

Improving Crimson Seedless grape quality

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Abstract: The present study was executed at Behaira Governorate, Egypt during two consecutive seasons 2019 and 2020 on Crimson Seedless grape which is still ranking well among favorable cultivars in Egypt thus it's worthy to raise its quality; definitely the coloration. For this purpose, ethephon and ethanol at various combinations were sprayed on clusters at two stages; pre veraison (a week earlier) and at veraison to determine the optimum treatment.

Results show that the pre veraison sprays did not affect coloration or even enhanced berries physical or chemical characteristics so it was ignored later. As for veraison stage sprays, the physical characteristics except for berry firmness did not influenced by different treatments; however, the high concentrations reduced berries adherence force (AF). Regarding chemical characteristics, soluble solids content (SSC) and anthocyanin content were significantly affected by the treatments. The best results of the study characteristics such as; SSC, SSC/acid ratio, firmness and anthocyanin content were gained from combining ethephon and ethanol sprays at veraison stage. Whereas, treatments that contains either solely ethephon or ethanol, as well the highest concentrations combination (500 ppm ethephon +400 ml L⁻¹ ethanol), have recorded lower values for almost studied characteristics.

At the end, presence of ethanol at a moderate dose of 200 ml L⁻¹ in the formula boost 250 ppm of ethephon role in enhancing berries physical and chemical characteristics especially coloration.

Key words: Crimson Seedless, grape, ethephon, ethanol.

I. INTRODUCTION

Grapes (*Vitis vinifera*) are one of the most valuable and economical crops worldwide. More importantly in Egypt, expanding desert cultivation as area or as cultivars; means the future to convoy different developing needs. In recent years, there is an increasing concern about climate change impact on viticulture, particularly, regarding its influence on grape maturity and quality. Subsequently, seeking for the best horticultural practices under unsuitable environmental conditions is needed to improve its productivity and quality to meet both local and global markets demand. According to Portu *et al.* (2015), grapes anthocyanin content is partially genetically determined but also climate and soil factors contribute to the final composition and content. So that, some viticultural practices are able to increase phenolic compounds including anthocyanins.

Working on Crimson Seedless, Dokoozlian *et al.* (1995) improved its color *via* ethephon applied at the fruit ripening initial stages; it resulted in a small decrease in berry firmness.

In this respect, Human and Bindon (2008) applied 200 ppm of ethephon a week post-veraison on grapes and they showed that it increased berries anthocyanins concentration significantly. Moreover, Human (2010) demonstrated that 'Crimson Seedless' anthocyanin was significantly increased by ethephon application, and was not altered by sunlight. Ethephon more consistently increased berry anthocyanins concentration than did sunlight. An *In vitro* study on *Vitis vinifera* done by Heidi Riedel *et al.* (2012) pointed out that elicitors such as; ethephon can be used to enhance phenolic compounds synthesis in cell suspension cultures due to speedy biomass increment. In those respects, (Abdel Aal, 2013 and Gehan Sabry *et al.*, 2013) revealed that spraying Crimson Seedless and Flame Seedless with ethephon ranging from 100 to 300 ppm at veraison hadn't affect cluster or berry weight and dimensions, while it was very effective in enhancing red berries %, anthocyanins content, T.S.S % and T.S.S/ acid. Ethephon application tended to reduce total acidity %, pink and green berries %. Also, Roberto *et al.* (2013) reported that applying 500 mg L⁻¹ of ethephon combined with ABA 7 days after veraison and 15 days before harvest provided the highest color coverage and intensity of 'Rubi Seedless' grape. Furthermore, Venburg *et al.* (2015) marked an inconsistent ethephon efficacy in Crimson Seedless; as multiple high rates applications reaches 400 mg/ vine from veraison till a week before harvest may be required to achieve desired coloration. In addition, ethephon alone can cause berry softening, cracking and splitting thus additional treatments needed, so combining it with ABA yielded more harvestable clusters (greater weight and brix) and avoided excessive firmness losses. In another study, Ferrara *et al.* (2016) used 2890 mg/ L of ethephon to induce abscission of Crimson Seedless grapes. Ethephon affected fruit retention force and gave slightly darker berries but less uniform in color, while it did not affect SSC, acidity and firmness also it has safe residues limits. Working on Flame Seedless grapevines, combining different NPK fertilizers or growth regulators with 250 or 400 ppm of foliar ethephon at veraison advanced ripening and improved yield, cluster and berry color quality significantly. Also it resulted in the lowest uneven berries color and the lowest acidity (Hussein and Abd-Elall, 2017 and Mahawer *et al.*, 2017). In this respect, Porika *et al.* (2018) evaluated some viticulture techniques effects on marketability and economic returns of Crimson Seedless. They measured the highest bunch weight, better compactness ratio, berry weight and diameter when bunches dipped in 500 ppm ethephon, berries thinned and basal leaf removed. Moreover, Barba-Espín *et al.*

