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Acceleration of *Veraison* and Enhancement of Berry Quality of "Crimson" Grapes Bypreharvest Treatments with Lysophosphatidylethanolamine, Protone and Magnesium

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Abstract

This study was conducted during the two successive seasons 2015 and 2016 on "Crimson" grapevine grown in a private orchard at Nobariya district, Beheira governorate, Egypt. The vines were under the standard cultural practices, uniform, healthy and free of visible disorders. The vines were sprayed with one of the following treatments that included water (control), lysophosphatidylethanolamine (lisophos) at 200 ppm and 400 ppm, lisophos at 200 ppm + $MgNo_3$ at 1% (w/v), lisophos at 400 ppm + $MgNo_3$ at 1% (w/v), ABA (ProTone as the trade name) at 100 ppm and 400 ppm, the combination of ProTone at 100 ppm + $MgNo_3$ at 1% (w/v), at 200 ppm + $MgNo_3$ at 1% (w/v), in addition to $MgNo_3$ alone at 1% (w/v). The non-ionic surfactant Tween 80 was added to all treatments at 0.05% (v/v). Four vines per treatment were sprayed to the run off point by a hand sprayer on Aug. 1st and 5th during the two seasons, respectively. First sampling was done ten days after spray while the second sampling followed the first one by ten more days. The data provided evidences about the possibility of accelerating the *veraison* stage in a consistent manner especially in the second season. As shown by the color initiation at the first sample after ten days of spray and at harvest especially carotene and anthocyanin and the reduction of chlorophylls a and b in particular with the combinations of lisophos at 400 ppm plus $MgNo_3$ at 1% as well as ProTone at 200 ppm plus $MgNo_3$ at the same above concentration in addition to the significant increase in the TSS to acidity ratio at the first picking in both seasons. Moreover, juice acidity and tannins content were significantly reduced with the above two formulations and with the individual treatment of many other treatments at the first picking and at the final harvest such as lisophos at 200 ppm and 400 ppm and ProTone at 100 or 200 ppm. Meanwhile, berry size did not significantly vary among used treatments at the first sampling time but increased significantly by many treatments at the second picking. The main conclusion of this study can recommend using the formulation containing lisophos at 400 ppm plus magnesium nitrate at 1% (w/v) as well as ProTone at 200 ppm plus $MgNo_3$ at 1% to accelerate *veraison* and enhance the berries quality of "Crimson" grapevine under field conditions.

Keywords: *Lysophosphatidylethanolamine, ProTone, Magnesium, Veraison, Coloration*

Introduction

Grapevine is an important perennial crop cultivated in many countries. Grape berry composition, which is important for the grape growers and consumers is mainly determined by sugars, organic acids, and various secondary metabolites (such as, tannins, flavonols, anthocyanins, aroma precursors and volatile compounds) (Conde *et al*., 2007). Many factors and environmental conditions have been controlling grape berries quality. The accumulation of these components throughout berry development and ripening depends on the genotype and on the environment (Jackson and Lombard, 1993). Climate change already affects the physiology of the grapevine (Schultz, 2000), causing increased sugar concentration, (Duchêne and Schneider, 2005; Bock *et al*., 2013), consequently reduced organic acids and anthocyanins (Barnuud *et al*., 2013, 2014). The mechanisms controlling the accumulation of quality-related metabolites in grapes must be better understood. This will allow promoting innovative viticultural practices resulting in easier adaptation to climate change (Van Leeuwen *et al*., 2013). Among the different viticultural practices affecting berry composition (Keller, 2010a; Dai *et al*., 2011; Kuhn *etal*.,2014), source-sink modulation by summer pruning (i.e., leaf removal or shoot and cluster thinning)is an important tool that may control the relationship between yield and quality, and adjust the complex chemical composition of grape berry (Kliewer and Dokoozlian, 2005). For example, the berry sugar concentration is often positively correlated with leaf area-to-yield ratio when the ratio is below a threshold value of about $1m^2$/Kg of fruit mass (Kliewer and Dokoozlian, 2005; Duchêne *et al*., 2012). Above this value, the sugar concentration usually reaches a plateau and becomes less responsive to source-sink modulation (Kliewer and Dokoozlian, 2005). The responses of organic acids to source-sink modulation have been less thoroughly studied, and contradictory reports showed that a lower leaf area-to-yield ratio caused either an increase (Ollat and Gaudillere,1998), decline (Bravdo *et al*.,1985), or lack of response (Reynolds *etal*.,1994; Parker *et al*., 2015) of organic acids compared with a high leaf to yield ratio. In addition to primary metabolites, secondary metabolites (e.g. tannins, flavonols, anthocyanins, aroma precursors, and volatile compounds). Particularl, anthocyanins are responsible for grape color. Grape anthocyanins derive from five anthocyanidins: cyaniding (Cy), delphinidin (Dp), peonidin (Pn), petunidin (Pt) and malvidin (Mv). The fine- tuning of anthocyanin composition has important impacts on the color hue and color stability (Mazza, 1995). Grape growers and producer have been searching for practical methods that could be adopted on a field scale to accelerate *veraison* and to enhance berry quality especially under arid agriculture. However, other authors recently showed that a post-*version* source limitation resulted from either shoot trimming removal of leaves above the clusters (Palliotti *et. al*., 2013b; Poni *et. al*., 2013) or late-season application of anti-transparent (Palliotti *et. al*., 2013a) that all significantly reduced the speed of sugar

