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Effect of cow manure on fruit quality and antioxidant activities of sweet pepper (*Capsicum annuum* L.)

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Abstract—**To determine the effect of cow manure on antioxidant compounds and fruit quality of sweet pepper (*Capsicum annum* L.), an experiment was conducted in the open field. Cow manure was supplied in four levels (0, 5, 10, and 15 t ha^{-1}). The results indicated that fruit quality factors were influenced by cow manure. The pH significantly increased in response to cow manure treatments and the highest values were obtained from the most level of cow manure treatment (15 t ha^{-1}). Moreover, the titratable acidity of the fruit was significantly lower when treated in cow manure. It was observed that cow manure applied to at 10 t ha^{-1} resulted in the highest total soluble solids contents of the fruit, while the lowest values were recorded in the control. Also, cow manure at the lowest level (5 t ha^{-1}) had the highest ascorbic acid contents. Cow manure treatments positively affected fruit antioxidant compounds. Cow manure applied to at 15 t ha^{-1} resulted in the highest antioxidant activities, and carbohydrate contents, while the lowest values were recorded in the control. Total flavonoid contents significantly increased in response to cow manure treatments and the highest values were obtained from the 5 t ha^{-1} of cow manure treatment. Therefore, these results indicated that cow manure has a strong effect on the quality and antioxidant compounds of sweet pepper plants under field conditions.**

Keywords-antioxidant activities; carbohydrate; flavonoid; fruit quality

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1. Introduction

Bell pepper (*Capsicum annuum* L.) commonly known as Sweet pepper or Shimla mirch is an essential solanaceous vegetable crop cultivated throughout the world. It occupies a pride place among the vegetables in Indian cuisine because of its savory taste and pleasant flavor coupled with rich ascorbic acid and other minerals and vitamins [49]. Pepper is a good source of several antioxidant compounds. For example, pepper fruit contains more than 20 different carotenoids, abundant phenolic compounds (including flavonoids), and vitamin C [8, 31, 45]. India contributes almost one-fourth part of world production of capsicum with covered 0.885 million ha and annual mean production of 0.9 million tons with standard productivity of 1266 kg ha^{-1} [3]. According to the Food and Agriculture Organization (FAO), the production of global chili and peppers was 3.45×10^7 tons in 2016 and had an increasing tendency in recent years. China, Mexico, and Turkey are the major producers, the productions of which were 1.75×10^7, 2.74×10^6, and 2.46×10^6 tons, respectively [16].

Fertilization is the main method for the utilization of livestock manure, which is an important biomass material [24]. Untreated livestock manure can affect the soil, crops, and environment, eventually, impacting human health through the food chain [44]. Cow manure, containing a great amount of phosphorus, nitrogen, and potassium, has become a major environmental pollutant and endangers human health due to insufficient utilization. Aerobic composting could reinstate organics in livestock manure and minimize their negative effects by bacteria and fungi [33, 34, and 41].

Many studies have found that peppers from organic production are characterized by a higher content of biologically active compounds [20, 22, 47, and 55]. Similarly, Ibrahim et al. [26] observed that the use of organic manure enhanced the production of total phenolics, flavonoids, ascorbic acid, saponin, and glutathione content in *Labisia pumila* Benth. & Hook. f., compared to the use of inorganic manure. Daneshian et al. [10] reported the positive effect of cow manure (30 t ha^{-1}) on the quantitative and qualitative yield of basil in Guilan conditions. Therefore, in this study, we determine the influence of cow manure application on quality and antioxidant compounds of sweet pepper under field conditions.

2. Material and methods

The research was performed 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). A sample of soil (0-30 cm depth) was taken with a drill once the site has had prepared for cultivation. The sample was analyzed for chemical and physical attributes using standard laboratory procedures as explained by Mylavarapu & Kennelley [40], and data are showed in Table 1. The experimental farm was cleared, plowed, harrowed, and divided into plots. Pepper seeds (*Capsicum annuum* L.) were established in large trays with a 1:1 mixture of sand and peat (1:1 v/v) within a greenhouse. Irrigation was performed after sowing when necessary. Seven-week-old sweet pepper plants were hand-transplanted into well-prepared beds in the field. The plants were spaced at 50 and field 35 cm (respectively) between rows and plants on the row. All necessary cultural practices and plant protection measures were followed uniformly for all the plots during the entire period of the experiment.

