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THE EFFECTS OF AMINOETHOXYVINYLGLYCINE AND METHYL JASMONATE ON BIOACTIVE COMPOUNDS AND FRUIT QUALITY OF 'NORTH WONDER' SWEET CHERRY

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Abstract

Background: AVG is an organic ethylene inhibitor. AVG treatments retarded the ripening process, increased fruit sizes and delayed loss of post-harvest fruit flesh firmness by inhibiting ethylene. MeJA is a natural plant growth regulator and plays a regulatory role in various metabolic reactions. Apart from its role in fruit ripening, cell activities like anthocyanin and carotenoid synthesis and inhibitive role in aromatic material formation, chlorophyll and lycopene production.
Materials and Methods: The present study was conducted to determine the effects of pre-harvest aminoethoxyvinylglycine (AVG) and methyl jasmonate (MeJA) treatments on bioactive compounds, mineral nutrients and other fruit quality characteristics of 'North Wonder' sweet cherries variety in 2011. AVG was sprayed on experimental trees at 125 mg L^{-1} dose in two different periods i.e. 3 weeks and 2 weeks before the anticipated harvest date. MeJA was applied 3 week before the anticipated harvest date at a dose of 2240 mg L^{-1}.
Results: MeJA significantly increased fruit weight and geometric mean diameter. AVG significantly decreased fruit weight and flesh/stone ratio, significantly increased L*, chroma and hue angle values. Effects of both AVG and MeJA on flesh firmness were also found to be significant. While soluble solids concentration and pH values significantly decreased with AVG treatment, titrate acidity significantly increased. Both AVG and MeJA treatments significantly decreased total phenolics (TP), total antioxidant capacity (TAC) and total anthocyanin (TA). Effects of AVG on such decreases in TAC and TA were more efficient than MeJA. While the effects of both AVG and MeJA on iron content were significant, effects of only MeJA on nitrogen and phosphor contents were found to be significant.
Conclusion: Growth regulators significantly decreased bioactive compounds. AVG was more effective in such decreases than MeJA.

Key words: Antioxidant, color, flesh firmness, mineral nutrients, phenolics, *Prunus avium* L.

Introduction

Sweet cherry is one the most important stone fruits of Turkey, constituting 19.57% of world production with an annual production capacity of 438 550 tons (FAO, 2011). Turkey with such a high production capacity is the most significant supplier of European Union countries, Russian Federation and Middle East countries in particular. However, short harvest period and shelf life, rapid post-harvest quality losses create significant economic losses both in local and international markets.

Peel color, flesh firmness and fruit size have direct impacts on consumer preferences (Sloulin, 1990). Sweet cherry is a non-climacteric fruit and therefore rapidly losses peel color and flesh firmness because of higher post-harvest respiration rates. Despite some contrary opinions (Remon et al., 2003), various researchers (Estia et al., 2002; Shafiq et al., 2013) reported that such losses in peel color and flesh firmness were related to phenolics, antioxidants, anthocyanins and pectins in fruits (Crisosto et al., 2001). These characteristics vary based on growth period, soil and ecological conditions, plant nutrients, type of production (organic or inorganic), harvest time and other cultural practices (Measham, 2011). Other researchers (Zhang and Whiting, 2011; Shafiq et al., 2013) reported direct impacts in growth regulators on physical, mechanical and bio-chemical properties of the fruits.

Researchers use growth regulators [AVG, gibberellins, jasmonates, prohexadione calcium, synthetic auxins (Chlorophenoxyacetic acid), salicylic acid and 1-methylcyclopropene (1-MCP)] with positive impacts on fruit quality characteristics to supply market-demanded fruits (Zhang and Whiting, 2011).