accumulation but did not affect the concentration of berry anthocyanin at harvest. As the source-sink modulation techniques also bring about concomitant modifications in the fruit zone microclimate (such as, light and temperature regimes). It is well established that temperature significantly affects anthocyanin accumulation (Spayd *et. al*., 2002; Pereira *et al*., 2006; Mori *et. al*., 2007). Therefore, experiments that were more precisely controlled and avoided confounding between the effects of source-sink relationship and microclimate are needed to quantify the actual response of anthocyanin to carbon availability. The accumulation of carbon in primary and secondary metabolites is inter connected and results from a complicated metabolic network. For instance, sugar levels positively correlate with total anthocyanin levels (Vitrac *et al*., 2000; Dai *et al*., 2014), yet negatively correlate with organic acids (Keller, 2010b). Thus, treatments that conserve water in mature leaves could result in increasing the biosynthesis of sugars as well as the abundance of more nutrients that enhance the pumping of carbohydrates from source to sinks by a nutrient such as magnesium. Lisophos (the commercial product of lysophosphatidylethanolamine is a natural compound that has been considered an effective growth regulator that initiated a new era of plant growth substances. This compound has been shown to retard leaf senescence, reduce electrolyte leakage of leaf and fruit tissue and enhance its storability and shelf life. It was the first inhibitor of the enzyme called phospholipase D (the senescence enzyme).

On the other hand, magnesium has been involved in chlorophyll biosynthesis, carbohydrate formation and increasing the rate of sugar pumping or export from the source, (mature grape leaves) to various sinks in the vine such as berries, buds, branches, trunk and roots.

ProTone, the trade or commercial name of the plant growth regulator abscissic acid has been considered the main phyto-hormone involved in grape berries coloration.

The timing of ProTone application under field conditions was studied by many research groups (Peppi, *et al*., 2006).

In spite of the intensive studies on grapevines all over the world, but they lack reporting the influence of either ProTone or lisophos as individual treatments or in combination with magnesium.

Thus, the objectives of this study were to utilize both lisophos and magnesium as compared with PorTone to accelerate the stage of *veraison* of "Crimson" grapevines and to enhance the berry quality especially color intensity at harvest.

Materials and Methods:

This research was conducted during two successive seasons 2015 and 2016 on ten years old "Crimson" Seed less grapevines grown in a private orchard at Nobarya district, El-Beheira governorate. The vines were grown on their own root, spaced at 2 x 3 m, irrigated with drip irrigation system, the vines were uniform, healthy, cane pruned and supported by the Spanish trellis system. Each vine bore ten canes that were shortened to 10 buds with a total number of clusters adjusted to 45vine and

regularly received the same horticultural practices adopted in this orchard. Grape bunches, distributed over four vines per replication were sprayed to the run off using a hand sprayer on Aug.1st 5th for the two seasons 2015, 2016. Respectively, before *veraison* stage. First harvest was done ten days after spray while the second harvest was ten days later. Fourteen vines were selected in a randomized block complete design the treatments included water as (the control), LPE at (200 ppm) and (400 ppm), ABA at (100 ppm) and (200 ppm), LPE at (200 ppm) + $MgNo_3$ at 1%(w/v), LPE at (400 ppm) + $MgNo_3$ at 1%(w/v), ABA at (100 ppm) + $MgNo_3$ 1% (w/v), ABA at (200ppm) + $MgNo_3$ at 1% (w/v), addition to $MgNo_3$ 1% (w/v). The non-ionic surfactant Tween 80 at 0.05% (v/v) was added to all treatments to reduce the surface tension and to increase the contact angle of sprayed droplets. ProTone was the commercial name of abscissic acid in all treatments.

1- Fruit quality parameters:

1.A Physical characteristics:

The fruit parameters were measured at harvest. A sample of three canes from each replication was measured to assess the following vegetative parameters:

Number of leaves per shoot, shoot length, short length containing mature leaves, second internode length.

The berries parameters were measured at harvest. Fruit size was determined by displacement in cylindrical tube containing water.

Chlorophyll content was estimated as the method described by *Goodwine* (1965) is extracted in 80% acetone and the absorption at 663nm and 645nm were read in spectrophotometer using the absorption coefficients, the amount of chlorophyll was calculated as:

mg chlorophyll a/g tissues=

$$12.7\ (A_{663})\text{-}2.69\ (A_{645})\times(v/(1000\times w)$$

mg chlorophyll b/g tissues=

$$22.9\ (A_{645})\text{-}4.68\ (A_{663})\times(v/(1000\times w)$$

Where:

A=absorbance at specific wave length.

V= final volume of chlorophyll extracted in 80% acetone.

W= fresh weight of tissues extracted.

For estimation of total carbohydrates; 0.1 g of each air dried sample was submerged overnight in 10 ml of 80 % (v/v) ethanol at 25 °C with periodic shaking. The ethanolic mixture was filtered and the ethanolic filtrate was made up to known volume. Carbohydrates were first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose was dehydrated to hydroxymethyl furfural. This compound forms with another one a green coloured product with an absorbtion maximum at 630 n.m. standard curve was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of glucose. Amounts of carbohydrate present in 100 g of the sample = mg of glucose / volume of test sample x 100 (Hedge and Hofreiter 1962).

Reducing sugar was estimated by Nelson-Somogy method as described by *Naguib* (1964). The reducing sugar was heated with alkaline copper tartrate to reduce the copper from the cupric to cuprus state and thus cuprous oxide was formed. When cuprous oxide was treated with arsenomolybdic acid; the reduction of molybdic acid to molybdenum blue takes place. The intensity of blue colour was measured by spectropho-

tometer at wave length 620 nm. The amount of reducing sugar in the samples was calculated through the calibration standard curve using of pure D-glucose.

Total soluble solids (TSS %) was estimated using Galli 110 refractometer according to (A.O.A.C.; 1975).

Ascorbic acid (vitamin C) was determined by titration with 2.6 diclorophenol indophenol blue dye according to the method reported in (A.O.A.C.; 1975).