Table1. Soil characteristics of experimental field

N (%)	P (ppm)	K (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)	pH	OM (%)	Silt (%)	Sand (%)	Clay (%)	Texture
0.101	15.7	184	4.42	1.06	17.0	1.02	7.68	1.46	53	25	22	Silty loam

Cow manure was applied at four levels (treatments): Cow0= 0, Cow1= 5, Cow2= 10, and Cow3= 15 t ha^{-1}. Cow manure was applied and mixed with soil from the top 15 cm layer to form experimental beds. The four experiment treatments were arranged in a completely randomized block design (CRBD) with three replications. So, the field experiment consisted of 15 unit plots.

Pepper fruit was harvested at the red mature stage. There were three plots per treatment, and three replicates per plot were prepared. Each replicates comprised of twenty peppers, which were harvested from ten different randomly selected plants. The fruit was weighed and washed with distilled water. Part of the samples was directly used for some analyses (pH, titratable acidity, total soluble solids, and ascorbic acid contents) and the other part was freeze-dried and ground for antioxidant analysis determination and stored at -18 ˚C before chemical analysis started.

Sweet pepper fruit from each treatment was cut into small slices and pooled. Samples were homogenized in a blender and portions of the homogenate were used to determine the fruit quality. Titratable acidity (TA) was determined by titration with 0.1 N NaOH until pH= 8.1 was reached and reported as g L^{-1} of citric acid fresh weight using citric acid as a control [25]. The pH amount of fruit was measured with a pH meter at 20 ˚C. Total soluble solids contents (TSS) were specified at 20 ˚C with a refractometer and reported as ˚Brix. Vitamin C or ascorbic acid contents were evaluated by the classical titration method using 2, 6-dichlorophenol indophenol solution, and reported as mg 100 g^{-1} fresh weight [38].

Methanol extracts of freeze-dried fruit were prepared for the determination of antioxidant activity and total phenolic contents. Weighed pepper samples (5 g) were located in a glass beaker and homogenized with 50 mL of methanol at 24 ˚C overnight. The homogenate was filtered and next centrifuged at 6000 rpm for 15 min. The free radical scavenging activity of the samples was defined using the 2,2,-diphenyl-2-picryl-hydrazyl (DPPH) method of Turkmen et al. [59]. A fractional 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 vortex mixed for 30 s and then to stand at room temperature in the dark for 20 min. The absorbance was determined at 517 nm, using a spectrophotometer (Bio Quest, CE 2502, UK). The antioxidant activity was estimated using the following equation: Antioxidant activity (%) = 1 - A Sample (517 nm) / A Control (517 nm) ×100. The total phenolic contents in methanol extracts were determined using Folin– Ciocalteu's reagent [53]. Each methanol extract solution with 0.5 mL was mixed with 6 mL of distilled water and 0.5 mL of Folin–Ciocalteu's phenol reagent. Then 5 min, 2 mL of 20 g L^{-1} sodium carbonate solution was added and the mixture was vortexed vigorously. The same method 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 a spectrophotometer. Results were expressed as mg of gallic acid equivalents 100 g^{-1} on the dry weight.

The flavonoids contents were determined spectrophotometrically using a method based on the formation of a flavonoid– aluminum complex [65]. Each sample with 2 g was extracted with 10 mL methanol for 24 h. One milliliter 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 was also added to the mixture, followed by the addition of 2.1 mL distilled water. Absorbance at 510 nm was read immediately. Quercetin was chosen as a standard and the levels of total flavonoid contents were determined in triplicate and expressed as quercetin equivalents in mg 100 g^{-1} on the dry weight.

Carbohydrate contents were measured according to the method of Yemm and Willis [64] 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 of extract and 5 mL of anthrone reagent which was boiled at 100 °C for 30 minutes. Absorbance was determined at 620 nm. The carbohydrate contents are expressed as mg g^{-1} on the dry weight. Data were analyzed using SAS (SAS Institute, 2000) and means were compared through Duncan's multiple range test (DMRT) at a 5% level of confidence.

