Indian J. Hort. 68(3), September 2011: 322-327

Effect of pre-harvest ethylene application on phyto-chemicals and antioxidant activity of sour cherry

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ABSTRACT

The study was carried out to determine the effect of pre harvest ethephone spray on fruit quality and nutritional compounds of 'Cigany' sour cherry (*Prunus cerasus*). Trees were sprayed with 250 ppm ethephon one week before anticipated commercial harvest. Fruits from ethephon-sprayed trees had significantly lower soluble solids concentration (SSC), anthocyanin content, antioxidant activity, and firmness than those from non-sprayed control. Ethephon treatment did not affect antioxidant activity and total phenolic content, although it tended to be higher in fruits from non-sprayed control. Titratable acidity (TA), pH and SSC/TA ratio were not affected by ethephon spray. There was a significantly positive correlation between anthocyanin content and SSC (r = 0.99).

Key words: Anthocyanins, antioxidant activity, phenolics, *Prunus cerasus*.

INTRODUCTION

Sour cherry (*Prunus cerasus*) is one of the most benefit fruits containing phyto-nutrients such as anthocyanins, chlorogenic acid, gallic acid, *p*-coumaric acid, and quercetin, which have antioxidant activity. Anthocyanins as one of the flavonoids belong to phenolic compounds which make a dark red color and attract pollinators. The combination of various aglycones, glycosylations, and acylations results in more than 635 anthocyanins in nature. Their aglycone structures undergo reversible transformation at different pH (He and Monica Giusti, 13). Ethephon (2-chloroethylphosphonic acid), an ethylene releasing compound, is usually used to reduce the fruit retention force of mature cherries to enable removal without stems so that harvesting with shakers or hands can be easily done in a shorter time. It also promotes early uniform fruit maturation while minimizing detachment damage (Peterson and Wolford, 20). Glozer *et al*. (10) reported that application of ethephon in sweet cherry did not affect the fruit colour. Further, they demonstrated that after storage, firmness tended to be reduced by ethephon.

The sweet cherry cultivar 'Windsor' showed increasing weight and colour when ethephon is applied at rates of 500 ppm or greater (Bukovac *et al*., 4). The reaction appears to be cultivar-dependent because subsequent research showed no significant changes in fruit quality (Bukovac, 3). Eck (7) showed that ethephon promote the blueberry fruit maturity and the harvest period, however the treated berries had lower total soluble solids content and acidity than control. Ban *et al.* (2) found that ethephon application stimulated the decrease in titratable acidity, accumulation of anthocyanin and fruit softening of rabbit's eye blueberry during the growth period. Because of having more anthocyanins, tart cherries award far more benefits than sweet cherries. The stability of anthocyanins is determined intrinsically by the types of glycosylation and acylation, which is affected externally by the pH, temperature, light intensity, enzyme, and the presence of other compounds interacting with anthocyanin molecules (He and Monica Giusti, 13).

Cherry fruits are non-climacteric because fruit produced neither respiratory nor ethylene peaks near maturity. It cannot be excluded that the low ethylene production may have a role in the ripening process of some but not all non-climacteric fruits (Goldschmidt, 11). More recent works have revealed that some aspects of non-climacteric ripening may be associated with ethylene responses (Giovannoni, 9). Effects of pre-harvest ethephon on inner compounds of sour cherry have not been completely investigated yet. In this study we evaluated whether ethephon treatment on anthocyanin content, antioxidant activity, total phenolic content and other qualities.

MATERIALS AND METHODS

'C**i**gany' sour cherry cultivar grafted on 'Mahaleb' rootstock was planted in a commercial orchard of Mashhad, Iran used for experiment. Uniform 15-year-old trees were sprayed (1,250 l per ha about 5 l/tree) with ethephon (Ethrel 48%, Hockley Company, UK) at 250 ppm and control trees receiving only water. Spraying was conducted one week before anticipated commercial when a ting of red was appeared on fruit.