The acidity in the fruits juice was calculated as citric acid by titration with 0-1 N sodium hydroxide after adding a few drops of phenolphthalein as an indicator according to (A.O.A.C.; 1975).

Total carotenoides were extracted with acetone-hexane mixture and determined with a spectrophotometer at wave length 440 n.m as described by Dubois *et al.*, (1956).

Determination of anthocyanins: Total anthocyanins content was determined colorimetrically according to the procedure described by Du and Francis (1973) where a known volume of the filtered extract was diluted to 100 ml with the extracting solvent. The colour intensity was measured at wave length of 535 nm for acidified ethanol using spectrophotometer (model T80 x UVNIS Spectrometer PG Instruments Ltd). The total anthocyanins content referred to cyanidin-3-glucoside was calculated using the following equation: Total anthocyanins (mg/100g) = Absorbance X dilution factor X 100 Sample weight X 55.9 .

Determination of tannin

Five grams of each part was milled into powder. The powder was extracted with 100 ml acetone–water (70/30, V/V), and the mixture was stirred continuously for 72 h at room temperature. Then, the mixture was filtrated and evaporated under vacuum at 400C to remove acetone. The washed with 30 ml dichloromethane to remove lipid soluble remaining solution was substances. After that, the solution was further extracted with ethylacetate at a ratio of 30/30 (V/V). The water layer was separated and extracted twice more similarly. Then the resulting water layer was evaporated to dryness, and the resulting substance was weighed. (SY Zhang *et. al.,* 2008).

2. Statistical analysis:

Data were analyzed as a split plot arrangement where the treatment arranged as main plot and time was in sub-plot at randomized complete block design with four replicates. Comparisons among means were made via the Least Significant Differences according to (Sendecor and Cochran 1980). The data were analyzed using SAS (2000) program.

Results

I. The Treatment Factor:

I. A. Physical Characteristics of Vegetative Growth:

The reported results in (Table 1) revealed the influence of various applied treatment on some vegetative characteristics during the two seasons 2015 and 2016. The results indicated that, in general, the number of leaves per shoot was similar whether in the control or treatments with few exceptions in the first season such as lisophos treated vines at 400ppm and magnesium nitrate at 1% since the

two treatments had smaller number of leaves per shoot as compared with the control (Table1).Moreover, the shoot length results showed a similar trend of results to the previous property since the control and the treatments didn't significantly vary in their shoot length with the exception of lisophostreated vines at 400ppm in the first season only when compared with the control. Meanwhile, the shoot length containing mature leaves in the first season was greater than that found in the second season. However almost all treatments and the control had similar shoot length of mature leaves area except the control in the second season that was slightly longer in the length that contained mature leaves (Table1). The second internode length was also reported in Table1. There were variations between the two seasons. In the second season, this length was smaller in all used treatments than the control while this trend was found with lisophos (400ppm) plus magnesium nitrate 1% (w/v), ProTone 200ppm plus magnesium nitrate, in addition to magnesium nitrate alone.

I.B. Chemical Characteristics of Vegetative Growth:

Chemical characteristics of treated leaves in response to various applied treatments were reported in (Table2).It was evident from the data that chlorophyll a in treated leaves didn't show a consistent trend when comparing both seasons. However, the application of ProTone at 200ppm plus magnesium nitrate at 1% resulted in a significant increase in chlorophyll a content as compared with the control and all other treatments except with lisophos at 400ppm alone or with its combination with magnesium nitrate in the second season. The results of chlorophyll b, however, were more consistent and indicated to a significant increase in chlorophyll b by the application of either lisophos alone at 400ppm or its combination with magnesium nitrate. That was the case also with ProTone at 200ppm in the presence of magnesium nitrate as compared with the control in the two seasons. Meanwhile, applications such as lisophos at 200ppm, ProTone at 100ppm plus magnesium, in addition to magnesium nitrate alone led to a significant reduction of chlorophyll b relative to the control in both seasons.

With regard to chlorophyll a+b, the data in (Table 2) showed that lisophos at 400ppm whether alone or when was combined with magnesium nitrate resulted in a significant increase in both types of chlorophylls in the two seasons when compared with the control. In addition, ProTone at 200ppm plus magnesium nitrate resulted in a significant increase also in chlorophyll a+b. Meanwhile, ProTone alone at 100ppm or when was combined with magnesium nitrate resulted in a reduction of chlorophyll a+b relative to the control. Furthermore, total carbohydrates in the leaves was increased especially in the first season, by many treatments such as lisophos at 400ppm or its combination with magnesium nitrate in addition to ProTone at 200ppm plus magnesium nitrate. Changes in leaf starch in response to various applied treatments was also reported in (Table2). The data indicated to a reduction in such character by many treatments such as lisophos at 400ppm alone or

when combined with magnesium nitrate, in addition to ProTone at 200ppm plus magnesium nitrate while the application of magnesium nitrate alone resulted in a significant increase of starch content in the leaves relative to the control. On the other hand, reducing sugars were influenced by various applied treatments since the treatment of ProTone at 200ppm plus magnesium nitrate resulted in the greatest increase in these sugars as compared with the control and all other treatments. However, ProTone at 100ppm plus magnesium nitrate had similar influence on reducing sugars to that found in the control in both seasons. In a similar manner, lisophos at 400ppm resulted in a significant increase in reducing sugars whether alone or when it was combined with magnesium nitrate. Moreover, magnesium nitrate alone resulted in a significant reduction in this type of sugars relative to the control as well as the individual treatment of lisophos at 200ppm (Table2).

