African Journal of Biotechnology Vol. 11(68), pp. 13179-13185, 23 August, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.1507
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Fulvic acid affects pepper antioxidant activity and fruit quality

M. H. Aminifard[1]*, H. Aroiee[1], H. Nemati[1], M. Azizi[1] and Hawa Z. E. Jaafar[2]

[1]Department of Horticultural Science, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
[2]Department of Crop Science, Faculty of Agriculture, University Putra Malaysia, Selangor, Malaysia.

Accepted 10 August, 2012

Fulvic acid has been considered as a valuable fertilizer for sustainable agriculture. The present investigation was undertaken to evaluate the effect of fulvic acid (FA) on antioxidant compounds and fruit quality of pepper under field conditions. Plants were grown in the Department of Horticulture Farm, Ferdowsi University of Mashhad, Iran (latitude 36° 17′ N, longitude 59° 35′ E and 985 m elevation). The experiment was designed in randomized block design with three replications. Treatments consisted of five levels of fulvic acid (0, 25, 100, 175 and 250 mg kg^{-1}). The results indicate that fruit antioxidant activity, total phenolic, carbohydrate, capsaicin and carotenoids contents were influenced by fulvic acid, but total flavonoid and ascorbic acid contents were not affected significantly by fulvic acid treatments applications. FA applied at 25 mg kg^{-1} resulted in the highest carbohydrate content, lycopene and β-carotene contents, while the lowest values were recorded in the control. Fulvic acid treatments positively affected fruit quality (total soluble solids and titratable acidity); total soluble solids and titratable acidity significantly increased in response to FA treatments. These results confirm that the use of fulvic acid have a positive effect on antioxidant activity and quality of hot pepper under field conditions.

Key words: Fulvic acid, antioxidant activity, quality, pepper.

INTRODUCTION

Humic and fulvic acids are the most significant constituents of organic matter in both soils and municipal waste compost, and have a relevant role in the cycling of many elements in the environment and in soil ecological functions (Senesi et al., 1996). The fulvic acid is an important fraction of soil organic matter, an important portion of the dissolved organic C pool in soils (Van-Hees et al., 2005), and generally show higher chemical and physico-chemical activity compared with humic acid (Stevenson, 1994). Also, fulvic acids are recognized to play an important role in the acid–base buffering capacity of soil, and in the retention and release, biological availability, and mobility of metal ions and organic chemicals in soil (Senesi and Miano, 1995).

*Corresponding author. E-mail: aminifard_mh55@yahoo.com. Tel: +989151608215. Fax: +985118787430.

Fruits and vegetables are good sources of natural antioxidants, containing many different antioxidant components that provide protection against free radicals and are associated with health-promoting properties (Velioglu et al., 1998). Pepper is considered an excellent source of bioactive nutrients. Ascorbic acid (vitamin C), carotenoids and phenolic compounds are its main antioxidant constituents (Marin et al., 2004). The levels of vitamin C, carotenoids and phenolic compounds in peppers and other vegetables depend on several factors, including cultivar, agricultural practice (organic or conventional), maturity and storage conditions (Lee and Kader, 2000). Asami et al. (2003) reported significantly higher total, phenolics in marionberries grown with organic agricultural methods as compared with conventional method. Olsson et al. (2006) also demonstrated that strawberries grown organically had higher levels of all antioxidants including total phenolic, ellagic acid, and flavonols, than the conventionally grown strawberries.

Table 1. Soil characteristics of experimental field.

Texture	Clay (%)	Sand (%)	Silt (%)	OM (%)	pH	Zn (ppm)	Mn (ppm)	Cu (ppm)	Fe (ppm)	K (ppm)	P (ppm)	N (%)
Silty loam	22	25	53	1.46	7.68	1.02	17	1.06	4.42	184	15.7	0.101

Moreover, it was reported that cabbages from organic management presents higher phenolics content than cabbages from the conventional management (Sousa et al., 2005). Karakurt et al. (2009) reported that humic acid application significantly influenced total carbohydrate content and total yield of pepper. Also, increasing nitrogen fertilisation has been found to decrease ascorbic acid concentration in several fruits and vegetables (Lee and Kader, 2000).

Although positive influences of fulvic acid on plant growth and development have been well established for plants, their effects on fruit antioxidant activity and quality have not received much attention. Therefore, in this study, we determined the influence of fulvic acid application on fruit antioxidant activity and quality of pepper under field conditions.

