone reagent which was boiled at 100 °C for 30 minutes. Absorbance was determined at 620 nm. The carbohydrate content is expressed as mg/g dry weight.

pH, total soluble solids and titratable acidity

Pepper fruits from each treatment were cut into small slices and pooled. Samples were homogenized in a blender and portions of

the homogenate were taken to determine the fruit quality. The pH of fruit was measured using a pH meter at 20 °C. Titratable acidity (TA) was determined by titration with 0.1 N NaOH until pH 8.1 was reached and reported as g/l of citric acid fresh weight using citric acid as a control (Horwit, 1975). Total soluble solids (TSS) was determined at 20 °C with a refractometer and reported as °Brix.

Ascorbic acid contents and fruit firmness

Ascorbic acid contents (vitamin C) was measured by classical titration method using 2, 6-dichlorophenolindophenol solution, and expressed as mg/100 g fresh weight (Miller, 1998). Fruit firmness was determined for five fruits from each sample using a Chattillon hand penetrometer (Model DPP 1000) with a 0.6 mm probe. Measurements were performed at the center of each fruit. The maximum force (N) required to reach the bio yield point was recorded.

Data analysis

Data were analysed using SAS Software (version 9.1.3., 2000) and means were compared by Duncan's multiple range test (DMRT) at 5% level of confidence.

Results and Discussion

Fruit yield

The result indicated that fruit yield was affected by application of vermicompost (Table 3). The highest fruit yield with 21.87 kg m² was obtained by application of the lowest level of vermicompost (5 t/ha); while the control (without vermicompost application) had the lowest fruit yield with 14.69 kg m², however, no significant difference was found between three vermicompost treatments. Arancon *et al.* (2005) reported positive effects of vermicompost on the yield of peppers in field experiments and partly attributed this positive effect due to increased microbial biomass and activity, and addition of macronutrients such as phosphorus.

However, they suggested that the major contribution might have been the addition of plant growth regulators, such as humic acids and plant growth hormones adsorbed onto the humic acids. Plant growth regulators, such as auxins, gibberellins, cytokinins, abscisic acid, and ethylene, are signal molecules that regulate many processes of plant development including fruit development, i.e., formation of mature fruit or viable mature seeds (Ozga and Reinecke, 2003). In our experiment, the vermicompost treatments (10 and 15 t/ha) reduced the fruit yield of pepper compared to the 5 t/ha treatment, probably due to plant stress by its high soluble salt concentration. Similar results have been reported in tomatoes that were grown in soil treated with pig-manure vermicompost (Atiye *et al.*, 2001).

Antioxidant activity and total phenolic content

Antioxidant activity was significantly improved by vermicompost treatments (Fig. 1A). The highest fruit antioxidant activity was obtained by application of 10 t/ha vermicompost (80.7%), while the lowest value was recorded in the control (73.4% ; Fig. 1A). These findings are in agreement with those reported by Donghong *et al.* (2010). A number of factors (light intensity, temperature and cultivar) can influence total antioxidant capacity of plant tissues; furthermore, the type of soil and content of humic compounds in the soil can have a decisive effect: in a way that the higher the content of humic compounds in soil, the stronger antioxidant activity (Rimmer, 2006). Therefore, plant growth media and organic fertilizers can influence antioxidant content (Ahn *et al.*, 2005). The decreases in antioxidant capacity, when the concentration of vermicompost in the medium approached 15 t/ha, could be as a result of the high concentrations of soluble salts in the manure vermicompost, poor porosity or poor aeration, which negatively

affect root growth and proliferation and result in reduced antioxidant capacity and yield. Accordingly, Atiyeh *et al.* (2000) showed decrease in yield and fruit antioxidant activity in tomato plants at substitution rates of pig manure vermicomposts greater than 60% of the medium. Furthermore, Shiralipour *et al.* (1992) reported similar plant responses to substitution of container media with composted urban waste. In their experiment, plant growth and antioxidant capacity were decreased by increased proportion of the compost in the medium.

Vermicompost application significantly changed total phenolic content. Pepper plants grown in the lowest level of vermicompost (5 t/ha) had the most total phenolic content (95.2 mg/ 100 g), while the least value was observed in the control (71.6 mg/ 100 g; Fig. 1B). However, the differences between vermicompost

treatments were not significant (Fig. 1B). These results are in agreement with those observed by Asami *et al.* (2003) and Estiarte *et al.* (1994). It has been reported that plants cannot simultaneously allocate resources to growth and defence. There is competition between plant proteins and phenolics in plants for the common precursors involved in their biosynthesis (Riipi *et al.* 2002). These results led us to presume that pepper plants may benefit from vermicompost fertilizer for their protein synthesis and growth development. Also, organic acids and organic fertilizers (such as vermicompost) act as precursors or activators of phytohormones and growth substances and secondary compounds in plants (Vernieri *et al.*, 2006). On the other hand, the extra C could allocate for synthesis of C-based secondary compounds like phenolics in plants that treated with the organic fertilizers (Toor *et al.*, 2006).