I.C. Chemical Characteristics of berries:

With regard to the Chemical Characteristics of berries at harvest, the results in (Table 3) showed that there was a significant reduction in chlorophyll a by all applied treatments in a consistent way, however, these treatments varied in their effectiveness. For example, lisophos at 400ppm plus magnesium nitrate resulted in less chlorophyll a than that obtained with lisophos alone at 400 ppm. Meanwhile, ProTone at 200ppm plus magnesium nitrate was also more effective on reducing chlorophyll a than ProTone alone at 200ppm. The highest chlorophyll a content was found in the control berries followed by the sole application of magnesium nitrate (Table 3).

This trend of results was repeated again with chlorophyll b in the berry skins since all treatments led to a significant reduction of chlorophyll b relative to the control in both seasons. However the magnitude of such reduction was lower with the application of lisophos at 200ppm followed by the application of magnesium nitrate alone at 1% (w/v) (Table 3). Meanwhile, many treatments resulted in a remarkable reduction in chlorophyll b such lisophos at 400ppm alone or in combination with magnesium nitrate, in addition to ProTone at 200ppm plus magnesium nitrate, and finally ProTone at 100ppm. Moreover, changes in chlorophyll a+b were reported also in (Table 3) as affected by various applied treatments. The results showed that there was almost atypical pattern of since all treatments caused a significant reduction in chlorophyll a +b while the greatest influence on that reduction was obtained with the application of lisophos at 400ppm alone or when combined with magnesium nitrate as well as the application of ProTone at 200ppm plus magnesium nitrate on a consistent manner in both seasons and finally ProTone alone at 200ppm. With regard to the response of carotene to various applied treatments in both seasons, it was found that carotenes were increased by all applications when compared with the control. Meanwhile, some applications achieved more increase in such pigment as was found with the application of lisophos at 400ppm alone or in

combination with magnesium nitrate. The use of ProTone at 200ppm plus magnesium nitrate again was effective on increasing the biosynthesis of carotene followed by lisophos at 400ppm plus magnesium nitrate. The individual treatment of ProTone at 200ppm was also capable of increasing berry-skins carotene (Table 3).

The changes in anthocyanin in the berry skins as influenced by various applied treatments was also reported in (Table 3). The data revealed that many treatments were effective on increasing anthocyanin since the greatest increase was obtained with the application of ProTone at 200 ppm plus magnesium nitrate in both seasons followed by treating the berries with lisophos at 400 ppm plus magnesium nitrate as compared with the control. This later treatment resulted even in significantly greater anthocyanin content in "Crimson seedless" berries when compared with lisophos at 200 ppm plus magnesium nitrate ProTone alone, on the other hand, was effective on increase anthocyanin but with greater positive influence at 200 ppm more than 100 ppm relative to the control. The sole application of lisophos at 400 ppm or at 200ppm had apositive influence on the formation of anthocyanin in a concentration dependent manner in both seasons.

Table 1. Physical characteristics of "Crimson" grape shoot as influenced by various applied treatments during the two seasons 2015and 2016.

Treatments	Leaves /shoot (cm)		Shoot length (cm)		Shoot length containing mature leaves (cm)		Internode length (2^{nd}) (cm)	
	2015	2016	2015	2016	2015	2016	2015	2016
Control	23.4 ab	15.8 ns	153.7 abc	152.1 a	148.0 abc	92.4 ns	6.0 a	6.6 a
Lisophos 200 ppm	22.7 abc	16.0 ns	145.3 bcd	124.1 ab	141.0 abc	84.5 ns	5.8 a	5.0 b
Lisophos 400 ppm	18.0 e	14.6 ns	123.9 d	113.9 ab	118.9 c	79.2 ns	5.5 ab	4.7 b
ProTone 100 ppm	22.5 abc	16.4 ns	151.1 abcd	132.3 ab	147.2 abc	92.0 ns	5.5 ab	4.8 b
ProTone 200 ppm	22.0 abcd	16.5 ns	178.5 a	149.2 ab	159.8 a	96.1 ns	6.2 a	4.9 b
Lisophos 200 ppm + $MgNo_3$ 1%	19.6 cde	14.9 ns	140.7 bcd	99.1 b	137.8 abc	65.4 ns	4.7 b	5.2 b
Lisophos 400 ppm + $MgNo_3$ 1%	18.2 e	15.7 ns	133.6 cd	107.1 ab	127.0 bc	69.6 ns	5.5 ab	5.2 b
ProTone 100 ppm + $MgNo_3$ 1%	21.1 bcde	17.4 ns	167.3 ab	140.1 ab	154.4 ab	98.6 ns	5.9 a	5.3 b
ProTone 200 ppm + $MgNo_3$ 1%	24.9 a	20.3 ns	179.3 a	132.2 ab	161.7 a	92.7 ns	4.8 b	5.1 b
$MgNo_3$ 1%	18.8 de	16.4 ns	172.2 cd	141.6 ab	117.5 c	88.7 ns	3.9 c	5.2 b

* Values, with a column, of similar letters are not significantly different according to the least significant difference (LSD) at 0.05 level.

Table 2. Chemical characteristics of "Crimson" grape leaves as influenced by various applied treatments during the two seasons, 2015 and 2016.