3. Results and discussion

3.1. pH of fruit

Fruit pH was significantly affected by cow manure treatments as shown in Table 2. A comparison of the means indicated that there was a significant difference between the levels of cow manure. So that 15 t ha^{-1} of cow manure had the maximum fruit pH (6.89) and control treatment had the minimum (5.94) (Table 2).

This result is the same trend with the findings of Mauromicale et al. [36], and Toor et al. [57]. The pH of fruit is associated with acidity and acid contents and citric acid is the primary organic acid found in most fruit [62].

3.2. Titratable acidity

According to the results of sweet pepper, the effect of fertilizer treatments on fruit acidity was significant. Among cow manure treatments, the highest fruit acidity (10.53 g L^{-1}) was obtained in the control treatment and the lowest (7.66 g L^{-1}) in the treatment of 15 t ha^{-1} of cow manure was obtained (Table 2).

Shehata et al. [50] and Singh et al. [51] reported in separate experiments that vermicompost fertilizers did not have a significant effect on the acidity of the strawberry fruit, which did not agree with our results. It is likely that to maintain the C: N ratio in the plants supplied with organic fertilizer, the extra C may have been used for the production of organic acids like citric acid and malic acid, which are responsible for the acidity of fruit [57].

3.3. Total soluble solids

The contents of total soluble solids in sweet pepper were affected by fertilizer treatments, and the use of cow manure showed a significant increase in the total soluble solids of the fruit compared to the control. The maximum total soluble solids contents of fruit (5.87 °Brix) in the treatment of 10 t ha^{-1} of cow manure and the minimum (4.82 °Brix) were observed in the control (Table 2).

The average recorded for TSS exceeded by at least 27.36% to the average value reported by Tzortzakis et al. [60] at the fruit of pepper cv. Oregon, in evaluating the effects of municipal solid waste compost (MSWC) mixed with soil, in different ratios, without fertigation. Additionally, this average was similar to the mean value of 4.26 ˚Brix, determined by Aminifard et al. [2] in the fruit of sweet pepper cv. California Wonder, developed under in field, whose fertilization consisted of four levels of compost (0, 5, 10, and 15 t ha^{-1}) and they concluded was that fruit harvested from plants that obtained compost fertilizer had significantly higher TSS than those harvested from the mineral manure plots. similarly, the mean of 4.16 ˚Brix was analogous to an average of 4.6 ±

0.3 and 4.7 ± 0.4 ˚Brix, recorded by Chassy et al. [4] in the fruit of bell peppers, cv. California Wonder and Excalibur, respectively, grown under organic situation over a 3-year period. Equally, the mean of TSS found in this experiment was relatively low in comparison to the range, 5.2 to 6.6 ˚Brix, obtained by Abu-Zahra [1] in a study of sweet pepper fruit, cv. Barotte, produced in conventional and organic systems. Similar behavior was reported by Fawzy et al. [17] with sweet pepper cv. California Wonder, whose TSS ranged between 5.2 and 6.6 ˚Brix, developed with a different mixture of mineral nitrogen, organic (chicken manure) as well as biofertilizer (Microbin and Biogen), under field conditions. The differences that were registered in the contents of TSS could be due to differences between the used cultivars. The possible reason depicted in the increase in TSS may be due to differences in mineralization, continuous availability of more nutrients in higher amounts, and better utilization by plants [7]. Another possible reason for TSS may be the release of fixed nitrogen, hence increasing the concentration and availability of nutrients in the root zone [17].

3.4. Ascorbic acid contents

Application of cow manure significantly influenced ascorbic acid contents (vitamin C) in sweet pepper fruit, and there was a significant difference between treatments. Cow manure at the lowest level (5 t ha^{-1}) had the highest (140.60 mg 100 g^{-1}) and control treatment had the lowest contents of vitamin C (90.00 mg 100 g^{-1}) (Table 2).