MATERIALS AND METHODS

Plant preparation

The investigation was conducted during the 2011 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). The temperature during the experimental period varied from 16 to 39.1 8°C and relative humidity varied between 55 ± 5.5%. Soil sample (0-30 cm depth) was taken with auger after the site had been prepared for cultivation. The sample was analyzed for physical and chemical properties using standard laboratory procedures described by Mylavarapu and Kennelley (2002) and data are shown in Table 1. The experimental field was cleared, ploughed, harrowed and divided into plots. Pepper seeds (*Capsicum annuum* L.) were established in a greenhouse in large trays with a 1:1 mixture of sand and peat (1:1 v/v) on 17 March 2011. Irrigation was done after sowing when necessary. Seven-week-old pepper plants were hand-transplanted into well-prepared beds in the field on 28 April 2011, and the plants were spaced at 50 and 35 cm between rows and plants on row, respectively. During the growing period, plants were drip irrigated as needed. There were no insecticide and fungicide treatments in experiments; weeds were controlled by hand. All necessary cultural practices and plant protection measures were followed uniformly for all the plots during the entire period of experimentation.

Experimental design and treatments

The experiment was arranged in a completely randomized block design (CRBD) with five treatments and three replications. Fulvic acid fertilizer (containing 70.0% fulvic acid) consisted of five levels: FA0= 0, FA1=25, FA2=100, FA3= 175 and FA4= 250 mg kg^{-1} (providing 0, 50, 200, 350 and 500 kg ha^{-1} FA). Drip irrigation system was used. Fulvic acid solutions prepared in distilled water were applied as a drench to the plant's root area four times during the vegetative period at 10-day intervals three weeks after planting.

Measurements

Harvesting was performed at red mature stage starting from on the 24 August 2011. There were three plots per treatment, and three replicates per plot were collected. Each replicate comprised twenty peppers harvested from ten different randomly selected plants. Fruits were weighed and washed with distilled water. Part of the samples was immediately used for some analyses (titratable acidity, pH, total soluble solids and ascorbic acid) and the other part was freeze-dried and ground for antioxidant analysis determination and stored at -18°C before chemical analysis commenced.

pH, total soluble solids, titratable acidity and ascorbic acid content

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 value of fruit was measured with 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^{-1} of citric acid fresh weight using citric acid as a control (Horwitz, 1975). Total soluble solids content (TSS) was determined at 20°C with a refractometer and reported as °Brix. Ascorbic acid contents (vitamin C) was measured by classical titration method using 2,6-dichlorophenol indophenol solution, and expressed as mg 100 g^{-1} fresh weight (Miller, 1998).

Antioxidant activity and total phenolic (extraction and analysis)

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 the 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 vortexed for 30 s and left to stand at room temperature in the dark for 20 min. Next, the absorbance was measured at 517 nm, using a spectrophotometer (Bio Quest, CE 2502, UK). The antioxidant activity (%) was calculated using the following equation: $1 - A_{\text{Sample (517 nm)}} / A_{\text{Control (517 nm)}} \times 100$.

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 of distilled water and 0.5 ml of Folin–Ciocalteu's phenol reagent. After 5 min, 2 ml of 20 g L^{-1} sodium carbonate solution was added and the mixture was vortexed vigorously. 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 was measured at 750 nm using spectrophotometer. Results were expressed as mg of gallic acid equivalents (GAE) 100 g^{-1} on dry weight.

Total flavonoid content

The flavonoid content was determined spectrophotometrically using a method based on the formation of a flavonoid–aluminium complex (Yoo et al., 2008). Each sample (2 g) was extracted with 10 ml methanol for 24 h. One millilitre 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) aluminium chloride ($AlCl_3$) was added and then at 6 min, 2 ml of 1 M sodium hydroxide (NaOH) were 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 content were determined in triplicate and expressed as quercetin equivalents in mg 100 g^{-1} on dry weight.

Carbohydrate content

Carbohydrate content was measured according to the method of Yemm and Willis (1954), 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 min. Absorbance was determined at 620 nm; the carbohydrate content was expressed as mg g^{-1} on dry weight.