AVG is an organic ethylene inhibitor. It is well known that ethylene is a hormone which promotes fruit ripening and influences fruit quality. It was reported for various fruits that pre-harvest AVG treatments retarded ripening, increased fruit sizes and delayed loss of post-harvest fruit flesh firmness by inhibiting ethylene biosynthesis (Rath and Prentice, 2004). MeJA is a natural plant growth regulator (Rudell et al., 2005) and plays a regulatory role in various metabolic reactions. It enhances fruit ripening and cell activities like anthocyanin and carotenoid synthesis and inhibiting role in aromatic material formation, chlorophyll and lycopene production (Kondo et al., 2001). Some researchers (Kondo et al., 2001; Wang and Zheng, 2005) also reported that MeJA took place in reactions altering the bioactive compounds of fruits (anthocyanins, carotenoids, phenolic compounds, antioxidants, ascorbic acid and flavonoid contents). Rudell and Mattheis (2009) noticed a synergetic or additive response between ethylene and MeJA for regulation of apple peel pigment synthesis pathway.

The present study was conducted to determine the effects of pre-harvest AVG and MeJA treatments on physico-mechanical, biochemical characteristics and mineral nutrients of 'North Wonder' sweet cherry fruits.

Materials and Methods

Plant material

. Four-years old uniform 18 sweet cherry trees (*Prunus avium* L. cv. 'North Wonder', voucher No. 148-1) trees grafted on Gisela 6 rootstock at Research station of Horticulture, Department of Gaziosmanpasa, University of Agricultural Faculty, 40 ,20 , 02.19" N latitude , 36 , 28' 30.11"E longitude and 623 m) in the middle Black Sea Region of Turkey were selected for the study

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Treatments design and management

The planting density was 4.0 m x 2.0 m. The trees were trained by Vogel Central Leader system and grouped into three blocks of 6 trees in randomized block design based on proximity in orchard and crop load. Standard practices (pruning, fertilization, irrigation and etc.) were regularly implemented.

In each block, 2 trees were selected for control treatment, 2 trees for 2240 mg L^{-1} MeJA (Sigma-Aldrich, USA) treatment and 2 trees for 250 mg L^{-1} AVG treatment. 'ReTain' (ValentBioSciences Crop, Libertyville II, USA) containing 15% AVG was used in AVG treatments. 'ReTain' was sprayed to experimental trees at 125 mg L^{-1} dose in two different periods as of 3 weeks (24 May 2011) and 2 weeks (31 May 2011) before the anticipated harvest date. MeJA was applied 3 week (24 May 2011) before the anticipated harvest date at a dose of 2240 mg L^{-1}. Sylgard-309 [0.05%, (Dow Corning, Toronto, Canada)] surfactant was used in all experimental solutions to improve the effectiveness of the applied solutions. Only the surfactant solution was sprayed to control trees. Solutions were sprayed with a low-pressure hand sprayer in a day without wind and precipitation.

Fruit quality properties

Fruits were manually harvested early in the morning at anticipated harvest date (14th of June, 2011). Harvested fruits were placed into polyethylene bags to prevent water loss and directly transported to laboratory for analyses. Fruit weight, flesh/stone ratio, geometric mean diameter, color characteristics and flesh firmness values were measured over 50 fruits harvested from 2 trees of each treatment (25 fruits from each tree). Fruit and stone weights (g) were measured with a digital balance (±0.01 g) (Radvag PS 4500/C/1, Poland).

By using fruit and stone weights, flesh/stone ratio was calculated with the following equation specified by Mohsenin (1980);

$$\text{Flesh / stone ratio} = \frac{W_f - W_s}{W_s}$$

W_f : fruit weight (g)

W_s : stone weight of the same cherry (g)

