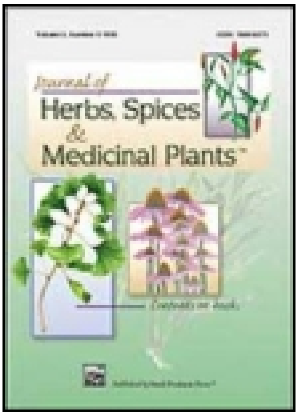

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Effect of Humic Acid on Antioxidant Activities and Fruit Quality of Hot Pepper (*Capsicum annuum* L.)

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*The effect of humic acid (HA) on antioxidant compounds and fruit quality of hot pepper (*Capsicum annum var. *Red chili) was determined in an open-field study. Pepper plants were treated with HA (0, 25, 100, 175, and 250 mg.kg^{-1}). HA treatments affected antioxidants in the fruits (antioxidant activity, total flavonoid, capsaicin, lycopene, and β-carotene). There was no difference in total phenolic and carbohydrate between HA and control treatments. It was observed that HA applied at 100 mg kg^{-1} resulted in the highest capsaicin and lycopene contents, and the lowest values were observed in control group. Total soluble solids and titratable acidity increased in response to HA treatments, and the highest values were obtained from the highest HA treatment (250 mg kg^{-1}).*

KEYWORDS *Humic acid, antioxidant activities, red chili*

INTRODUCTION

Humic and fulvic acids constitute 65% to 70% of organic matter in soils and are the subject of study in soil chemistry, fertility, and plant physiology

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as well as environmental sciences because of the multiple roles played by these acids in plant growth and fruit quality (1). Pepper (*Capsicum annum*, Solananceae) is known for its versatility as it is consumed both as fresh vegetables or dried for use as a spice (2). It is an excellent source of bioactive nutrients and antioxidant constituents including ascorbic acid, carotenoids, and phenolic compounds (3). Antioxidant concentrations in fruit are dependent upon environmental and genetic factors (4). Increasing nitrogen fertilization decreased ascorbic acid concentration in several fruits and vegetables (5). While plant growth media and fertilizer regime may influence antioxidant concentrations (6), Karakurt et al. (7) reported that humic acid (HA) application influenced total carbohydrate content and total yield of pepper. Asami et al. (8) reported higher total phenolics in marion berries grown with organic agricultural practices compared to conventional method. Strawberries grown organically had higher levels of antioxidants including total phenolic, ellagic acid, and flavonols than conventionally grown strawberries (9). Cabbages from organic management had higher phenolics content than those from conventional management (10). Although positive influences of HA acid on plant growth and development have been established in plants, their effects on fruit antioxidant activity and quality have not received much attention. The aim of this study was to assess the efficacy of HA on antioxidant compounds and fruit quality of red pepper under field conditions.

MATERIAL AND METHODS

Field and Plant preparation

The study was conducted during the 2010 growing season at the experimental field of agricultural faculty, Ferdowsi University of Mashhad, Iran. Soil sample (0–30 cm depth) was taken with an auger after the site had been prepared for cultivation. Total N was determined by the regular Kjeldahl distillation method, and P, Fe, Cu, Mn, and Zn were measured by atomic absorption spectroscopy (11). Soil K was measured by a flame photometer (PEP7/C, Jenway, Bibby Scientific Limited, Straffordshire, UK). The pH of soil samples was measured in distilled water [soil: water, 1:2.5 (v/v) ratio] after shaking the solution for 30 min by means of a pH meter (CD 510, WPA) fitted with a glass electrode. The soil mixture used in the study had a loamy texture; pH 7.68, N 0.1%; 15.7 ppm P; and 184 ppm K. The soil used contained 1.02 ppm Zn, 17 ppm Mn, 4.42 ppm Fe, and 1.06 ppm Cu. The experimental field was cleared, plowed, harrowed, and divided into plots. Pepper seeds (*Capsicum annuum* var. Red chili.) were established in a greenhouse in large trays with 1:1 mixture of sand and peat (1:1 v/v) and irrigated after sowing and as needed. Seven-week-old pepper plants were hand-transplanted into well-prepared beds in the field. The plants were spaced at 50 cm and 35 cm between rows and plants, respectively. All cultural practices and plant protection measures followed were similar in all the plots during the entire period of experimentation.