Capsaicin content

Capsaicin content in the samples was estimated by spectrophotometric measurement of the blue coloured component formed as a result of reduction of phosphomolybdic acid to lower acids of molybdenum (Sadasivam and Manikkam, 1992). Two grams of freeze-dried sample was extracted with 10 ml of dry acetone using pestle and mortar. The extract was centrifuged at 10,000 rpm for 10 min and 1 ml of supernatant was pipetted into a test tube and evaporated to dryness in a hot water-bath. The residue was then dissolved in 0.4 ml of NaOH solution and 3 ml of 3% phosphomolybdic acid. The contents were shaken and allowed to stand for 1 h. Afterward, the solution was filtered to remove any floating debris and centrifuged at 5000 rpm for 15 min. Absorbance was measured for the clear blue solution, thus obtained, at 650 nm using reagent blank (5 ml of 0.4% NaOH + 3 ml of 3% phosphomolybdic acid). Capsaicin content calculated from the standard curve was expressed as mg kg^{-1} on dry weight.

Carotenoids (lycopene and β–carotene)

Sixteen millilitres of acetone–hexane (4:6) solvent were added to 1.0 g of pepper homogenate and mixed in a test-tube. Automatically, two phases were separated and an aliquot was taken from the upper solution for measurement of optical density at 663, 645, 505 and 453 nm in spectrophotometer. Lycopene and β-carotene contents were calculated according to the Nagata and Yamashita (1992) equations:

Lycopene (mg 100 ml^{-1} of extract) = $-0.0458 * A_{663} + 0.204 * A_{645} + 0.372 * A_{505} - 0.0806 * A_{453}$.

β -Carotene (mg 100 ml^{-1} of extract) = $0.216 * A_{663} - 1.22 * A_{645} - 0.304 * A_{505} + 0.452 * A_{453}$.

Lycopene and β -Carotene were finally expressed as mg kg^{-1} on dry weight using the fruit water content.

Statistical analysis

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

RESULTS

The data on analysis of variance as shown in Tables 2 and 3 indicate that fulvic acid fertilization significantly affect pepper antioxidant activity and fruit quality.

Effect of fulvic acid on fruit antioxidant activity and quality of pepper

Fulvic acid applications significantly affected fruit antioxidant activity (Table 4). Results indicate that fruit antioxidant activity decreased with increasing fulvic acid levels. The most fruit antioxidant activity was obtained from 100 mg kg^{-1} FA with 81.7%, while the least fruit antioxidant activity was recorded at 250 mg kg^{-1} FA with 67.3%. The results also showed increasing fulvic acid concentrations resulted in a significant enhancement in total phenolic content as compared with the control, but as the concentration of fulvic acid increased further, the total phenolic content decreased, especially in response to 175 and 250 mg kg^{-1} FA treatments (Table 4). Results indicated that addition of fulvic acid did not change the fruit total flavonoid content, although the pepper plants grown in the 175 mg kg^{-1} FA treatment had most total flavonoid content with 199 mg 100g^{-1} (Table 4). As shown in Table 4, fulvic acid application significantly increased carbohydrate content. The highest carbohydrate content was achieved at 25 mg kg^{-1} FA with 192.9 mg g^{-1}, while the least carbohydrate content was observed at control with 132.5 mg g^{-1}.

The levels of FA application significantly influenced capsaicin content (Table 5). Fruit capsaicin content increased with increasing fulvic acid levels, and the highest fruit capsaicin content was obtained from the more elevated level of fulvic acid treatment (FA4) with 453.9 mg kg^{-1}, while control (without fulvic acid application) had the lowest capsaicin content with 201 mg kg^{-1}. It was also found out that fulvic acid affected on lycopene and β –carotene content (Table 5). Carotenoids contents were improved by all fulvic acid treatments. The maximum values of lycopene and β–carotene contents were obtained in FA1 (25 mg kg^{-1}) treatment with

Table 2. Analysis of variance of selected parameters of pepper (antioxidant activity) as affected by fulvic acid fertilizer treatments.