Treatment	Chlorophyll a (mg/g F.W)		Chlorophyll b (mg/g F.W)		Chlorophyll a + b (mg/g F.W)		Total carbohydrates (%)		Starch (%)		Reducing sugar (%)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Control	0.70 c	0.72 bc	0.56 c	0.58 c	1.3 c	1.3 c	36.7 de	37.1 abc	1.4 de	1.5 de	6.0 de	5.9 c
Lisophos 200 ppm	0.68 e	0.69 de	0.53 e	0.54 f	1.2 f	1.2 ef	36.2 fg	36.2 bc	1.7 b	1.7 b	5.5 g	5.2 d
Lisophos 400 ppm	0.73 b	0.74 ab	0.57 b	0.60 b	1.3 b	1.3 b	37.4 b	36.2 bc	1.2 f	1.2 g	6.3 b	6.3 ab
ProTone 100 ppm	0.69 d	0.70 cde	0.54 b	0.55 e	1.2 e	1.3 de	36.5 ef	36.6 abc	1.5 cd	1.6 c	5.8 f	5.7 c
ProTone 200 ppm	0.71 c	0.72 bcd	0.56 c	0.57 c	1.3 c	1.3 c	37.0 c	36.4 abc	1.4 e	1.3 f	6.0 cd	6.0 bc
Lisophos 200 ppm + $MgNo_3$ 1%	0.70 c	0.72 bcd	0.55 c	0.57 d	1.3 d	1.3 cd	36.9 cd	37.1 abc	1.4 e	1.4 e	6.0 cd	5.9 c
Lisophos 400 ppm + $MgNo_3$ 1%	0.73 b	0.74 ab	0.58 b	0.59 b	1.3 b	1.3 b	37.2 bc	37.7 ab	1.2 f	1.2 g	6.1 bc	6.3 ab
ProTone 100 ppm + $MgNo_3$ 1%	0.70 d	0.68 e	0.54 d	0.56 e	1.2 e	1.2 ef	36.7 de	36.8 abc	1.5 cd	1.5 d	5.8 ef	5.8 c
ProTone 200 ppm + $MgNo_3$ 1%	0.75 a	0.76 a	0.59 a	0.61 a	1.3 a	1.4 a	37.8 a	38.2 a	1.0 g	1.0 h	6.5 a	6.5 a
$MgNo_3$ 1%	0.67 f	0.68 e	0.52 f	0.53 g	1.2 g	1.2 f	36.0 g	35.9 c	1.8 a	1.9 a	5.4 g	5.4 d

*Values, with a column, of similar letters are not significantly different according to the least significant difference (LSD) at 0.05 level.

Table 3. Chemical characteristics of "Crimson" grape fruits as influenced by various applied treatments during the two seasons, 2015 and 2016.

Treatment	Chlorophyll a (mg/g F.W)		Chlorophyll b (mg/g F.W)		Chlorophyll a + b (mg/g F.W)		Carotene (mg/100g)		Anthocyanin (mg/100g)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Control	0.36 a	0.38 a	0.26 a	0.28 a	0.62 a	0.66 a	0.75 j	0.72 i	27.3 j	26.2 i
Lisophos 200 ppm	0.34 c	0.37 c	0.24 c	0.26 bc	0.58 c	0.63 c	0.85 h	0.81 g	29.6 h	28.0 g
Lisophos 400 ppm	0.30 h	0.32 h	0.20 g	0.22 gh	0.50 h	0.53 g	1.1 c	1.0 b	35.8 c	31.9 b
ProTone 100 ppm	0.33 d	0.35 d	0.23 d	0.25 cd	0.57 d	0.60 d	0.91 g	0.86 f	30.9 g	28.7 f
ProTone 200 ppm	0.31 g	0.33 g	0.20 f	0.23 efg	0.51 g	0.56 f	1.1 d	1.0 c	34.5 d	31.1 c
Lisophos 200 ppm + $MgNo_3$ 1%	0.33 e	0.35 e	0.22 e	0.24 de	0.55 e	0.59 e	0.95 f	0.91 e	32.1 f	29.5 e
Lisophos 400 ppm + $MgNo_3$ 1%	0.29 i	0.30 i	0.19 h	0.20 h	0.48 i	0.51 h	1.2 b	1.1 a	37.1 b	32.6 a
ProTone 100 ppm + $MgNo_3$ 1%	0.31 f	0.34 f	0.22 e	0.23 ef	0.53 f	0.57 e	1.0 e	0.96 d	33.4 e	30.2 d
ProTone 200 ppm + $MgNo_3$ 1%	0.28 j	0.31 i	0.18 i	0.22 fgh	0.46 j	0.52 g	1.2 a	1.1 a	38.3 a	32.6 a
$MgNo_3$ 1%	0.35 b	0.38 b	0.25 b	0.27 ab	0.60 b	0.64 b	0.80 i	0.78 h	28.4 i	26.9 h

*Values, with a column, of similar letters are not significantly different according to the least significant difference (LSD) at 0.05 level.

Juice acidity at harvest as influenced by applied treatments was reported in (Table 4). It was evident that all treatments resulted in reducing such acidity while the magnitude of that reduction varied among used

treatments. The greatest reduction in juice acidity was obtained with lisophos at 400 ppm when combined with magnesium nitrate followed by ProTone at 200 ppm when combined with magnesium nitrate. Furthermore, both lisophos concentrations when applied individually, were able to reduce juice acidity in a concentration dependent pattern. This was also case with ProTone at 200 ppm and at 100 ppm since both concentrations were effective on reducing acidity with greater reduction by 200 ppm than that treated with ProTone at 100 ppm in both seasons. Moreover, all applied treatments were able to cause a significant increase in total soluble solids (TSS) with varying degree of success. The greatest TSS values were obtained with application ProTone at 200 ppm plus magnesium nitrate. Such increase in TSS was even greater than that found with the same combination but at 100 ppm for ProTone. Similar trend was found with lisophos alone at 400 ppm which resulted in greater increase in TSS than that found by lisophos at 200 ppm. In addition, the application of lisophos at 400 ppm combined with magnesium nitrate resulted in a greater increase in TSS than the sole application of lisophos at 400 ppm or at 200 ppm in both seasons as compared with the control.