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Aryanpooya and Davarynejad (1) did similar experiment on 'Érdi Jubileum' sour cherry cultivar in the same period of time. They applied different concentrations and best results were obtained at 250 ppm. Nugent (18) recommended the use of ethephon, 7 to 14 days before anticipated harvest. Fruits were harvested at commercial maturity from ten trees within a single plot at the end of June 2009 and sent in an ice flask to the laboratory. SSC was measured using a digital refractometer (model RFM340, Bellingham & Stanley, Kent, UK). TA was determined by titration with 0.1 N NaOH to reach to pH 8.1 and expressed as percent from malic acid in juice. The pH value was measured by using a digital pH meter. The flesh/pit ratio of fruits was obtained by the following equation:

$$\text{flesh/pit} = \frac{W_T - W_P}{W_P}$$

Where W_T = total weight of fruit, W_P = pit weight.

The flesh firmness was measured by a firmness tester machine (Shinwa-MARUTO, Japan) and expressed as a rate of fruit deformation (mm) is caused by one minute impact of a weight (110 g) on fruit surface. This device consists of a 110 g weight and a graded ruler with 0.5 mm accuracy. Measurements were conducted in three replications and each replication consisted of ten fruits. For measurement of anthocyanin content, antioxidant activity and total phenolic content, the samples were kept at -20°C until extraction. Fruits were stoned and homogenized in a blender. Fifty gram of mixed cherry which was covered with foil had macerated in 100 ml methanol 70% containing 0.1% hydrochloric acid and placed on the shaker for 24 h. Then the extract was filtered over Whatman No. 1 paper under vacuum, and the residue was again extracted with the same solvent until it was colourless (total solvent volume was 200 ml). The extracted solutions were concentrated at 45°c to dryness under reduced pressure by a rotary evaporator (Heidolph, Germany) and dried by oven until it obtained constant weight.

Total anthocyanins were determined by the pH differential method (Wrolstad, 24). The resultant cyanidin-3-glucoside was expressed as milligram per 100 g of fruit. Total phenolic content was determined using the Folin-Ciocalteu colorimetric method. In this case, 0.1 ml of the methanolic extract solution was mixed with distilled water to reach 0.5 ml, then 0.25 ml Folin-Ciocalteu reagent (1 N) was added and mixed well. After 3 min. 7.5% Na_2CO_3 was added and the mixture was vortexed. After remaining for 45 min. at room temperature, the absorbance of the solution was read at 725 nm by using a UV spectrophotometer (CE2502, BioQuest & BioAquarius Series, Cecil Instr. Inc., Cambridge, UK). Tannic acid was used as a standard. Determination of antioxidant activity was done by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity (Brand-Williams *et al.*, 5). To 3.9 ml methanolic DPPH solution (0.004%, w/v), 0.1 ml of methanolic extract at various concentrations was added, mixed thoroughly and left in a dark place. After 30 min. the absorbance was read at 517 nm against control without the extract. DPPH radical scavenging activity was obtained with the following equation: Radical scavenging activity (%) = $(A_0 - A)/A_0 \times 100$; Where A_0 = control absorbance; and A = sample absorbance. Results were analyzed according to a completely randomized experimental design. Statistical analysis was carried out using MSTAT-C software. The results were calculated by one-way analysis of variance (ANOVA). Significant differences were *P*-values<0.01.