Source of variance	df	Antioxidant activity	Total phenolic content	Total flavonoid content	Carbohydrate content	Capsaicin content	Lycopene	β –Carotene
Block	2	24.89^{Ns}	10.58^{Ns}	299.1^{Ns}	181.36^{Ns}	4003^{Ns}	134^{Ns}	914^{Ns}
Fulvic acid	4	97.12**	162.14*	2125.4^{Ns}	1687.24**	29538**	3362**	7302**
Block × fulvic acid	6	73.04**	111.62*	1516.6 ^{Ns}	1185.28*	21026**	2286**	5173**
Error	8	10.67	26.25	1383.2	239.89	2326.91	127.3	674.35

Ns = Non significant; * and ** = Significant at 5% and 1% probability level, respectively.

Table 3. Analysis of variance of selected parameters of pepper (fruit quality) as affected by fulvic acid fertilizer treatments.

Source of variance	df	pH	Titratable acid	Total soluble solid	Vitamin C
Block	2	0.005^{Ns}	5.72^{Ns}	1.200 ^{Ns}	3197.9**
Fulvic acid	4	0.0536*	33.03**	0.527*	200.7^{Ns}
Block × fulvic acid	6	0.0359^{Ns}	23.92**	0.751 ^{Ns}	1199.8*
Error	8	0.0230	2.59	0.279	276.05

Ns = Non significant; * and ** = significant at 5 and 1% probability level, respectively.

Table 4. Effect of fulvic acid (FA) on fruit antioxidant activity of pepper.

Treatment (fulvic acid)	Fruit antioxidant activity (%)	Total phenolic content (mg 100 g^{-1})	Total flavonoid content (mg 100 g^{-1})	Carbohydrate content (mg g^{-1})
FA0 (control)	70.1 ± 6.9^{cd}	40.3b ± 1.5^{c}	137.8 ± 23.4^{a}	132.5 ± 5.8^{c}
FA1 (25 mg kg^{-1})	77.1 ± 1.7^{ab}	50.8 ± 6.4^{a}	164.1 ± 45.0^{a}	192.9 ± 10.0^{a}
FA2 (100 mg kg^{-1})	81.7 ± 3.3^{a}	48.7 ± 6.7^{ab}	151.7 ± 52.5^{a}	157.8 ± 11.0^{bc}
FA3 (175 mg kg^{-1})	74.6 ± 1.8^{bc}	36.0 ± 2.3^{c}	199.0 ± 11.73^{a}	142.8 ± 18.9^{bc}
FA4 (250 mg kg^{-1})	67.3 ± 1.0^{d}	34.6 ± 4.5^{c}	132.0 ± 22.04^{a}	170.6 ± 22.8^{ab}

Within each column, same letter indicates no significant difference between treatments at 5% levels.

191 and 316 mg kg^{-1}, while the minimum values were recorded in the control with 121 and 196 mg kg^{-1}, respectively. Data cited in Table 6 show a significant effect of fulvic acid on the fruit titratable acidity of pepper. Titratable acidity increased with increasing fulvic acid levels, and the highest fruit acidity (26.5 g L^{-1}) was obtained from the FA3 (175 mg kg^{-1} FA), while the least value (19.7 g L^{-1}) was related to the control. In addition, the pH of the fruits was significantly lower in fulvic acid treatments (Table 6). The least pH value was obtained from the application of 250 mg kg^{-1} FA with 4.60, while the most value was in FA1 (25 mg kg^{-1} FA) with 4.98. Our result indicated that total soluble solid (TSS) of fruit was affected by fulvic acid (Table 6). The maximum total soluble solid was observed in FA2 (100 mg kg^{-1} FA) with 10.75 °Brix, while the minimum TSS was recorded at

Table 5. Effect of fulvic acid on capsaicin content and Carotenoids (lycopene, β –carotene) of pepper.

Treatment (fulvic acid)	Capsaicin content (mg kg $^{-1}$)	Lycopene (mg kg $^{-1}$)	β –Carotene (mg kg $^{-1}$)
FA0 (control)	201.0 ±1 2.6^{d}	121.3 ± 7.0^{b}	196 ±15.2^{c}
FA1 (25 mg kg^{-1})	238.6 ± 18.6^{cd}	191.6 ± 16.6^{a}	316 ± 18.1^{a}
FA2 (100 mg kg^{-1})	349.4 ± 56.5^{b}	122.0 ± 5.4^{b}	246 ± 45.0^{b}
FA3 (175 mg kg^{-1})	297.8 ± 71.5^{bc}	174.3 ± 3.5^{a}	277 ± 22.8^{ab}
FA4 (250 mg kg^{-1})	453.9 ± 68.0^{a}	179.3 ± 16.5^{a}	310 ± 22.1^{a}

Within each column, same letter indicates no significant difference between treatments at 5% levels.