Tannins in the fruit were also affected by various used treatments (Table 4), as compared with the control. The data proved that all treatments resulted in reducing tannin content relative to the control. However, the magnitude of such reduction varied among treatments in both seasons since the greatest reduction in tannin content was obtained with the application of lisophos at 400 ppm plus magnesium nitrate followed by the application of ProTone at 200 ppm plus magnesium nitrate. Moreover, the individual application of lisophos at 400ppm was able to reduce tannins in a remarkable way which was greater than the reduction of tannins obtained by lisophos at 200 ppm. ProTone at 200 ppm resulted in comparable values of tannins to that obtained with lisophos at 400 ppm .Even the sole treatment with magnesium nitrate resulted in a significant reduction of tannins when compared with the control in both seasons.

Regarding the response of vitamin C to various applications in the two seasons, it was noticed again that all treatments resulted in a significant increase in vitamin C in "Crimson seedless" grape berry juice (Table 4). The greatest vitamin C values were found with the application of ProTone at 200 ppm plus magnesium nitrate in both seasons. However, proTone alone at 200 ppm was able to significantly increase vitamin C content but less than that obtained with the later combination. Meanwhile, the application of lisophos at 400 ppm plus magnesium nitrate was very effective on increasing vitamin C followed by the sole application of lisophos at 400 ppm but with a significant difference between both treatments (Table 4).

Furthermore, the reducing sugars in berries of "Crimson seedless" were also increased in response to various applications. The highest increase was found with the application of ProTone at 200 ppm plus magnesium nitrate followed by application

of lisophos at 400 ppm plus magnesium nitrate. A slight increase in reducing sugars was found with the individual treatment of magnesium nitrate. The sole application of lisophos at 400 ppm gave comparable values to that found with the application of ProTone at 200 ppm. However, the relatively lower concentration of lisophos at 200 ppm resulted in a significant increase in reducing sugars when compared with the control in both seasons (Table 4).

II-The Time Factor:

II-A. Vegetative Physical Characteristics:

The changes between the first and the second time of assessments, the data in (Table 5) indicated to a significant increase by the second time of assessment in studied properties such as the number of leaves per shoot, shoot length and the length of shoot containing mature leaves and internode length.

II- B. Vegetative_Chemical Characteristics:

The characteristics of "Crimson" leaves as affected by the picking time were documented and reported in (Table 7). The data revealed that even at the second picking time, both of chlorophyll a and b were significantly greater than that obtained at the first picking in a consistent manner in both season. That was also reflected on the summation of both chlorophylls a + b of the leaves at the second picking when compared with the first one in both seasons. In line with the mentioned trends, it was found also that total carbohydrates in the leaves at the second picking was still greater than that obtained at the first picking while starch and reducing sugar content at the second picking were lower than that found at the first one.

II-C. Berry Characteristics:

The effect of the time factor on "Crimson" berries characteristics in the two seasons was reported in (Table 6). It was obvious that there was a significant reduction in chlorophylls a, b and their summation by the second picking as compared with the first one in both seasons. Meanwhile, juice acidity was reduced by the second picking and TSS was significantly increased which was reflected on the ratio of both especially in the first season. In addition, reducing sugars content was significantly increased in the second picking as compared with the first picking in both seasons. That was also the trend with vitamin c in the berry juice in the second picking that was significantly greater than that obtained at the first picking.

Table 4. Chemical characteristics of "Crimson" grape fruits as influenced by various applied treatments during the two seasons, 2015 and 2016.

Treatments	Acidity (%)		TSS (%)		TSS/Acidity		Vitamin C (mg/100g)		Tannins (%)		Reducing Sugar (%)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Control	0.63 a	0.87 a	16.4 j	15.5 g	26.2 g	17.7 g	9.4 j	9.7 h	2.3 a	2.2 a	8.6 j	7.9 i
Lisophos 200 ppm	0.57 b	0.76 c	17.7 h	16.4 f	31.2 f	21.6 ef	9.7 h	10.0 f	2.1 c	2.0 c	9.0 h	8.3 g
Lisophos 400 ppm	0.46 fg	0.58 ef	20.5 c	18.6 c	45.7 b	32.1 c	10.4 c	10.6 c	1.6 h	1.6 f	9.9 c	9.0 cd
ProTone 100 ppm	0.54 c	0.73 c	18.2 g	16.9 e	34.3 e	23.3 e	9.9 g	10.2 e	2.0 d	1.9 d	9.1 g	8.6 f
ProTone 200 ppm	0.48 ef	0.56 f	20.0 d	18.5 c	41.9 c	33.8 c	10.3 d	10.6 c	1.7 g	1.6 f	9.7 d	9.2 c
Lisophos 200 ppm + $MgNo_3$ 1%	0.51 de	0.61 de	18.7 f	17.8 d	37.7 d	29.2 d	10.0 f	10.4 d	1.9 e	1.7 e	9.4 f	8.8 e
Lisophos 400 ppm + $MgNo_3$ 1%	0.45 gh	0.49 g	21.1 b	19.4 b	47.8 b	39.6 b	10.6 b	10.8 b	1.5 i	1.4 g	10.0 b	9.5 b
ProTone 100 ppm + $MgNo_3$ 1%	0.52 cd	0.62 d	19.3 e	18.0 d	37.5 d	29.3 d	10.1 e	10.4 d	1.8 f	1.7 e	9.5 e	8.9 de
ProTone 200 ppm + $MgNo_3$ 1%	0.43 h	0.48 g	21.6 a	19.9 a	51.5 a	42.2 a	10.7 a	11.0 a	1.5 j	1.3 h	10.2 a	9.7 a
$MgNo_3$ 1%	0.59 b	0.81 b	17.0 i	16.1 f	29.3 f	19.7 g	9.5 i	9.9 g	2.2 b	2.1 b	8.8 i	8.1 h

*Values, with a column, of similar letters are not significantly different according to the least significant difference (LSD) at 0.05 level.