Dimensional characteristics [length (L), width (W) and thickness (T)] were measured with a digital caliper (± 0.01 mm) (Model No: CD-6"CSX, Mitutoyo, Japan) and geometric mean diameter was calculated by using the equation of (Dg) = (L. W .T)$^{1/3}$ (Mohsenin, 1980). Color characteristics were measured with a colorimeter (Minolta, model CR–400, Tokyo, Japan) from the mid-section over each side of the fruit. Fruit peel color were expressed as L*, Chroma and hue angle. Chroma was calculated by $(a^{*2}+b^{*2})^{1/2}$ and hue angle by [h^o= $\tan^{-1}$ x b^*/a^*] equations. Chroma value indicates the color saturation. While undertones have low chroma values, vivid colors have high chroma values. Hue angle is a color circle in which red-purple tones have an angle between 0^o - 360^o, yellow has 90^o and blue-green tones have angles between 180^o - 270^o (McGuire, 1992). Flesh firmness (Newton- N) was determined as the maximum force applied to penetrate into the fruit vertically. Measurements were performed in Zwick Z0.5 (Zwick/Roell Z0.5, Germany) universal test device with a maximum capacity of 500 N and 1.8 mm stainless steel tip at 0.5 mm s^{-1} testing speed and until 5 mm penetration.

For soluble solids concentration (SSC), pH and titrate acidity measurements, 45 fruits were harvested from 2 trees of each treatment of each block and fruits were divided into 3 groups each with 15 fruits. Stones of each fruit were removed and fruit juices were extracted with an electrical fruit juice extractor. A digital refractometer (PAL-1, McCormick Fruit Tech., Yakima, Wash., USA) was used to measure SSC (%); a pH meter (Hanna, model HI9321, USA) was used to measure the pH of extracts. For titrate acidity, 10 ml extract was taken from each sample, 10 ml distilled water was added and the value corresponding to consumed sodium hydroxide (NaOH) during the titration with 0.1 mol L^{-1} sodium hydroxide to increase the pH of samples to 8.1 was expressed in malic acid (g malic acid 100 g^{-1}).

Bioactive compounds

For each fruit sample, seeds were removed and homogenized in a standard food blender. Several fruits (50 individual fruits) were used to analyze naturally occurring fruit-to-fruit variation. TP content was measured according to the Singleton and Rossi (1965) procedure. Briefly, fruit slurries were extracted with a buffer containing acetone, water and acetic acid (70:29.5:0.5 v/v) for 2 hours at dark. Samples were replicated three times. Extracts were combined with Folin-Ciocalteu's phenol reagent and water, and incubated for 8 minutes followed by the addition of 7% sodium carbonate. After 2 hr, the absorbance at 750 nm was measured in an automated UV-Vis spectrophotometer (Model T60U, PG Instruments). Gallic acid was used as the standard. The results were expressed as mg Gallic acid equivalents (GAE) kg^{-1} fw.

Trolox equivalent antioxidant capacity (TEAC) method was used to determine total antioxidant capacity. For the standard TEAC assay, 10 mmol/L ABTS (2,2-azino-bis- 3-ethylbenzothiazoline-6-sulfonic acid) was dissolved in acetate buffer and prepared with potassium persulfate as described in Ozgen et al. (2006). The mixture was diluted using an acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700± 0.01 at 734 nm for longer stability (Ozgen et al., 2006). For the spectrophotometric assay, 2.90 mL of the $ABTS^+$ solution and 100 µL of fruit extract were mixed and incubated for 10 min. The absorbance at 734 nm was then determined. The results were expressed in mmol TE kg^{-1} fw. Total anthocyanin levels were measured by pH differential method described in Giusti et al**.** (1999). Sample extracts were combined in a 1:20 ratio (v:v) with potassium chloride and with sodium acetate buffers (pH 1.0 and 4.5, respectively) in separate vessels. After an equilibration period (15 min), the raw absorbance of each solution was measured at 533 and 700 nm. A corrected absorbance value was calculated as [(A520 – A700) pH 1.0 – (A520 – A700) pH 4.5]. The anthocyanin content was calculated using the molar absorptivity (ϵ) and molecular weights (MW) of cyanidin 3-glucoside (ϵ = 26.900; MW = 449.2). Results are expressed as micrograms of cyanidin 3-glucoside equivalents (µg cy-3-glu g^{-1} fw).