Our results conform to those of Taiwo et al. [56], who showed that compost application at different concentrations improved vitamin C of fruit. Kim et al. [31] found significantly higher contents of vitamin C in organic than in conventional pepper fruit. Wang et al. [61] and Singh et al. [52] also found that vermicompost can increase the levels of vitamin C in plants. Furthermore, it was reported that tomatoes grown in 100% vermicompost have a 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 [48]. An increase in ascorbic acid contents in the fruit with the application of vermicompost is supported by the findings of Densilin et al. [11] and Chauhan [5], who reported high ascorbic acid contents in the fruit of chili supplied with organic fertilizers. Also, higher ascorbic acid contents were reported in tomato with the application of vermicompost [37]. Besides, the results of Zaller [66] in the tomato plant and Singh et al. [51] in the strawberry plant showed that with the application of vermicompost, as well as its various levels, increased the contents of vitamin C in the fruit. The increase in ascorbic acid may be due to the slow but continuous supply of all major and micronutrients, which might have helped in the assimilation of carbohydrates and turn synthesis of vitamin C [28]. The increased activity of ascorbic acid oxidase enzyme in the presence of micronutrients may be concerned with another reason for the increase in ascorbic acid contents [35]. Vitamin C levels in vegetables depend on numerous factors, including cultivar, plant nutrition, production practice, and maturity [45]. Therefore, our investigation confirmed previous results that the level of vitamin C in organically grown sweet peppers was consistently higher than that in conventionally grown sweet peppers.

Table 2 .The effect of cow manure on the qualitative traits in sweet pepper (California wonder cultivar)

Treatments	pH	Titratable acidity (g L^{-1})	Total soluble Solid (˚Brix)	vitamin C (mg 100 g^{-1})
Cow 0 (Control)	5.94^{b}	10.53^{a}	4.82^{b}	90.00^{b}
Cow 1 (5 t ha^{-1})	6.04^{b}	9.80^{a}	4.93^{b}	140.60^{a}
Cow 2 (10 t ha^{-1})	6.59^{a}	8.40^{b}	5.87^{a}	129.33^{a}
Cow 3 (15 t ha^{-1})	6.89^{a}	7.66^{b}	5.20^{ab}	107.33^{b}

Different letters within columns indicate significant differences among treatments ($P \leq 0.05$)

3.5. Antioxidant activities

According to the results, the effect of fertilizer treatments on the percentage antioxidants of sweet pepper fruit was significant. The results of comparing the averages showed that the maximum rate of this index (80.30%) in the treatment of 15 t ha^{-1} of cow manure and the minimum (59.90%) was obtained in the control treatment (Table 3).

Cow manure with a positive effect on the physical and chemical properties of the soil as well as the plant's ability to access more nutrients increases the antioxidant activity of mortar [42]. Following this result, the researchers reported that the highest antioxidant contents in thyme were due to the use of animal manure [15]. Also, increasing the content of antioxidants in sage has been reported under the influence of animal manure use [58]. In accord with Díaz-Méndez et al. [12], the use of vermicompost has proven its potential as an alternative nutritional source for organic production of cucumber (*Cucumis sativus* L.) with high antioxidant capacity, under greenhouse conditions. Furthermore, Hallmann and Rembialkowska [21] determined that an organic growing system, compared with the conventional system, increased the level of antioxidant compounds in fruit of sweet bell pepper. Other research had similar findings of this higher antioxidant activity, which recorded when organic fertilization compared to mineral fertilization in fresh jujubes [63]. Reganold et al. [46] also reported that the use of compost had a significant effect on the antioxidant contents of strawberry fruit. Antioxidant activity of bell peppers, and other fruit and vegetables, depends on numerous factors counting production techniques used (plant growth regulators, date of harvest, etc.), genetic environmental condition (light, water, temperature, and nutrient availability), and postharvest storage conditions [14, 27].

3.6. Total phenolic contents

The results showed that the studied studies did not have a significant effect on the total phenol contents of sweet pepper fruit (Table 3).

3.7. Total flavonoid contents

Data from Table 3 showed that the application of cow manure significantly increased the total flavonoid contents of sweet pepper fruit. The treatment with 10 t ha^{-1} cow manure resulted in a higher concentration of total flavonoids (131.185 mg 100 g^{-1}) than the other treatments.