RESULTS AND DISCUSSION

Total soluble solids content was significantly lower in ethephon treated sour cherry (Table 1). Micke *et al.* (17) demonstrated that the soluble solids content of treated 'Royal Ann' sweet cherry decreased as the concentration of ethephon was increased. As reported by Delgado *et al.* (6), ethephon application decreased the total soluble solids content of 'Tempranillo' grapes at harvest time in relation to the control. Ethephon spray did not have significant effect on pH, total acidity (TA) and SSC/TA ratio (Table 1). Szyjewicz *et al.* (22) mentioned that the effect of ethephon on fruit composition varied with cultivars, and timing, concentration, and method of application, so contradictory results have been noted about its effects on SSC, TA, and pH. Aryanpooya and Davarynejad (1) reached the same result about pH, TA, and SSC/TA ratio in 'Érdi Jubileum' sour cherry treated with various concentrations of ethephon which was not different from control. Lombard *et al.* (15) reported that SSC and TA levels of 'Flame Seedless' grape decreased or tended to decrease by increasing ethephon dosages above 100 mgl^{-1}. The higher dosages having significantly lower SSC and TA levels than the control.

As shown in Table 2, ethephon treatment significantly influenced cherry weight, flesh/pit ratio, and fruit firmness ($P < 0.01$). The harvested fruits that receiving ethephon were heavier and softer than the control. Smith and Whiting (21) indicated that ethephon-treated 'Chelan' sweet cherry was significantly heavier and darker than non-treated fruit. Bukovac *et al.* (4) showed increased fresh weight and increased pigment formation in 'Windsor' sweet cherry cultivar at a rate of 500 ppm. Average berry mass was influenced by dosage and tended to reach a maximum at 200 ppm (Lombard *et al.*, 15). Firmness of 'Chelan' sweet cherry

Table 1. Effect of pre-harvest ethephon spray on chemical attributes of 'Cigany' sour cherry.

Treatment	SSC (°Brix)	pH	TA (g malic acid/100 ml)	Soluble solids content (SSC/TA)
Control	18.1az	3.09a	2.38a	7.60a
Ethephon sprayed	15.87b	3.08a	2.04a	7.84a

[z]Mean separation within columns by Student's *t*-test at P = 0.01; SSC = Soluble solids content, TA = Total acidity

Table 2. Effect of preharvest ethephon spray on physical properties of 'Cigany' sour cherry.

Treatment	Cherry weight (g/fruit)	Flesh/pit	Deformation of fruit (mm)
Control	2.81bz	8.92b	5.02b
Ethephon sprayed	3.08a	9.88a	6.54a

[z]Mean separation within columns by Student's *t*-test at P = 0.01.

Table 3. The effect of pre-harvest ethephon spray on radical scavenging activity (10 mg/ml) and EC50 of 'Cigany' sour cherry extract.

Treatment	Free radical scavenging activity (%)	EC50 (mg sample/ml)
Control	62.313az	7.475a
Ethephon sprayed	74.307a	6.333a

[z]Means followed by the same letters are not significantly different at 1% level of probability.

cultivar was reduced by ethephon applications (Smith and Whiting, 21).

Anthocyanin content was lower in treated fruits than control (p<0.01) (Fig. 1). Glozer *et al.* (10) mentioned that ethephon-treated fruit was not significantly different than the untreated control with respect to color at harvest in 'Bing' sweet cherry. Wicks and Kliewer (23) found variable responses for two table grapes cultivars 'Ribier' and 'Emperor' in response to ethephon application. In 'Emperor', ethephon increased anthocyanin concentration in sun exposed fruit skin, conversely the same treatment in 'Ribier' had negligible effect on anthocyanin concentration. Ban *et al.* (2) found that ethephon application accelerated the anthocyanin accumulation and greatly increased its final concentration compared to the control in rabbiteye blueberry (*V. ashei*). The significantly high positive correlation between SSC and anthocyanin content (Fig. 3) was specified with a correlation coefficient (r = 0.99, *P* <0.01), while a high negative correlation existed between anthocyanin content and fruit weight (r = -0.95, *P* <0.01) (Fig. 4). Gholami (8) showed that