Mineral elements

Fruit samples were rinsed through 0.01% HCl solution and distilled water, dried in an oven at 70°C for 2 days and grinded in a hand blender. Grinded samples were then dry ashed in an ashing oven in accordance with the method specified by Kacar and İnal (2008). Resultant extracts were used for phosphorus (P), potassium (K), calcium (Ca), iron (Fe), zinc (Zn) and manganese (Mn) analyzed with ICP-OES (Perkin Elmer-2100DV optima, USA) device. Kjeldahl distillation method was used for nitrogen (N) analysis (Bremner, 1965).

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Statistical analysis

Experiments were carried out in randomized complete block design. All statistical analyses were performed with SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA). Data were analyzed by means of analysis of variance. Main effects and interactions were analyzed and means were compared by Duncan's multiple range tests at a significance level of 0.05.

Results and Discussion

Effects of AVG and MeJA treatments on different fruit characteristics of sweet cherry are provided in Table 1. Compared to control treatment, fruit weight and geometric mean diameter significantly increased with MeJA treatments. Effects of MeJA on flesh/stone ratio were similar to control treatment. Effects of plant growth regulators on fruit quality characteristics were also reported by Fan et al. (1998). Rudel et al. (2005) indicated increased fruit sizes in apples with MeJA through promoting cell division and Shafiq et al. (2011) reported positive effects of MeJA treatments on apple quality characteristics. However, the effects of MeJA on quality characteristics of non-climacteric fruits are not well-known (Mukkun and Singh, 2009).

Table 1: The effects of AVG and MeJA treatments on some fruit characteristics of 'North Wonder' sweet cherry fruit.

Treatment	Fruit characteristics		
	Fruit weight (g)	Geometric mean diameter (mm)	Flesh/stone ratio
Control	7.40 b	22.92 b	17.05 a
250 mg L^{-1}, AVG	6.33 c	22.61 b	13.07 b
2240 mg L^{-1}, MeJA	7.87 a	24.60 a	15.06 ab

n=150 (50 fruit x three replicates) for fruit characteristics. Means in columns with the same letter do not differ, according to Duncan's Multiple Range test, P<0.05.

Compared to the control treatment, fruit weight and flesh/stone ratio significantly decreased with AVG treatments but AVG did not have any significant effects on geometric mean diameter (Table 1). Current findings agree with the findings of Webster et al. (2006) reporting insignificant effects of AVG on geometric mean diameter of sweet cherry. It was also pointed out that rootstock and cultivar genetics, fruit load and environmental conditions had significant effects on fruit size and quality of sweet cherry (Whiting and Lang, 2004).

It was reported that MeJA affected color formation in several climacteric fruits by promoting anthocyanin biosynthesis (Fan et al., 1998). Rudell and Mattheis (2009) claimed that a synergism may exist between MeJA and ethylene for regulation of pigment synthesis pathways in apple peel. However, such a case has not been clearly identified for non-climacteric fruits (Kondo et al., 2001). In the present study, MeJA did not cause a significant change in fruit peel color parameters (Figure 1).