The results were similar to the findings of Wang et al. [61], who presented a significant increase in total flavonoid contents in response to vermicompost use in Chinese cabbage. Moreover, Mitchell et al. [39] found that organic crop management practices can cause an increase in the flavonoids content in tomatoes and an increase in the levels of all antioxidants that analyzed in strawberry. The results of Karimi et al. [30] also showed that the application of 5 t ha^{-1} of cow manure increases the flavonoids content of *Ziziphora cliniopodiodes*. Our results agreement with Häkkinen and Törrönen [19], who reported the addition of organic fertilizer had a significant effect on total flavonoid. Hargreaves et al. [23] also showed that the content of flavonoids in the fruit increased with the use of organic fertilizers. These reports confirm the results of this study. So, our study confirmed the results obtained from previous studies that showed a positive effect of cow manure on total flavonoid content. Researchers showed that the high content of flavonoids in plants is due to the role of organic fertilizers in the biosynthesis of substances that induce the path acetate Shkmyk and thus produce more flavonoids and phenolics [54]. According to the results of many researchers, flavonoids are among the secondary metabolites whose biosynthetic pathways are affected by environmental conditions and fluctuate during plant development and environmental conditions [32]. It also found that soil fertility and the use of organic fertilizers had a positive effect on plant flavonoid levels, which researchers report confirms this [32].

3.8. Carbohydrate contents

Data in sweet peppers showed that when cow manure was used, the contents of fruit carbohydrate increased significantly compared to control. However, the application of high amounts of cow manure (especially with 15 t ha^{-1}) with 176.08 mg g^{-1} caused an increase in carbohydrate contents (Table 3).

The obtained results were in agreement with Karakurt et al. [29] and Dorais et al. [13], who demonstrated that applying organic fertilizers increased the sugar contents of plants. Also, Chen and Avaid [6] pointed out the role of humic acid aid in the enhancement of photosynthesis potential as well as increasing total carbohydrate contents in plants. The obtained results for carbohydrate contents were in agreement with Parthasarathi et al. [43], who showed that applying vermicompost increases the carbohydrate contents of plants. Similar to the results of this experiment, Pérez-López et al. [45] reported that the carbohydrate contents of pepper fruit were affected by the use of organic fertilizer. Copetta et al. [9] and Gutiérrez-Miceli et al. [18] in an experiment on tomatoes also reported that the use of compost and vermicompost had a significant effect on fruit carbohydrate levels. Wang and Lin [62] have described that total soluble solids and carbohydrate contents in strawberry fruit were positively correlated. They confirmed that sugar and organic acids are important for the sensory quality of fruit, i.e., fruit with low sugar and acid contents taste unsavory.

Table 3 .The effect of cow vermicompost on the antioxidant activity of fruit in sweet pepper (California wonder cultivar)

Treatments	Fruit antioxidant activity (%)	Total phenolic contents (mg 100 g^{-1})	Total flavonoid contents (mg 100 g^{-1})	Carbohydrate contents (mg g^{-1})
Cow 0 (Control)	59.90^{b}	52.33^{a}	96.13^{c}	134.83^{c}
Cow 1 (5 t ha^{-1})	61.96^{b}	63.73^{a}	102.76^{bc}	152.16^{bc}
Cow 2 (10 t ha^{-1})	70.46^{ab}	58.40^{a}	131.18^{a}	158.50^{ab}
Cow 3 (15 t ha^{-1})	80.30^{a}	45.53^{a}	115.15^{ab}	176.08^{a}

Different letters within columns indicate significant differences among treatments ($P \leq 0.05$)

4. Conclusions

The use of organic fertilizers while maintaining the health of the environment can increase the quantitative and qualitative performance of plants. Organically harvested plants have higher levels of antioxidant compounds such as phenol and flavonoids than non-organic crops, as pesticides and non-organic substances have reduced the amount of these compounds, but manure and organic fertilizers used in organic farming increase their value. So, the cow manures enhance the quality of the fruit of pepper developed under field conditions. Moreover, cow manure treatments positively affected fruit antioxidant compounds counting antioxidant activity, total flavonoid, and carbohydrate contents.

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