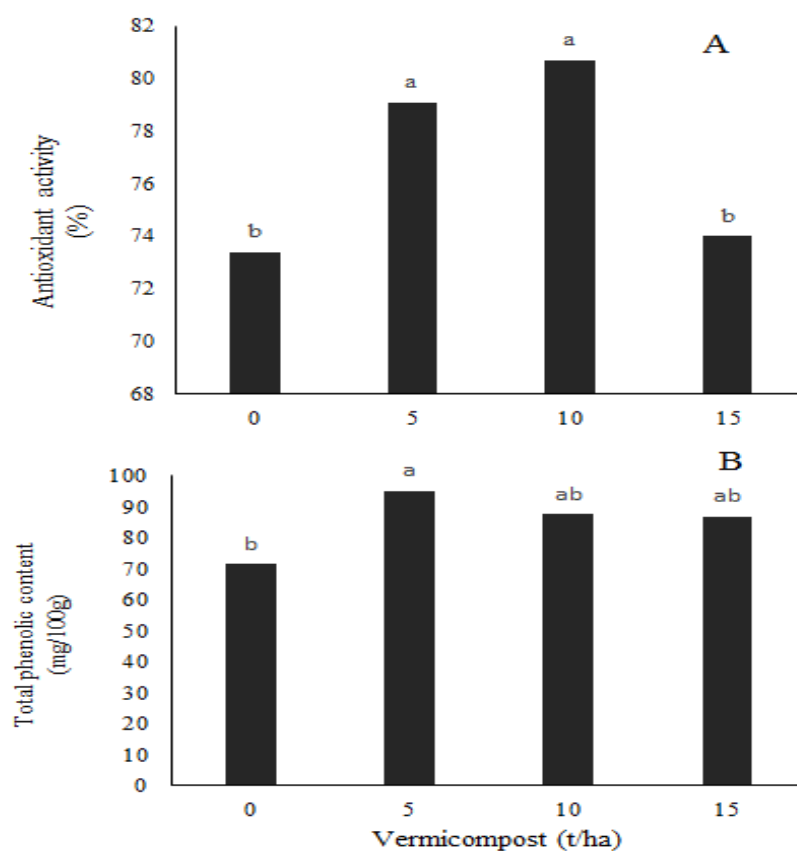


Fig. 1. Effect of application of vermicompost in soil on antioxidant activity (A) and total phenolic content, (B) of pepper

Total flavonoid content

It was found out that the effect of vermicompost on total flavonoid content was positive and significant (Table 3). The treatment with 15 t/ha vermicompost was resulted in higher concentration of total flavonoids (159 mg/ 100 g) than the other treatments. However, no significant differences were found among vermicompost treatments. The results were similar to the findings of Donghong *et al.* (2010) who showed a significant increase in total

flavonoid content in response to vermicompost application in Chinese cabbage. Moreover, Mitchell *et al.* (2007) found that organic crop management practices can cause increase in the flavonoids content in tomatoes and increase in the levels of all antioxidants that analysed in strawberry. Therefore, our study confirmed the results obtained from previous studies that showed positive influence of vermicompost on total flavonoid content.

Table 3. Effect of application of vermicompost in soil on fruit antioxidant compounds of pepper

Vermicompost (t/ha)	Fruit yield (Kg/m ²)	Total flavonoid content (mg/100 g)	carbohydrate content (mg/g)	pH
0 (Control)	14.69c	100.3 ^b	173.5 ^b	5.18 ^b
5	21.87a	147.3 ^a	194.0 ^{ab}	5.32 ^b
10	18.75abc	131.0 ^{ab}	229.4 ^a	5.66 ^a
15	20.22ab	159.0 ^a	182.2 ^b	5.23 ^b

Same letters within each column indicates no significant difference among treatments at 5% levels.

Carbohydrate content

Applications of vermicompost were resulted in a significant increase in carbohydrate content when compared to its content in the control. However, application of high amounts of vermicompost (especially with 15 t/ha) caused a decrease in carbohydrate content (Table 3). The obtained results for carbohydrate content were in agreement with Parthasarathi *et al.* (2008) who showed that applying vermicompost increases carbohydrate content of plants. Wang and Lin (2002) have reported that carbohydrate content and total soluble solids in strawberry fruits were positively correlated. They confirmed that sugar and organic acids are important for the sensory quality of fruits, i.e., fruits with low sugar and acid content taste unsavoury.