Experimental Design and Treatments

The experiment was laid out in a completely randomized block design with five treatments and three replications. HA fertilizer (prepared from leonardite containing 80.0% HA (JH Biotech, Inc. Co., Ltd. Ventura, California, USA) consisted of five levels—$HA_{0=0}$, $HA_{1=25}$, $HA_{2=100}$, $HA_{3=175}$, and $HA_{4=250}$ mg kg^{-1} (providing 0, 50, 200, 350, and 500 Kg ha^{-1} HA, respectively). HA solutions prepared in distilled water were applied as a drench to the plant root area four times during the vegetative period at 10-day intervals 3 weeks after planting.

Measurements

Pepper fruits were harvested at the red mature stage. There were three plots per treatment, and three replicates per plot were collected. Each replicate was composed of 20 peppers harvested from 10 randomly selected plants. Fruits were weighed and washed with distilled water. Part of the samples were immediately used for analysis of titratable acidity, pH, total soluble solids, and ascorbic acid, and the other part was freeze-dried, ground, and stored at –18°C until chemical analysis for antioxidant analysis, which commenced in 30 days.

pH, Total Soluble Solids, and Titratable Acidity and Ascorbic Acid Contents

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. pH value of fruit was measured with a pH meter (Jenway, 3320, Bibby Scientific Limited, Straffordshire, UK) at 20°C. Titratable acidity was determined by titration with 0.1 N NaOH until reaching pH 8.1 and reported as $g.L^{-1}$ of citric acid fresh weight using citric acid as control (12). Total soluble solids content (TSS) was determined at 20°C with a refractometer (RFM340, Bellingham and Stanley Ltd., Turnbridge wells, UK) and reported as °Brix. Ascorbic acid concentration was measured by extracting the fruit homogenate (10 mL) with metaphosphoric acid 3% (50 mL) and acetic acid solution 8% (50 mL) and then titrated against 2,6-dichlorophenol-indophenol (13).

Antioxidant Activities and Total Phenolic (Extraction and Analysis)

Methanol extracts of freeze-dried fruits were prepared for determination of antioxidant activity and total phenolic content. Pepper fruit samples (5 g) were placed in a glass beaker and homogenized overnight with 50 mL methanol at 24°C, were filtered, and then were centrifuged at 6,000 rpm for 15 min. Free radical scavenging activity of the samples was determined by 2,2,-diphenyl-2-picryl-hydrazyl method (14). Total phenolic content of pepper samples was determined by the Folin-Ciocalteu method (15).

Total Flavonoid Content

Flavonoids were determined spectrophotometrically based on the formation of a flavonoid–aluminum complex (16). A 2-g sample was extracted with 10 mL methanol for 24 h. One mL of the extracts was added to a 10-mL volumetric flask, made up to 5 mL with distilled water, and 0.3 mL of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.6 mL of 10% (w/v) $AlCl_3$ was added and then, at 6 min, 2 mL of 1M NaOH was added to the mixture, followed by 2.1 mL distilled water and absorbance read at 510 nm.

Carbohydrate Content

Carbohydrate content was measured using anthrone reagent as described earlier (17).

Capsaicin Content

Capsaicin content in the samples was estimated by spectrophotometric measurement of the blue component formed as a result of reduction of phosphomolybdic acid to lower acids of molybdenum (18).

Carotenoids Contents (Lycopene and β–Carotene)

Sixteen mL of acetone-hexane (4:6) solvent was added to 1.0 g of pepper homogenate and mixed in a test tube. When the two phases separated, an aliquot was taken from the upper solution for measurement of optical density at 663, 645, 505, and 453 nm in a spectrophotometer (BioQuest CE 2502, Cecil Instruments Ltd., Cambridge, UK), and the lycopene and β-carotene contents were calculated (19).

Statistical Analysis

Data were analyzed using SAS (SAS Institute, 2000) and means compared by Duncan's multiple range test ($p < 5\%$).

RESULTS

Fruit antioxidant activity increased with increasing HA levels (Table 1). The highest fruit antioxidant activity was obtained from the highest HA treatment (250 mg.Kg^{-1}) with 85.7%, while the control had the lowest fruit antioxidant activity with 70.1%. Total phenolic concentration was not affected by the HA concentrations although HA_3 had the highest total phenolic content (see Table 1).