(2020) set a protocol of black carrot hairy root cultures for anthocyanins and antioxidants *In vitro* production depending on ethephon. They noticed that adding ethephon to the culture increased anthocyanin content by 82 % and by > 20 % in two examined lines. İşçi *et al.* (2020) mentioned that, Crimson Seedless does not show an adequate color in warm climate. So they combined 500 mg/ L of ethephon with 400 mg/ L PGRs and S- ABA and revealed better maturity results such as; °Brix, SSC/ TA and color.

In an interesting study, Ramadan and Omran (2005) sprayed Flame Seedless grapevines with aqueous methanol 30 % and recorded an increase in pigments, TSS, TSS/ acid ratio and anthocyanins, but decreased total acidity. Furthermore, a highly significant positive correlation detected between total yield, chlorophyll and carbohydrates content. Working on lettuce, applying foliar ethanol at 20 and 40 % led to a significant increase of head size, whereas leaves and roots dry weights were not affected significantly (Guvenc and Kaymak, 2006). Another study enhanced tomato growth, fruit color and yield significantly using pre- harvest foliar sprays of 10 % ethanol. However, 20 % ethanol gave the least TSS and titratable acidity and the highest firmness thus it prolonged the shelf life (Abbasi *et al.*, 2011). In another study on Crimson Seedless grape, Farag *et al.* (2012b) found that adding glycerol to 0.05 % ethephon significantly increased red berries % and TSS, while decreased green berries %. Treatments hadn't affect physical characteristics like berry weight, size, diameter and length. Furthermore, hydro- alcoholic solutions of ethanol, methanol and their mixture influence on different plants at 5 % or 15 % were investigated. Results showed that applications increased photosynthesis, thus promoted plant growth, biomass by 90 %, TSS, pigments, fruit weight and total yield, but lowered acidity. Alcoholic solutions affected the majority of the characters, moreover ethanol effects on plant growth and photosynthesis were significantly better than methanol effects (Zhao *et al.*, 2013; Zeinab Yavarpanah *et al.*, 2015 and Nourafcan and Zahra Kalantari, 2017). Also, Samadimatin and Hani (2017) studied ethanol effects at four levels and pointed that, 10 % had the highest positive effect. Ethanol increased yield, photosynthetic pigments and free sugar and improved morphological characteristics generally. Lamia Mehrabani (2019) compared effects of methanol and ethanol foliar applications at (0, 10 and 20 %) under salinity stress on geranium. Both alcohols significantly affected proline, protein, pigments and root dry weight. Ethanol increased proline content significantly rather than methanol and control treatments.

So, this study aimed to improve Crimson Seedless grape color *via* a safe practice by lowering ethephon concentration used by combining it with ethanol to maintain sufficient yield and quality and avoid undesirable effects of ethephon.

II. MATERIALS AND METHODS

Crimson Seedless (*Vitis vinifera*) grapevines aged eight year- old, trellised as open Gable system and caned at 12 eyes for each cane with a total bud load of 84 eye/ vine and subjected to all vineyard practices. Uniform vines were selected for this trial in both seasons of 2019 and 2020. This work was held in a private orchard lies in Cairo- Alexandria desert road, El Khatatba region at Behaira Governorate, Egypt.