Table 6. Effect of fulvic acid on fruit quality characteristics of pepper.

Treatments (fulvic acid)	pH	Titratable acidity (g L^{-1})	Total soluble Solid(°Brix)	vitamin C (mg 100 g^{-1})
FA0 (control)	4.93 ± 0.07^{ab}	19.76 ± 2.6^{b}	9.65 ± 0.6^{b}	132 ± 25^{a}
FA1 (25 mg kg^{-1})	4.98 ± 0.21^{a}	20.44 ± 0.8^{b}	10.15 ± 1.1^{ab}	151 ± 22^{a}
FA2 (100 mg kg^{-1})	4.80 ± 0.16^{ab}	25.73 ± 2.8^{a}	10.75 ± 0.7^{a}	132 ± 14^{a}
FA3 (175 mg kg^{-1})	4.74 ± 0.08^{ab}	26.54 ± 0.4^{a}	10.10 ± 0.1^{ab}	141 ± 33^{a}
FA4 (250 mg kg^{-1})	4.66 ± 0.09^{b}	26.09 ± 0.5^{a}	10.50 ± 0.2^{ab}	136 ± 42^{a}

Within each column, same letter indicates no significant difference between treatments at 5% levels.

control with 9.65 °Brix. Vitamin C in fulvic acid treatments was consistently greater, but not statistically different from those in control treatment (Table 6). The highest vitamin C was FA1 with 151 mg 100 g^{-1}, while the least value occurred in the control with 132 mg 100 g^{-1}.

DISCUSSION

In our study, fruit antioxidant activity improved with decreasing fulvic acid levels and FA2 (100 mg Kg^{-1} FA) had the most fruit antioxidant activity. However, no significant difference was found between FA1 and FA2 treatment, which was in agreement with the findings of Jiu et al. (1995) who also indicated that organic fertilizer significantly changed the antioxidant activity. A number of factors (light intensity, temperature and cultivar) can influence total antioxidant capacity of plant tissues and the type of soil and the content of humic compounds (humic acid and fulvic aid) in soil can have a decisive effect: the higher the content of humic compounds in soil, the stronger antioxidant activity (Rimmer, 2006); Therefore, plant growth media and organic fertilizer influence antioxidant concentrations (Ahn et al., 2005). Another hypothesis explaining increases of antioxidant compounds in organic foods is that since insecticide, fungicide and herbicide use is limited in organic agriculture (similarly, in our experiment), plants devote greater resources to fight pathogen attacks, which includes generation of antioxidant compounds (Winter and Davis, 2006). Thus, these findings reveal that fulvic acid had a positive influence on the antioxidant activity pepper fruits.

The statistical analysis of data showed significant effect of fulvic acid on the total phenolic content of fruit and FA1 (25 mg Kg^{-1} FA) had the highest total phenolic content. This result is in agreement with the study of Asami et al. (2003) who showed that the organic fertilizer applications significantly affected total phenolic content. It has been reported that plants cannot simultaneously allocate resources to growth and defence and that there is competition between 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 utilise benefits from fulvic acid fertilizer for their protein synthesis and growth development. On the other hand, organic acids (such as fulvic acid) and amino acids act as precursors or activators of phytohormones and growth substances, as well as secondary compounds in plants (Vernieri et al., 2006). However, since there was enough light for regular photosynthetic rates in our experiment, the extra carbon (C) could have been allocated for synthesis of C-based secondary compounds like phenolics in plants treated with the organic fertilisers (Toor et al., 2006). Hence, our study confirmed previous results that organic fertilizers influence the total phenolic content of fruit. Furthermore, the results indicated that addition of fulvic acid did not change the fruit total flavonoid content, although the control had the least total flavonoid content, while FA3 (175 mg kg^{-1} FA) had the most value. Conversely, our results disagree with those of Hakkinen and Torronen

(2004) who reported addition of organic fertilizer had significant effect on total flavonoid. Also, Mitchell et al. (2007) found that organic crop management practices increased the content of flavonoid in tomatoes. Therefore, this study unconfirmed earlier results that organic fertilizers influenced on total flavonoid content of fruit.