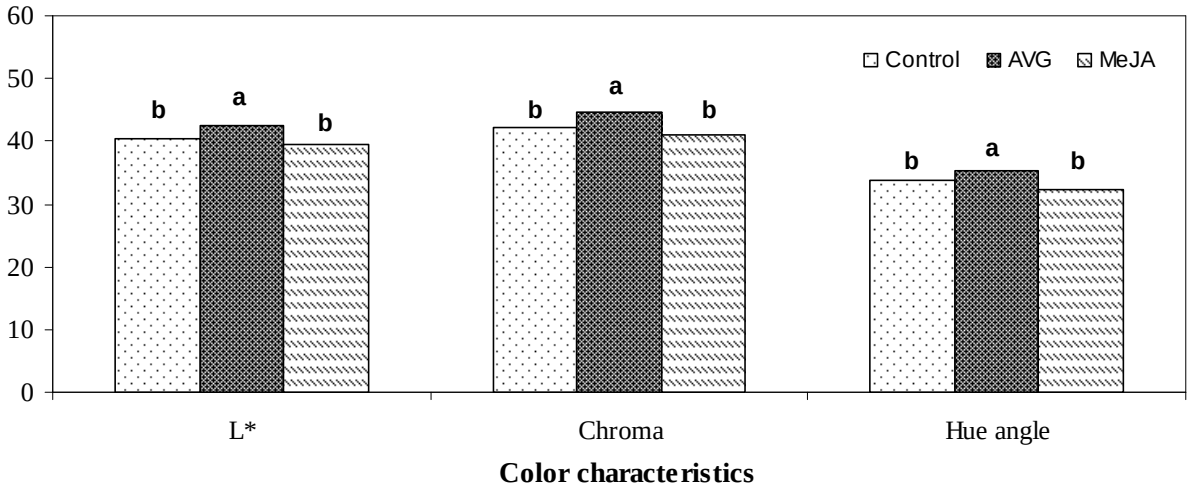

Figure 1: The effects of AVG and MeJA treatments on L*, a*, chroma and hue angle of 'North Wonder' sweet cherry fruit. n=300 (50 fruit x three replicates x two measurements for each fruit) for chromaticity values. Different letter above the bars indicate statistically significant differences, according to Duncan's Multiple Range test, at P<0.05.

L*, chroma and hue angle of insufficiently ripened fruits are usually higher than the values of fully-ripened fruits (Gonçalves et al., 2004). When compared to control treatment, L*, hue angle and chroma values significantly increased with AVG treatments (Figure 1). This situation clearly showed retarding effect of AVG on fruit ripening. Similarly, Webster et al. (2006) stated that AVG treatments retarded ripening and consequently the color formation of sweet cherry. Many researchers working on different fruit species reported that this effect of AVG was likely to be due to its inhibitory effect on ethylene biosynthesis (Jobling et al., 2003; Rath and Prentice, 2004).

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Although the possible role of MeJA in non-climacteric fruits is still unknown, Kondo and Fukuda (2001) reported that endogenous MeJA might stimulate abscisic acid (ABA) concentrations in grape berries since MeJA activated lipoxygenase that is involved in ABA synthesis from carotenoids. It has been reported that ABA, rather than ethylene, plays a role in the onset of fruit maturation in non-climacteric fruit (Kondo and Inoue, 1997). In grape berries, endogenous ABA concentration increased toward ripening and decreased from ripening toward harvest (Kondo and Kawai, 1998). It has been reported that endogenous MeJA in sweet cherries fruits was higher at the immature stage and steadily decreasing during fruit development (*Prunus avium* L.), and this decreasing of MeJA in sweet cherries during fruit ripening decreased fruit firmness dramatically (Kondo et al., 2000). In this study, exogenous MeJA applications increased fruit firmness in 'North Wonder' cv. sweet cherry (Figure 2). However, postharvest MeJA application decreased fruit firmness some Japanese plum cultivars (Khan and Shing, 2007). It has been reported that response to exogenous application of MeJA to strawberry are dependent on concentration and developmental stage at which of MeJA was applied (Mukkun and Singh, 2009; Yılmaz et al., 2007).

Exogenous application of AVG has been claimed to delay fruit ripening and flesh firmness losses becoming during ripening process (Jobling et al., 2003; Öztürk et al., 2012). The present study confirmed those finding for North Wonder sweet cherry fruits, with higher flesh firmness of AVG-treated fruits than fruits of control treatment (Figure 2). Similarly, with AVG treatments, 30-50% increase was observed in flesh firmness of peaches (Noppakoonwong et al., 2005), 7-58% increase in nectarines (Rath and Prentice, 2004) and 12-60% in stone-fruits (Launder and Jerie, 2000). Current findings supported by these previous findings.