The highest flavonoid concentration was achieved at the lowest HA (HA_1) with 192.3 mg.100 g^{-1}; no difference was found among the four treatments: HA_1, HA_2, HA_3, and HA_4 (see Table 1). The carbohydrate content did not differ between treatments. However, the highest level of HA (250 mg.Kg^{-1}) increased the carbohydrate content compared to control and had the highest carbohydrate content (133.9 mg.g^{-1}).

The control group had the lowest capsaicin content (168 mg.Kg^{-1}), and the HA_2 treatment with 281.7 mg.Kg^{-1} produced the highest capsaicin (Table 2). There was no difference between HA treatments.

Carotenoids were improved by all HA treatments (see Table 2). The maximum value of lycopene and β-carotene were obtained in HA_2 (100 mg.Kg^{-1}) and HA_3 (175 mg.Kg^{-1}) treatments, respectively, while the minimum values were recorded in the control.

TABLE 1 Effect of humic acid on fruit antioxidant compounds of hot pepper (*Capsicum annuum*, Var. Red Chilli)

Treatments (Humic acid)	Fruit antioxidant activity (%)	Total phenolics (mg.100 g^{-1})	Flavonoids (mg.100 g^{-1})	Carbohydrate (mg.g^{-1})
HA_0 (control)	70.1c	40.3a	137.8 b	113.9a
HA_1 (25 mg.Kg^{-1})	78.9b	40.7a	192.3a	96.4a
HA_2 (100 mg.Kg^{-1})	72.7c	39.2a	163.6ab	114.7a
HA_3 (175 mg.Kg^{-1})	83.8ab	41.5a	181.6ab	115.3a
HA_4 (250 mg.Kg^{-1})	85.7a	39.8a	140.9ab	133.9a

Note: Mean separation by Duncan's multiple range test. Within each column, means followed by the same letter are not different. $p < 5\%$.

TABLE 2 Effect of humic acid on capsaicin and carotenoids of hot pepper (*Capsicum annuum*, Var. Red Chili)

Treatments (Humic acid)	Capsaicin (mg.Kg^{-1})	Lycopene (mg.Kg^{-1})	β—Carotene (mg.Kg^{-1})
HA_0 (control)	168.8b	122d	149b
HA_1 (25 mg.Kg^{-1})	244.1ab	147c	302a
HA_2 (100 mg.Kg^{-1})	281.7a	225a	300a
HA_3 (175 mg.Kg^{-1})	230.9ab	172b	312a
HA_4 (250 mg.Kg^{-1})	261.7a	157c	165b

Note: Mean separation by Duncan's multiple range test.Within each column, means followed by the same letter are not different. $p < 5\%$.

TABLE 3 Effect of humic acid on fruit quality characteristics of hot pepper (*Capsicum annuum*, Var. Red Chili)

Treatments (Humic acid)	pH	Titratable acidity (g L^{-1})	Total soluble solid (°Brix)	vitamin C (mg.100 g^{-1})
HA_0 (control)	4.98a	24.7b	9.90cd	126a
HA_1 (25 mg.Kg^{-1})	5.04a	25.5b	9.60d	122a
HA_2 (100 mg.Kg^{-1})	4.70b	32.9a	10.15bc	131a
HA_3 (175 mg.Kg^{-1})	4.73b	33.1a	10.60b	146a
HA_4 (250 mg.Kg^{-1})	4.76b	34.6a	11.25a	117a

Note: Mean separation by Duncan's multiple range test. Within each column, means followed by the same letter are not different. $p < 5\%$.

Titratable acidity increased with increasing HA levels, and the highest level of HA (250 mg.Kg^{-1}) produced the highest fruit acidity of 34.6 g.L^{-1}), while the control had the lowest at 24.7 g.L^{-1} (Table 3). The pH of the fruits was lower in HA treatments compared to control. and the lowest pH value was obtained from the application of HA_2 treatment with 4.70 (see Table 3).

Among HA treatments, the lowest TSS was obtained in HA_1 (25 mg.Kg^{-1}) with 9.60° Brix, while the highest TSS was produced in the highest level of HA (HA_4) with 11.25° Brix. There was no difference in vitamin C content due to treatments, although HA_3 (175 mg.Kg^{-1}) produced higher vitamin C than the other treatments (see Table 3).