We found that addition of fulvic acid to soil also improved the carbohydrate content of fruit and FA1 (25 mg kg^{-1} FA) had the highest carbohydrate content other than treatment. The obtained results were in agreement with the results of Karakurt et al. (2009) and Dorais et al. (2008) who showed that applying organic fertilizers increased sugar content of plants. Wang and Lin (2002) have reported that carbohydrate content and total soluble solids in strawberry fruits were positively correlated and sugar and organic acids are important for the sensory quality of fruits; that is - fruits with low sugar and acid content taste flat. Therefore, it could be concluded from the results that fulvic acid had a positive effect on carbohydrate content of pepper.

Additionally, there were positive effect due to use of fulvic acid on pepper capsaicin content that value of FA4 treatment (250 mg kg^{-1} FA) was 125% higher than the control. Wang et al. (2010) reported that organic fertilizer increased capsaicin content of pepper. Also, Bajaj et al. (1979) reported an increase in capsaicin content of pepper pods with increasing phosphorus rates. The pungency levels in peppers are determined by the genetic make-up of the plant and how it interacts with the environment (Estrade et al., 1999). Practical concerns regarding hot pepper production have spurred interest in methods to environmentally enhance capsaicinoid production (Charles and Denis, 1996). Warm weather, poor soil, dry climates and high night temperatures are suggested to favour the production of high capsaicin concentrations (Charles and Denis, 1996). Thus, capsaicin content was affected by nutritional fertility and our experiment was at warm weather and dry climate; therefore, it seems these conditions along high rate of fulvic acid increased capsaicin content.

As shown in Table 5, FA application significantly increased lycopene content and β –carotene of pepper fruits so that FA1 (25 mg kg^{-1} FA) had the highest values (lycopene content and β –carotene) than other treatments. Carotenoids accumulation in plant tissue appears to be shaped by the physiological, genetic and biochemical attributes of a plant species, as well as by environmental growth factors such as light, temperature, and fertility (Kopsell and Kopsell, 2006). Organic fertilization seems to improve carotene accumulation. Archana et al. (2009) reported that total carotenoids increased with application vermicompost tea treatments.

Perez et al. (2005) indicated a significantly higher content of total carotenoids in organically grown sweet peppers than in integrated and conventional peppers. Thus, our study confirmed previous results that humic substances influenced on β –carotene content of fruit.

By increasing the fulvic acid fertilizer rate, fruit titratable acidity of pepper increased so that the most fruit acidity was produced in FA3 treatment (175 mg kg^{-1} FA) with 26.5 g L^{-1}, however, no significant difference was found between three treatments: FA2, FA3 and FA4. Similar result was also reported by Dogan and Demir (2004). It is likely that in order 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 (Toor et al., 2006). Therefore, our data confirmed previous reports that organic fertilizers increased levels of organic acids in pepper fruits. Unlike fruit titratable acidity, fulvic acid application significantly decreased the pH of pepper fruits, especially in FA4 (250 mg kg^{-1} FA). However, the difference between FA2, FA3 and FA4 treatments was not statistically significant. This result is the same trend with the findings of Giovanni et al. (2011). The pH of fruit is correlated with acidity and acid content and citric acid is the primary organic acid found in most fruits (Wang and Lin, 2002). On the other hand, fruits with low pH value (grown in organic fertilizers) indicate more citric acid, which is beneficial for human consumption (Wang and Lin, 2002). Additionally, fruits with low pH are more suitable for ripening while it also improves shelf life (Hernandez et al*.,* 2005).

We found that total soluble solid was significantly affected by FA application and the highest TSS was gained from FA2 (100 mg kg^{-1} FA), while the lowest was found in the control. Azarmi et al. (2009) observed that fruits harvested from plants that received organic fertilizer had significantly greater total soluble solid (TSS) than those harvested from the mineral fertilizer plot. The improvement of fruit quality may be attributed to better growth of plant at different rate of organic fertilizer, which might have favoured the production of better quality fruit (Rajbir et al., 2008). Vitamin C in fulvic acid treatments was consistently greater, especially in FA1 (25 mg kg^{-1} FA), but not statistically different from those in the control treatment. Our results disagree with those of Giovanni et al. (2011) and Dorais et al. (2001) who determined that humic substance application at different concentrations improved tomato vitamin C. Vitamin C levels in vegetables depend on several factors, including cultivar, plant nutrition, production practice and maturity. It can be concluded from the results that fulvic acid did not have a positive influence on the vitamin C content of pepper fruits.