While control and MeJA treatments had similar effects on SSC values, AVG treatments significantly decreased soluble solids concentration (Figure 2). Increasing SSC values with the progress of ripening were also reported in cherries by previous researchers (Erdem and Öztürk, 2012; Serradilla et al., 2012). However, Webster et al. (2006) reported that AVG retarded the ripening of sweet cherry. While the effects of AVG on pH and titrate acidity were significant, the effects of MeJA were similar to control treatment (Figure 2). Since AVG retards ripening, it consequently decreases SSC and increases titrate acidity (Gonçalves et al., 2004). Unripe fruits usually have lower SSC values than ripened fruits.

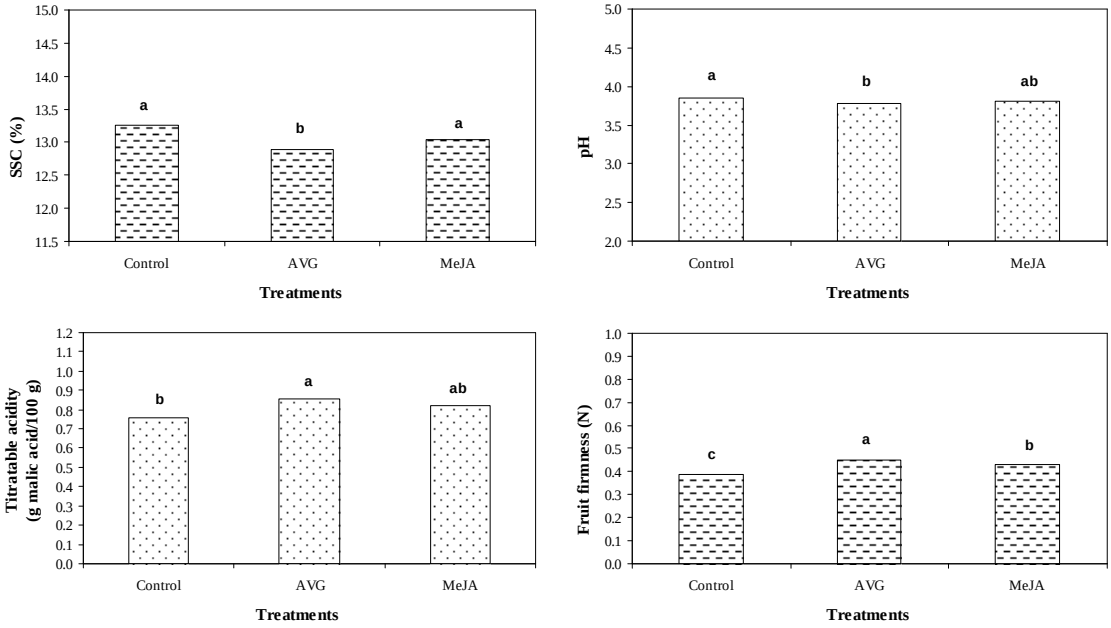

Figure 2: The effects of AVG and MeJA treatments on SSC, pH, titrate acidity and firmness of 'North Wonder' sweet cherry fruit. n=9 (three replicates x three different measurements for each replicate) for SSC, pH and titrate acidity. n=150 (50 fruit x three replicates) for flesh firmness. Different letter on same line indicate statistically significant differences, according to Duncan's Multiple Range test, at P<0.05.

Bioactive compound contents of sweet cherry are provided in Table 2. TP, TAC and TA values significantly decreased with both MeJA and AVG treatments.

Table 2: The effects of AVG and MeJA treatments on total phenolic and total antioxidant activity of 'North Wonder' sweet cherry fruit.