DISCUSSION

Fruit antioxidant activity improved with increasing HA levels, and HA_4 (250 mg.Kg^{-1}) treatment demonstrated the highest fruit antioxidant activity, which is consistent with previous studies that indicated that HA affected the antioxidant activity (20). The type of soil and the content of humic compounds in soil can have a decisive effect: the higher the content of humic compounds in soil, the stronger antioxidant activity (21). Another hypothesis explaining increases of antioxidant compounds in organic foods is that, since insecticide, fungicide, and herbicide use is limited in organic agriculture (as in this study), plants devote greater resources to fight pathogen attacks, including production of antioxidants (22).

Statistical analysis of data showed no effects of HA on total phenolic content of pepper fruit. This is in agreement with studies in organic lettuce (23) and greenhouse tomato (24) production, which showed that nutrient sources did not affected the total phenolic content. In this study, application of HA increased total flavonoid content of fruit compared to control but not within treatments as also reported earlier (25). Contrary to this, other studies reported increased flavonoid in tomatoes and antioxidants in strawberries due to organic crop management practices (26).

Addition of HA to soil did not change the carbohydrate content of fruit, with the highest achieved at the highest HA (HA_4), which was in agreement with those reported by Giovanni et al. (27); however, Karakurt et al. (7) and Dorais et al. (28) indicated that HA affected the carbohydrate content.

There were positive effects due to use of HA on pepper capsaicin content that value of HA_2 treatment, which was 59% higher than control. Pungency in peppers is determined by the genetic makeup of the plant and how it interacts with the environment (29). Practical concerns regarding hot pepper production have spurred interest in methods to environmentally enhance capsaicinoid production (30). Wang et al. (31) reported organic fertilizer increased capsaicin content of pepper. Bajaj et al. (32) reported an increase in capsaicin content of pepper pods with increasing phosphorus rates. Thus, capsaicin content was affected by nutritional fertility, and the present study confirmed previous results that capsaicin increased by organic fertilizer (HA) application.

HA application increased lycopene content and β-carotene of pepper fruits with medium levels of HA (HA_2 and HA_3) producing higher than other treatments (see Table 2). Carotenoid 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 (33). Archana et al. (34) reported that total carotenoids increased with application vermicompost treatments. Hernandez-Perez et al. (35) observed a higher total carotenoids in organically grown sweet peppers than in integrated and conventional peppers.

The highest fruit titratable acidity was obtained with highest HA (250 mg Kg^{-1} HA) treatment. A similar result was also reported by Dogan and Demir (36). It is likely that, in order to maintain the C:N ratio in the plants supplied with organic fertilizer, the additional C may have been used for the production of organic acids such as citric acid and malic acid, which are responsible for the acidity of fruit (24).

Unlike fruit titratable acidity, HA decreased the pH of pepper fruits, especially in HA_2 treatment (4.70), which is in line with earlier findings (27). The pH of fruit is correlated with acidity and acid content, and citric acid is the primary organic acid found in most fruits (37). Conversely, fruits with low pH value (grown in organic fertilizers) indicate more citric acid, which is beneficial for human consumption (37). Additionally, fruits with low pH are more suitable for ripening, as it improves shelf life (35).

The highest TSS was observed in fruits from HA_4 (11.25° Brix), which decreased in HA_1 slightly. Azarmi et al. (38) observed that fruits harvested from plants that received organic fertilizer had significantly greater TSS than those from the mineral fertilizer plot. Improvement of fruit quality may be attributed to better growth of plant with organic fertilizer, which might have favored the production of better quality fruit (39). Wang and Lin (37) reported that TSS and carbohydrate content in strawberry fruits were positively correlated, and sugar and organic acids are important for the sensory quality of fruits (i.e., fruits with low sugar and acid content taste flat). Vitamin C in HA treatments was consistently greater, although not different from those in control. This is not in agreement with other reports (9,12), which studied the effect of HA application on vitamin C in tomato. It is to be noted that vitamin C levels in vegetables depend on several factors, including cultivar, plant nutrition, production practice, and maturity.

In conclusion, although there were no differences between HA levels on almost all variables studied, fruit antioxidant compounds and quality of fruits were improved with HA application at all levels, even at the lowest level. The usage HA could be suggested as bio-treatment for improvement in the fruit antioxidant activities and quality of pepper.

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