Conclusion

Fruit antioxidant activity and quality of pepper were affected by different fulvic acid levels. Although there were no significant differences between FA levels in almost all cases of variables, however, fruit antioxidant compounds and quality of fruits were improved with lower fulvic acid level (25 mg Kg^{-1} FA) except capsaicin content that increased with highest fulvic acid treatment. Therefore, the usage fulvic acid could be suggested as bio-treatment for improvement in the fruit antioxidant activity and quality of pepper.

REFERENCES

Ahn T, Oke M, Schofield A, Paliyath G (2005). Effects of phosphorus fertilizer supplementation on antioxidant activities of tomato fruits. J. Agric. Food Chem. 53:1539 -1545.

Archana P, Theodore JK, Ngyuen VH, Stephen TT, Kristen AK (2009). Vermicompost extracts influence growth, mineral nutrients, phytonutrients and antioxidant activity in pak choi (*Brassica rapa* cv. Bonsai, Chinensis group) grown under vermicompost and chemical fertiliser. J. Sci. Food Agric. 89:2383-2392.

Asami DK, Hong YJ, Barrett DM, Mitchell AE (2003). Comparison of the total phenolic and ascorbic acid content of freeze-dried and dirdried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. J. Agric. Food Chem. 51:1237-1241.

Azarmi R, Giglou MT, Hajieghrari B (2009). The effect of sheep-manure vermicompost on quantitative and qualitative properties of cucumber (*Cucumis sativus* L.) grown in the greenhouse. Afr. J. Biotechnol. 8(19):4953-4957.

Bajaj KL, Kaur G, Singh J, Brar JS (1979). Effect of nitrogen and phosphorus levels on nutritive values of sweet peppers (*Capsicum annuum* L.). Plant Foods Hum. Nutr. 28(4):287-292.

Charles DJ, Dennis RD (1996). Nitrogen and potassium fertility affects Jalapeno pepper plant growth pod yield and pungency. HortScience 31(7):1119-1123.

Dogan E, Demir K (2004). Determination of yield and fruit characteristic of tomato crop grown in humic acids-added aggregate culture in greenhouse conditions. National Vegetable Symposium. Canakkale, Turkey, pp. 218-224.

Dorais M, Ehret DL, Papadopoulos AP (2008). Tomato (*Solanum lycopersicum*) health components: from the seed to the consumer. Phytochem. Rev. 7:231-250.

Estrade B, Pomar F, Diaz J, Merino F, Bernal MA (1999). Pungency level in fruits of the padron pepper with different water supply. Sci. Hortic. 81:385-396.

Giovanni M, Angela GL, Antonino LM (2011). The effect of organic supplementation of solarized soil on the quality of tomato fruit. Sci. Hortic. 129:189-196.

Hernandez-Perez T, Carrillo-Lopez A, Guevara F, Cruz- Hernandez A, Paredes-Lopez O (2005). Biochemical and nutritional characterization of three prickly pear species with different ripening behavior. Plant Food 8(19):4953-4957.

Hakkinen SH, Torronen AR (2000). Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: influence of cultivar, cultivation site and technique. Food Res. Int. 33:517-524.

Horwitz W (1975). Official methods of analysis of the Association of Official Analytical Chemist (AOAC), USA, Washington.

Jiu CF, Qi YD, Sheng WQ (1995). Physiological effects of humic acid on drought resistance of wheat. Chin J. Appl. Ecol. 6(4):363-367.

Karakurt Y, Unlu H, Halime U, Padem H (2009). The influence of foliar and soil fertilization of humic acid on yield and quality of pepper. Acta Agric. Scand., Sect. B. 59(3):233-237.

Kopsell DA, Kopsell DE (2006). Accumulation and bioavailability of dietary carotenoids in vegetable crops. Trends Plant Sci. 11:499-507.

Lee SK, Kader AA (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Biotechnol. 20:207-220.

Marin A, Ferreres F, Tomas-Barberan FA, Gil MI (2004). Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). J. Agric. Food Chem. 52:3861-3869.

Miller D (1998). Food chemistry: a laboratory manual, USA, New York: John Wiley and Sons Press.