II- D Berry Size:

The volume of berries indicated to an increase in such character by most treatments in the two seasons. The greatest increase was found with the application of lisophos at 400ppm alone or when combined with magnesium nitrate. Meanwhile, the least berry volume was obtained with the control. Furthermore, the response of berry volume was consistent with lisophos alone at 200 ppm or its combination with magnesium nitrate, in addition to ProTone at 100 ppm plus magnesium nitrate in the second season (Table 8).

The effect of the interaction between various applied a treatments and berry size at the second picking proved that there were many significant increases by the application of lisophos alone at 400 ppm or when combined with magnesium nitrate, since both treatments were equally effective on increasing berry size as compared with control in the second picking. However, the sole application of magnesium nitrate was not able to cause a significant difference in fruit size in the second picking. ProTone, on the other hand, whether at 100 ppm or at 200ppm caused a significant increase in berry size in the second picking. That was the trend when each ProTone concentration was applied in a combination with magnesium nitrate in both seasons.(Table 8).

On the other hand, the time factor indicated to the significant increase in berry volume during both seasons with the second picking as compared with the first one (Table 8).

Table 8. Volume of 10 berries as influenced by the treatments, the picking time and their interaction during the two seasons, 2015 and 2016.

Treatments	Season 2015 volume 10 berries (cm^3)		Mean *	Season 2016 volume 10 berries (cm^3)		Mean *
	First Picking	Second Picking		First Picking	Second Picking	
Control	19.00 f	40.67 de	29.84 bcd	19.67 m	56.67 f	38.17 h
Lisophos 200 ppm	18.00 f	39.33 e	28.67 d	23.67 gh	61.00 d	42.33 cd
Lisophos400 ppm	18.67 f	46.67 ab	32.67 a	22.00 ijk	63.33 b	42.67 c
ProTone100ppm	18.67 f	46.33 abc	32.50 a	24.00 g	62.33 bc	43.17 b
ProTone 200 ppm	18.33 f	44.00 bc	31.17 abc	22.33 ij	62.00 cd	42.17 d
Lisophos 200 ppm + $MgNo_3$ 1%	18.67 f	43.33 cd	31.00 abcd	20.67 lm	61.00 d	40.84 f
Lisophos 400 ppm + $MgNo_3$ 1%	18.33 f	48.00 a	33.17 a	22.67 hi	65.00 a	43.84 a
ProTone 100 ppm + $MgNo_3$ 1%	18.67 f	45.33 abc	32.00 ab	21.33 jkl	59.67 e	40.50 f
ProTone 200 ppm + $MgNo_3$ 1%	18.67 f	44.67 bc	31.67 abc	23.67 gh	59.33 e	41.50 e
$MgNo_3$ 1%	18.33 f	40.33 de	28.27 cd	21.00 kl	57.67 f	39.34 g
Mean	18.53 b	39.93 a		22.10 b	60.77 a	

* Values, with a column, of similar letters are not significantly different according to the least significant difference (LSD) at 0.05 level.

III- The Interaction between Treatments and The picking Time:

1- Vegetative characteristics of the fruits shoot:

1-A: at the first picking:

The assessment of the vegetative characteristics the fruiting shoots at the first picking proved that there no significant differences among treatments whether at the number of leaves per shoot, the shoot length, the length of shoot part containing mature leaves and the second internode length (Table 9).

1-B: at the second picking:

Similar trend of results regarding the vegetative characteristics of the fruiting shoot at the second picking since the statistical analyses showed no significant differences among applied treatments. They were all similar to the control which might indicate to the uniformity of treated shoots (Table 9).

2-Berry characteristics at the first picking:

2-A: Pigment contents:

Changes in chlorophyll a in the berry in response to various applied treatments at the first picking were reported in (Table 10). The data indicated to no significant difference among all treatments in chlorophyll a. However, there were significant differences in chlorophyll b especially in the second season, where there was a reduction in chlorophyll b by all treatments with varying degrees of efficacy. The greatest reduction in chlorophyll b was obtained with lisophos at 400ppm plus magnesium nitrate. In addition lisophos alone at 400ppm resulted in a significant reduction of chlorophyll b at the first picking. The application of ProTone at 200ppm plus magnesium nitrate ,however, was able to reduce chlorophyll b but in much lower magnitude than the above two mentioned treatments. Moreover, magnesium nitrate alone at 1% (w/v) didn't cause a significant change in chlorophyll b. Meanwhile, chlorophylls a +b in the first picking didn't change significantly by all used treatments in the first season. In the second season, however, there were significant breakdown of such chlorophylls especially with the application of lisophos at 400ppm plus magnesium nitrate followed by the individual use of lisophos at 400ppm. ProTone alone at 200ppm also resulted in reducing chlorophylls a +b in an equal magnitude to that obtained by lisophos (400ppm). The individual application of magnesium nitrate didn't cause a significant alternation in chlorophylls a +b relative to the control in the first picking. Carotene in the berries was also influenced by various applied treatments in the first picking. The greatest carotene content was found with application of ProTone at 200ppm plus magnesium nitrate followed by the application of lisophos at 400ppm plus magnesium nitrate. Lisophos alone at 400ppm was also able to increase carotene content in both seasons but less than its combination with magnesium nitrate especially in the first season. Concerning the changes in berry anthocyanin in response to preharvest application were reported in (Table 10). The data revealed that lisophos at 400ppm plus magnesium nitrate caused a significant increase in anthocyanin in grape berries in addition to the individual application of Pro-

Tone at 200ppm alone and the application of lisophos at 400ppm.