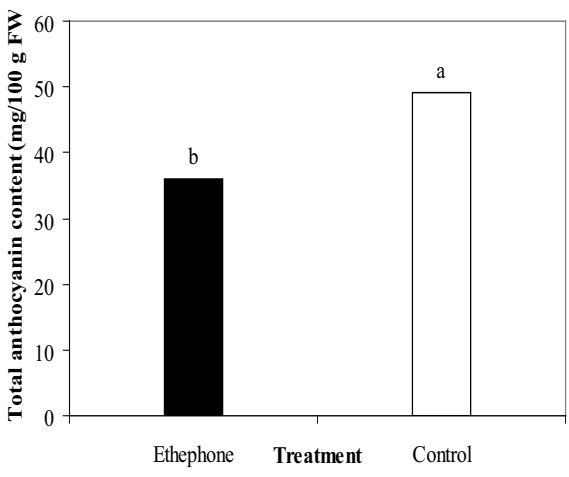

Fig. 1. Total anthocyanin content (mg cyanidin-3-glucoside /100 g FW) in ethephon sprayed and un-sprayed fruits.

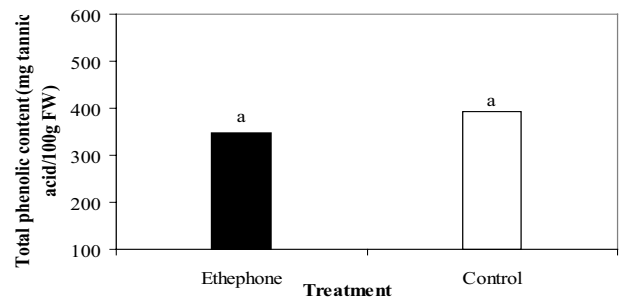

Fig. 2. Total phenolic contents in ethephon sprayed and un-sprayed fruits.

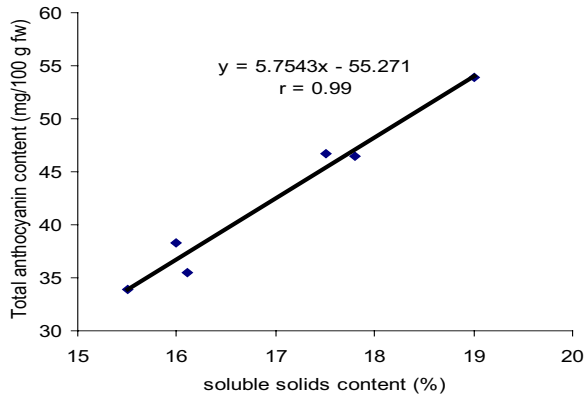

Fig. 3. Correlation between total anthocyanin and soluble solids content in sour cherry (r = 0.99).

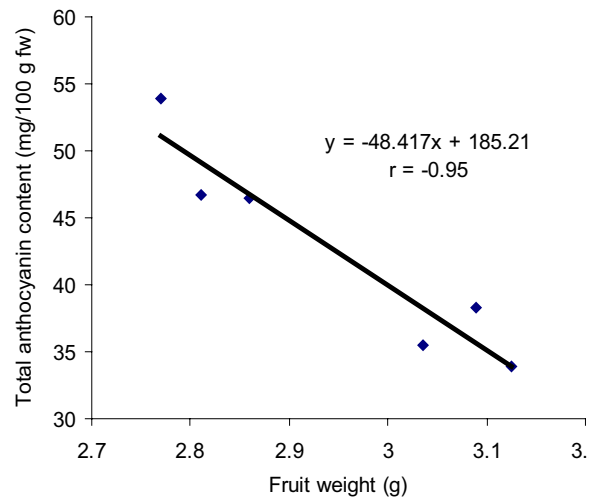

Fig. 4. Correlation between total anthocyanin and fruit weight of sour cherry (r = -0.95).

changes in total SSC closely accompanied changes in color or anthocyanin levels in the skin of Shiraz grape berries, but Wicks and Kliewer (23) found contrary result about grape berry.