Mitchell AE, Hong Y, Koh E, Barrett DM, Bryant DE, Denison RF(2007). Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. J. Agric. Food Chem. 55:6154-6159.

Mylavarapu RS, Kennelley DE (2002). Uf/if as extension soil testing laboratory (ESTL): Analytical procedures and training manual. Institute of food and agricultural sciences. Univ. Florida Gainesville, U.S.A.

Nagata M, Yamashita I (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. J. Jpn. Food Sci. Soc. 39:925-928.

Olsson ME, Andersson CS, Oredsson S, Berglund RH, Gustavsson K (2006). Antioxidant levels and inhibition of cancer cell proliferation in vitro by extracts from organically and conventionally cultivated strawberries. J. Agric. Food Chem. 54:1248-1255.

Perez-Lopez AJ, Lopez-Nicolas JM, Nunez-Delicado E, Del Amor FM, Carbonell-Barrachina AA (2005). Effects of agricultural practices on color, carotenoids composition, and minerals contents of sweet peppers. J. Agric. Food Chem. 5:8158-8164.

Rajbir S, Sharma RR, Satyendra K, Gupta RK, Patil RT (2008). Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (*Fragaria* × *ananassa*.). Bioresour. Technol. 99:8507-8511.

Riipi M, Ossipov V, Lempa K, Haukioja J, Ossipova S, Pihlaja K (2002). Seasonal changes in birch leaf chem. are there tradeoffs between leaf growth and accumulation of phenolics? Oecologia.130: 380- 390.

Rimmer DL (2006). Free radicals, antioxidants, and soil organic matter recalcitrance. Eur. J. Soil Sci. 57: 91-94.

Sadasivam S, Manikkam A (1992). Capsaicin. In Biochemical methods for agricultural sciences, India, New Delhi: Wiley Eastern Press.

Senesi N, Miano TM, Brunetti G (1996). Humic-like substances in organic amendments and effects on native soil humic substances. In: Piccolo, A. Ed, Humic Substances in Terrestrial Ecosystems. USA, New York: Elsevier.

Senesi N, Miano TM (1995). The role of abiotic interactions with humic substances on the environmental impact of organic pollutants. Natural Anthropogenic Organics. 1:311-335.

Singleton VL, Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. Am. J. Enol. Vitic. 16:144-158.

Sousa C, Valentao P, Range J, Lopes G, Pereira JA, Ferreres F, Seabra RM, Andrade PB (2005). Influence of two fertilization regimens on the amounts of organic acids and phenolic compounds of tronchuda cabbage (*Brassica oleracea* L. Var. costata DC). J. Agric. Food Chem. 53:91280-9132.

Stevenson FJ (1994). Humus Chemistry: Genesis, Composition, Reactions, USA, New York Wiley- Interscience Press.

Toor RK, Geoffrey PS, Anuschka H (2006). Influence of different types of fertilisers on the major antioxidant components of tomatoes. J. Food Compos. Anal. 19:20- 27.

Turkmen N, Sari F, Velioglu YS (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. J. Food Chem. 93:713 -718.

Van-Hees PA, Jones DL, Finlay R, Godbold DL, Lundstrom US (2005). The carbon we do not see the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil. Biol. Biochem. 37: 1-13.

Velioglu YS, Mazza G, Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food. Chem. 46:4113-4117.

Vernieri P, Borghesi E, Tognoni F, Ferrante A, Serra G, Piaggesi A (2006). Use of biostimulants for reducing nutrient solution concentration in floating system. Acta Hortic. 718:477-484.

Wang SY, Lin S (2002). Compost as soil supplement enhanced plant growth and fruit quality of strawberry. J. Plant Nutr. 25:2243-2259.

Wang WJ, Zhu Y, Wang HS (2010). Effect of Different Amount of Organic Fertilizer in the Yield of Organic Vegetables. North Hortic. p. 17.

Winter CK, Davis SF (2006).Organic foods. J. Food Sci. 71(9):117-124.

Yemm EW, Willis AJ (1954). The Estimation of carbohydrate in the plant extract by anthrone reagent. J. Biochem. 57:508-514.

Yoo KM, Lee CH, Lee H, Moon BK Lee CY (2008). Relative antioxidant and cytoprotective activities of common herbs. J. Food Chem. 106:929-936