A vine was set for a treatment since three clusters were considered replicates. Vines were sprayed at veraison with a hand sprayer to the run off with various concentrations and combinations of ethephon ((2-Chloroethyl) phosphonic acid), Ethrel® and ethanol (C₂H₅OH), (EtOH) and the treatments introduced as follows:

1. Water (control).
2. 250 ppm ethephon.
3. 500 ppm ethephon.
4. 200 ml L⁻¹ ethanol.
5. 400 ml L⁻¹ ethanol.
6. 250 ppm ethephon + 200 ml L⁻¹ ethanol
7. 500 ppm ethephon +200 ml L⁻¹ ethanol
8. 250 ppm ethephon + 400 ml L⁻¹ ethanol.
9. 500 ppm ethephon +400 ml L⁻¹ ethanol.

At harvest time (according to SSC and satisfying coloration), the following measurements were taken:

2.1. Clusters physical measurements

The measurements taken: Average cluster weight (g), average berry weight (g), berry size (cm³), berry firmness measured by a penetrometer (FT011) expressed as (g/ cm²) and berry AF with mechanical push-pull dynamometer (Model FD101) and expressed as (g/ cm²).

2.2. Berries chemical measurements

The chemical contents taken: Juice SSC measured using a hand refractometer, total titratable acidity as tartaric acid (%) was calibrated according to (A.O.A.C. 1990) and subsequently SSC/ acid ratio was calculated. Skin anthocyanin content (mg/ g fresh weight) was evaluated as described by Geza *et al.* (1983).

2.3. Experimental design and statistical analysis

Data were organized and statistically analyzed according a randomized complete block design. The statistical analysis of the data was carried out according to Snedecor and Cochran (1980). Comparisons among treatments means were held using the new L.S.D. values at 5 % level.

III. RESULTS

3.1. Plant physical measurements

The first three physical parameters measured weren't affected significantly by various treatments in both studied seasons as presented in Table (1) detailed below.

Regarding average cluster weight, the highest weights (515.3 g) and (503.0 g) were measured with the treatment 250 ppm ethephon + 200 ml L⁻¹ ethanol in the first and the second season, respectively. While, the lowest (448.4 g) was found with 500 ppm ethephon + 400 ml L⁻¹ ethanol treatment in the first season and (409.6 g) with the control in the second one. These results are in harmony with those of Farag *et al.*, 2012b; Zhao *et al.*, 2013; Zeinab Yavarpanah *et al.*, 2015 and Nourafcan and Zahra Kalantari, 2017.

Concerning average berry weight, data show that the heaviest berries (3.86 g) were weighed with 250 ppm ethephon + 400 ml L⁻¹ ethanol in the first season and (3.77 g) with the control in the second season. Whereas, the treatment 500 ppm ethephon gave the lightest weight (3.38 g) in the first season and the treatment 250 ppm ethephon + 400 ml L⁻¹ ethanol in the second season (3.01 g). Farag *et al.*, 2012b; Zhao *et al.*, 2013; Zeinab Yavarpanah *et al.*, 2015 and Nourafcan and Zahra Kalantari, 2017 recorded similar trends.

As for berry size, the largest berries (3.77 cm³) were recorded with 250 ppm ethephon + 400 ml L⁻¹ ethanol treatment in the first season and (3.78 cm³) with the control treatment in the second one. However, the smallest berries (3.13 cm³) were detected with 500 ppm of ethephon in the first season and (2.93 cm³) with 400 ml L⁻¹ of ethanol in the second season. These results agreed with those of Farag *et al.* (2012b).

Regarding berries firmness, data point out that the formula of 250 ppm ethephon + 200 ml L⁻¹ ethanol scored the most firm berries significantly in both seasons (412.5 g/cm²) and (460.0 g/cm²), by order. While, the control gave the lowest value (355.0 g/cm²) without significant difference in the first season but the softest berries significantly (351.7 g/cm²) in the second season. These results are in agreement with Abbasi *et al.* (2011) and Ferrara *et al.* (2016) findings.

Considering berry AF, it's clear that there was a non-significant decrement with the treatments in the two studied seasons since the control had the highest AF (605.8 g/cm²) and (666.7 g/cm²), successively. However, the 500 ppm ethephon + 400 ml L⁻¹ ethanol combination resulted in the lowest AF (309.2 g/cm²) and (466.7 g/cm²) in both seasons, respectively. These results are in harmony with those of Venburg *et al.* (2015) and Ferrara *et al.* (2016).