The increase in fruit size was due to increase in water content (lower SSC), so dilution effect might explain the inverse relationship between fruit size and anthocyanin content. Because ethephon treatment caused a significant increase in fruit weight but surface area increases less rapidly than the total volume of the fruit thus anthocyanins are diluted. Delgado *et al.* (6) indicated ethephone was generally found to increase the accumulation of highly methoxylated monoglucoside of peonidin (Pn) and malvidin (Mv) in the berry skin during ripening so increasing in red color without any effect on total anthocyanin content in the must of 'Tempranillo' grapevines seems to have been associated with a higher level of methylation in the anthocyanin molecules.

Antioxidant capacity was assayed by DPPH method (Table 3), which was scavenged by antioxidants through the donation of hydrogen, forming the reduced DPPH-H•. The color changed from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Effective concentration of sample that scavenging free radicl 50% (EC_{50}) was not affected by ethephon. Lower EC_{50} indicates higher scavenging activity which was lower in un-treated fruits. Ethephone treatment did not affect phenolic content (Fig. 2). There is little information about effects of ethylene on antioxidant activity and phenolic content.

Delgado *et al.* (6) demonstrated that the combined application of 700 ppm ethephon and 800 ppm of ABA at veraison increased the total polyphenol content of 'Tempranillo' grapevines must up to 16% in relation to the control. There was also a positive correlation

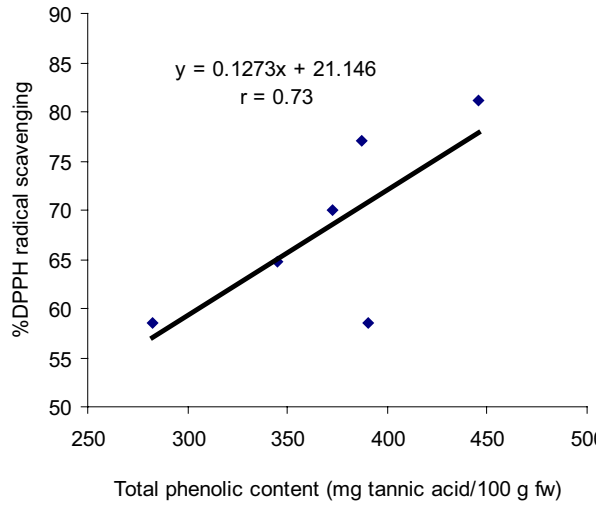

Fig. 5. Correlation between antioxidant activity and phenolic content of sour cherry (r = 0.73).

between total phenolic content and antioxidant capacity (Fig. 5) in all fruits (treated and untreated) (r = 0.73). Meyer *et al.* (16) mentioned that the antioxidant activity of polyphenols were strongly dependent on the test system and substrate in order to be protected by the antioxidant. There was a close correlation between FRAP (ferric reducing ability of plasma) and total phenolic content in sour cherry (Papp *et al.* 19). Similar result was obtained by Karlidag *et al.* (14) between total phenolic content and antioxidant activity (r = 0.76) in wild sweet cherries.

The regulation of ripening processes in non-climacteric fruit is still under question and not so detailed compared to climacteric fruit. Ban *et al.* (2)

concluded that ethephon application in fruits promoted ripening, but the stimulatory effect of ethephon on fruit ripening differed in degree for each fruit ripening character. Post harvest metabolic changes of sweet cherry polyphenols are not regulated by ethylene (Hartmann, 12) and ethylene has no role in ripening processes of sweet cherries at all.

Goldschmidt (11) expressed that ripening-related Chl breakdown in non-climacteric fruit is seemed to be either ethylene dependent or -independent depending on the type of fruit. More recent works have revealed that some aspects of non-climacteric fruit ripening may be associated with ethylene responses (Giovannoni, 9). We concluded that ethephon application did not affect anthocyanin accumulation of 'Cigany' sour cherry, but increased fruit weight, flesh/pit ratio, and fruit softening.

ACKNOWLEDGEMENT

We thank Shahd Iran Company's commercial orchard which let us to perform the research.

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Received: November, 2010; Revised: June, 2011; Accepted : July, 2011