pH, titratable acidity and total soluble solid

Fruit pH was significantly affected by vermicompost treatments as shown in Table 3. The highest value for pH was observed in fruits of plants treated with 10 t/ha vermicompost, while the lowest fruit pH was observed in control. This result is

in the line with the findings of Giovanni *et al.* (2011). Citric acid is the primary organic acid found in most fruits, pH of fruit is correlated with acidity and acid content (Wang and Lin, 2002). Fruits with low pH value (grown in organic fertilizers) have more citric acid (Wang and Lin, 2002). Additionally, fruit with low pH is more suitable for ripening and have better shelf life (Hernandez *et al.* 2005). Results presented in Table 4 indicate that vermicompost significantly affected titratable acidity of the fruits. Among vermicompost treatments, the highest titratable acidity was obtained in fruits of plants treated with 15 t/ha of vermicompost (9.8 g/L), while the lowest titratable acidity was obtained in control (7 g/L). However, no significant differences were found among vermicompost treatments. Our results regarding effects of vermicompost application on titratable acidity of fruits can be supported by the findings obtained by Maria *et al.* (2008). It is likely that in plants supplied with organic fertilizer to maintain the C:N ratio, the extra C can be used for the production of organic acids

like citric acid and malic acid, which are responsible for the acidity of the fruit (Toor *et al.*, 2006). Therefore, our data confirmed the results obtained from previous studies which indicated that application of vermicompost can increase levels of organic acids in pepper. Higher total soluble solid was observed in the plants treated with vermicompost treatments. However, this difference was not statistically different from those in control treatment (Table 4). The highest total soluble solid was observed in the highest level of vermicompost treatment (15 t/ha), while the lowest value was observed in the control. Rajbir *et al.* (2008) indicated that vermicompost treatments can significantly change the total soluble solid of fruit.

Ascorbic acid contents and fruit firmness

Application of vermicompost significantly influenced ascorbic acid contents (vitamin C) in pepper fruits (Table 4). The maximum vitamin C content was observed in the highest level of vermicompost application (15 t/ha) with 165 mg/ 100 g, while the minimum value was recorded at control with 102 mg/ 100 g. Vitamin C levels in vegetables depend on several factors, including cultivar, plant nutrition, production practice and maturity (Antonio *et al.*, 2007). Donghong *et al.* (2010) and Rajbir *et al.* (2008) found that vermicompost can increase the levels of vitamin C in plants. Organic fertilisation has been resulted in low fruit yield with high ascorbic acid content in tomatoes, whereas mineral or mineral+organic

fertilizers resulted in a high yield of fruit with low ascorbic acid contents (Dumas *et al.*, 2003). Furthermore, it was reported that tomatoes grown in 100% vermicompost have low concentration of ascorbic acid than plants grown in other media; the highest concentration of ascorbic acid was recorded in plants grown in 40% vermicompost (Paula *et al.*, 2007). Although, ascorbic acid is one of the components of antioxidant capacity but, it has been reported that zinc and manganese concentrations have key roles in enabling ascorbic acid oxidase enzyme (Bybordi and Malkouti, 2007), since vermicompost has sufficient amount of these elements, therefore, application of vermicompost in the soil (especially high amount vermicompost) resulted in improvement of elements uptake and concentration in plant, and as a result of increased the activity of the ascorbic acid oxidase enzyme the amount of vitamin C in plants will increase. Therefore, the level of ascorbic acid was increased by higher level of vermicompost (15 t/ha).

Fruit firmness was significantly higher in vermicompost treatments than control (Table 4). The highest fruit firmness was observed in plants treated with 15 t/ha vermicompost (9.15 N), while the lowest fruit firmness was in control (5.85 N). This result is in agreement with Riahi *et al.* (2009) and Mccollum *et al.* (2005) who reported that organically-grown tomato fruit are generally firm. Firm fruits do not lose too much juice when sliced and are less susceptible to physical damage in shipping.

Table 4. Effect of application of vermicompost in soil on fruit quality characteristics of pepper

Vermicompost (t/ha)	Titrateable acidity (g/L)	Total soluble solid (°Brix)	vitamin C (mg /100 g)	Fruit firmness (N)
0 (Control)	7.0 ^b	4.25 ^a	102 ^b	5.85 ^c
5	8.4 ^{ab}	4.50 ^a	143 ^{ab}	8.70 ^a
10	7.7 ^{ab}	4.40 ^a	120 ^{ab}	6.75 ^b
15	9.8 ^a	4.85 ^a	165 ^a	9.15 ^a

Same letters within each column indicates no significant difference among treatments at 5% levels.

In conclusion, antioxidant activities, fruit yield and quality of pepper can be affected by application of different levels of vermicompost. Although, there were no significant differences among vermicompost levels on almost all cases of variables, however, fruit antioxidant compounds, yield and quality of fruits were improved by application of low levels of vermicompost in the soil. Therefore, application of vermicompost can be suggested as an easy bio-treatment for improving pepper fruit quality and yield.

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