Table 1- Average cluster weight, berry weight, berry size, berry firmness and berry adherence as affected by different ethephon and ethanol treatments during 2019 and 2020 growing seasons.

Treatments	Cluster weight (g)		Berry weight (g)		Berry size (cm ³)		Berry firmness (g/cm ²)		Berry adherence (g/cm ²)	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
Cont.	455.4	409.6	3.55	3.77	3.57	3.78	355.0	351.7	605.8	666.7
250 Ethrel®	456.6	429.6	3.44	3.61	3.44	3.55	377.0	381.3	581.3	658.3
500 Ethrel®	473.3	457.6	3.38	3.67	3.13	3.71	367.2	415.8	542.3	602.5
200 EtOH	483.4	480.0	3.50	3.38	3.29	3.22	370.0	419.4	547.9	612.5
400 EtOH	483.2	463.7	3.76	3.01	3.52	2.93	375.0	396.7	487.9	541.7
250 Ethrel® + 200 EtOH	515.3	503.0	3.6	3.45	3.23	3.38	412.5	460.0	574.3	651.4
500 Ethrel® + 200 EtOH	507.6	488.6	3.57	3.15	3.28	3.23	400.4	449.2	558.1	602.5
250 Ethrel® + 400 EtOH	460.5	472.6	3.86	3.60	3.77	3.56	380.0	440.0	533.8	568.2
500 Ethrel® + 400 EtOH	448.4	474.9	3.74	3.46	3.48	3.47	365.0	366.7	309.2	466.7
L.S.D. (0.05)	25.11	30.81	0.22	0.47	0.94	0.46	24.2	29.0	96.2	102.8

3.2. Plant chemical contents

Data of Table (2) show a marked increase in berries SSC related to ethephon and ethanol presence in the spraying solutions. The maximum SSC (20.25) and (19.24) were recorded with the combination of 250 ppm ethephon + 200 ml

L⁻¹ ethanol followed by or equaled to the 500 ppm of ethephon + 200 ml L⁻¹ ethanol combination (19.91) or (19.24) in both seasons, successively without significant differences among them. Whereas, the minimum significant SSC readings (18.34) and (17.78) were taken from water sprays in the first and the second seasons, consecutively. These results goes

with those of Ramadan and Omran, 2005; Farag *et al.*, 2012b; Abdel Aal, 2013 and Gehan Sabry *et al.*, 2013, while Ferrara *et al.* (2016) reported opposite findings.

Regarding juice acidity, Table (2) demonstrate a slight effect of high ethanol concentrations but not significant as the most acidic juice (0.64 %) and (0.43 %) was titrated when 500 ppm of ethephon combined with 400 ml L⁻¹ of ethanol in both seasons, by order. While, the control treatment had the least juice acidic values (0.49 %) and (0.25 %) in the two studied seasons, successively. Similar results were obtained by Ramadan and Omran, 2005; Farag *et al.*, 2012b; Abdel Aal, 2013 and Gehan Sabry *et al.*, 2013.

Concerning SSC/ Acid ratio, the highest significant ratio (40.50) was calculated with the combination of 250 ppm ethephon + 200 ml L⁻¹ ethanol in the first season, while in the second season the highest non- significant ratio calculated was (71.42) with 250 ppm ethephon treatment. On the contrary, the lowest non- significant ratios calculated were (29.72) and

(42.07) with 500 ppm ethephon + 400 ml L⁻¹ ethanol combination in both seasons, respectively. Abdel Aal (2013) and Gehan Sabry *et al.* (2013) found matching results.

It is obvious from Table (2) that berries anthocyanin content differed significantly among various treatments as ethephon raised it and combining both solutions led to the maximum values, meanwhile the high concentrations inhibited coloration. The highest significant contents (32.98 mg/ g) and (29.15 mg/ g) were recorded with the combination 250 ppm ethephon + 200 ml L⁻¹ ethanol, consecutively in both seasons. Whereas, the lowest contents the non-significant (18.39 mg/ g) and the significant one (17.37 mg/ g) were measured when 500 ppm of ethephon combined with 400 ml L⁻¹ of ethanol in the two studied seasons, by order. These results are in harmony of those of Ramadan and Omran, 2005; Human and Bindon, 2008; Human, 2010; Farag *et al.*, 2012b; Abdel Aal, 2013 and Samadimatin and Hani, 2017. However, Venburg *et al.* (2015) mentioned opposite findings.