Treatment	Bioactive compounds		
	Total phenolic (mg GAE g^{-1} fw)	Total antioxidant capacity (µmol TE g^{-1} fw)	Total anthocyanin (µg cy-3-glu g^{-1} fw)
Control	542.1 a	9.73 a	21.8 a
250 mg L^{-1}, AVG	411.5 b	6.12 c	4.6 c
2240 mg L^{-1}, MeJA	433.2 b	7.73 b	18.0 b

n= 15 (three replicates x five different measurement for each replicate) for total phenolic, total antioxidant capacity and total anthocyanin. Means in columns with the same letter do not differ, according to Duncan's Multiple Range test, P<0.05.

During the ripening process of sweet cherry, total phenolics and anthocyanin activity exhibit a linear increase (Gündoğdu and Bilge, 2012; Serradilla et al., 2012). Anthocyanins are the pigments responsible for red color formation in sweet cherry (Mozetic et al., 2004). A color transition from green to red is observed with anthocyanin accumulation (Barrett and Gonzales 1994). In present study, anthocyanin contents decreased with MeJA and AVG treatments. Plant growth regulators may alter fruit biochemical composition (Khan et al., 2007). Similarly, Ozturk et al. (2012) reported decreasing TP, TAC and TA values in plums with AVG treatments and Ozturk et al. (2013) indicated again decreasing TP, TAC and TA values of sweet cherry with AVG and MeJA treatments.

http://dx.doi.org/10.4314/ajtcam.v12i2.17

Compared to control treatment with regard to micro nutrients, the effects of both growth regulators were found to be significant only on Fe contents (Table 3). With regard to macro nutrients however, only the effects of MeJA on N and P contents were found to be significant (Table 4).

Table 3: The effects of AVG and MeJA treatments on macro element concentration of 'North Wonder sweet cherry fruit.

Treatment	Mineral nutrients (g kg^{-1})			
	N	P	K	Ca
Control	1.20 a	0.11 a	0.84 a	0.69 a
250 mg L^{-1}, AVG	1.07 a	0.10 ab	0.76 a	0.79 a
2240 mg L^{-1}, MeJA	0.74 b	0.08 b	0.80 a	0.86 a

n=9 (three different measurement for each replicates x three replicates) for mineral nutrients. Means in columns with the same letter do not differ, according to Duncan's Multiple Range test, P<0.05.

Table 4: The effects of AVG and MeJA treatments on micro element concentration of 'North Wonder sweet cherry fruit.

Treatment	Mineral nutrients (mg kg^{-1})		
	Fe	Zn	Mn
Control	24.99 a	4.00 a	2.16 a
250 mg L^{-1}, AVG	14.21 b	3.83 a	2.35 a
2240 mg L^{-1}, MeJA	13.49 b	3.70 a	2.58 a

n=9 (three different measurement for each replicates x three replicates) for mineral nutrients. Means in columns with the same letter do not differ, according to Duncan's Multiple Range test, P<0.05.

Nickel (1978) reported that plant growth regulators played an important role on mineral element contents of plants. Moatshe (2011) reported significant effects of benzyladenine doses on micro and macro mineral elements of morula fruit (*Sclerocarya birrea* subspecies caffra). Rudell *et al*. (2005), on the other hand, reported that there were no significant relationships between pre-harvest MeJA treatments and mineral element concentrations in 'Fuji' apple.

Conclusion

The results of this study on the effect of AVG - an ethylene synthesis inhibitor, and MeJA on fruit characteristics, showed that both growth regulators might have had significant effects in fruit ripening and characteristics when applied before harvest. AVG treatment clearly increased fruit firmness and delayed fruit skin color development by retarding fruit ripening. While MeJA did not cause any significant change in color parameter of fruit, it increased fruit firmness. Both growth regulators significantly decreased bioactive compounds. AVG treatments decreased SSC, pH and titrate acidity values. Both MeJA and AVG had limited effects on mineral nutrients.

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