Table 2- Juice SSC, acidity and SSC/ acid ratio and skins anthocyanin contents as affected by different ethephon and ethanol treatments during 2019 and 2020 growing seasons.

Treatments	SSC (%)		Acidity (%)		SSC/ Acid ratio		Anthocyanin (mg/g dry skin)	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
Cont.	18.34	17.78	0.49	0.25	37.43	71.12	18.88	18.53
250 Ethrel [®]	19.56	18.57	0.50	0.26	39.12	71.42	23.96	23.44
500 Ethrel [®]	18.41	18.50	0.50	0.27	36.82	68.52	21.15	20.07
200 EtOH	19.60	17.88	0.53	0.29	36.98	61.65	20.10	21.01
400 EtOH	19.30	18.67	0.62	0.37	31.13	50.46	18.09	19.39
250 Ethrel [®] + 200 EtOH	20.25	19.24	0.5	0.31	40.50	62.06	32.98	29.15
500 Ethrel [®] + 200 EtOH	19.91	19.24	0.57	0.36	34.93	53.44	29.59	27.01
250 Ethrel [®] + 400 EtOH	18.99	18.27	0.63	0.38	30.14	48.08	19.45	18.79
500 Ethrel [®] + 400 EtOH	19.02	18.09	0.64	0.43	29.72	42.07	18.39	17.37
L.S.D. (0.05)	0.51	0.88	0.12	0.07	4.25	12.57	1.60	0.50

IV. DISCUSSION

This experiment gives strong evidence on the effect of viticultural practices especially ethephon and ethanol applications during veraison stage on grape quality. Ethephon is an ethylene- releasing compound, while degradation; the plant hormone that produces multiple compounds like antioxidants, phenolics *etc.* Subsequently, some enzymes activities increased in response to ethephon, which are related to anthocyanins synthesis and accumulation. Moreover, application at veraison sufficiently improved grape berry color, while late or excessive applications can soften berries as a response to elevating ethylene levels in plant tissues (Fidelibus and Vasquez, 2019; Barba-Espín *et al.*, 2020 and). Thus, higher ethephon concentrations that exceed 400 ppm

had lowered quality, as coloration, SSC% and firmness, significantly. Furthermore, ethephon can increase phenolics bioavailability (Maduwanthi and Marapana, 2021).

As for, alcoholic aqueous solutions like ethanol are growth stimulators that increasing CO₂ fixation (Rowe *et al.*, 1994). Ethanol synergized ethephon positive effects on grape due to vines biomass, transpiration rate, intercellular CO₂ concentration and photosynthesis increment (Zhao *et al.*, 2013 and Nourafcan and Zahra Kalantari, 2017).

Furthermore, acidification of ethanol with ethephon led to rich anthocyanin content according to (Annete Lima *et al.*, 2011; Farag *et al.*, 2011 and Farag *et al.*, 2012b). Knowing that there is only five mono- glucoside anthocyanins detected

in grapes usually, plus that their optimum extraction conditions were obtained with acidified hydro- alcoholic solution and temperature ranging between 36.5 and 40 °C, which are available in Egypt conditions, Rim Nabli *et al.* (2013).

Moreover, this study showed the effect of sunlight and day and night temperatures on anthocyanin production in grapes. More studies needed to understand how these factors may influence anthocyanin composition and content especially in desert.

V. CONCLUSION

Spraying Crimson Seedless grape at veraison with an ethephon 250 ppm and ethanol 200 ml L⁻¹ formula can solve a big problem faces grape growers definitely with this cultivar especially in desert which is; variable coloration plus giving more firm and sugary berries. Further studies are needed to figure out new alternatives of ethephon or lowering its concentrations; regarding satisfying berry quality and minor chemical residues.

Abbreviations: Ethrel[®]: ethephon; EtOH: ethanol; SSC: soluble solids content; AF: berries adherence force; PGRs: plant growth regulators.

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