2-2 Berry Characteristics at the second Picking:

2.A. Pigment Contents

The response of berry chlorophyll a to various applications at the second picking was reported in (Table 10). The data indicated to similar values of chlorophyll a in both seasons. However, chlorophyll b was only reduced in the second season by all used treatments in the second picking. These above trends were reflected on chlorophyll a+b that was reduced by all applications as compared with the control. With regard to berry carotene at the second picking, the data in (Table 10) also showed a significant increase by many used treatments especially with the combination of lisophos at 400ppm plus magnesium nitrate in both seasons, followed by the individual application of lisophos at 400ppm relative to the control. In addition, ProTone at 200ppm also resulted in a significant increase in carotene content at the second picking.

The changes in anthocyanin content in berries at the second picking also showed a significant increase by the application of ProTone 200 ppm plus magnesium nitrate and lisophos at 400ppm plus magnesium nitrate followed by the applying lisophos alone at 400ppm. However, the difference was significant between using lisophos in the presence of magnesium nitrate and its absence.Favoring the addition of magnesium nitrate to obtain further enhancement of anthocyanin formation. Furthermore, ProTone at 200ppm was able to enhance anthocyanin formation as compared with ProTone at 100ppmor the control in a consistent manner in both seasons. It was effective on anthocyanin formation when combined at 200ppm with magnesium nitrate (Table10).

2-B: Chemical characteristics at the first picking:

The data in (Table 11) regarding total soluble solids changes indicated that there was no significant change by any treatment at the first picking in the first season. However, in the second season there was a significant increase in TSS caused by ProTone at 200ppm plus magnesium nitrate followed by lisophos at 400ppm plus magnesium nitrate. This later combination resulted in similar TSS to that found by ProTone alone at 200ppm. Moreover, juice acidity in berry wasn't affected at the first season while in the second season acidity was the highest in the control berries relative to all other treatments again, the reduction in acidity was the greatest by the application of ProTone at 200ppm plus magnesium nitrate in addition to lisophos at 400ppm plus magnesium nitrate and the application of ProTone at 200ppm. Meanwhile, the sole application of lisophos 200ppm or 400ppm each concentration was able to significantly reduce juice acidity. As for as the ratio of TSS to acidity. Itwas affected by various used treatment in both seasons. For example, ProTone 200ppm plus magnesium nitrate. Furthermore, the combination of lisophos at 400 ppm plus magnesium nitrate resulted also in greater TSS/ acidity ratio than the control followed by the application of ProTone alone at 200ppm. Again, the sole treatment of magne-

sium nitrate was also able to cause a significant increase in TSS/ acidity ratio but less than the combination of both ProTone 200ppm plus magnesium nitrate.

Vitamin C content in the first picking relation to various applied treatments was reported in (Table 11). There was no significant difference between all treatments and the control. However, in the second season, all treatments resulted in a significant increase in vitamin C with varying degrees of efficacy. For example, ProTone at 200ppm plus magnesium nitrate caused a greater increase in vitamin c than the individual use of each component in the combination. In a similar manner, lisophos at 400ppm plus magnesium nitrate resulted in a significant increase in vitamin C more than the separate use of each component when compared with the control.

Tannins in berries as influenced by the used treatments in the first picking were detected then reported in (Table 11). The tannins data indicated that there was no significant variation in tannins during the first season. However, the same above trends were found since the lowest tannin content was obtained with the application of ProTone at 200 ppm plus magnesium nitrate followed by lisophos at 400ppm plus magnesium nitrate. Even lisophos alone at 200ppm or 400ppm was effective on reducing the tannins content in the berries with higher efficacy with the higher concentration. Finally, reducing sugars content in grape berries was also influenced in a significant manner only during the second season. It was also found that the highest reducing sugars was obtained with ProTone at 200ppm plus magnesium nitrate which resulted also in an efficacy similar to that found by lisophos at 400ppm plus magnesium nitrate. In addition, ProTone at 200ppm gave similar reducing sugars to its combination with magnesium nitrate at the first picking.

2-2.B. Chemical characteristics for the second picking:

The effect of the interaction between the treatments and the second picking was reported in (Table 11). The data showed no significant effect during the first season by any of the applied treatments. However, all treatment except magnesium nitrate alone, had a significant increase in TSS especially with applying ProTone at 200ppm combined with magnesium nitrate. Moreover, lisophos at 400ppm caused a significant increase in TSS in a similar magnitude to that obtained by the combination of lisophos (at 400ppm) plus magnesium nitrate. Similar trends of results were found with juice acidity in the second picking since there were no significant differences between treatments and the control in the first season, while in the second one all treatments resulted in a significant reduction in juice acidity relative to the control. The highest reduction in that acidity was achieved by lisophos alone at 400ppm followed by the combination of lisophos at 200ppm plus magnesium nitrate.

Even lisophos at 400ppm plus magnesium nitrate resulted in the most significant reduction in acidity along with ProTone at 200ppm plus magnesium nitrate.

On the other hand, there were significant changes in the TSS to acidity ratio in both seasons except with the individual application of magnesium nitrate at 1% (w/v) that had similar ratio to that found in the control. The greatest increase in TSS to acidity ratio was found with ProTone at 200ppm plus magnesium nitrate that was even greater than that obtained with lisohpos (400ppm) plus magnesium nitrate especially in the first season. The sole application of lisophos at 400ppm was also able to cause a significant increase in the TSS to acidity ratio in a remarkable manner Vitamin C in the berries at the second picking did not also show a significant change in the first season by any treatment. However, in the second season, many treatments were able to cause a significant increase in vitamin C with a greater influence obtained by ProTone at 200 ppm plus magnesium nitrate followed by lisophos at 400 ppm plus magnesium nitrate then the individual application of lisophos at 400 ppm such increases were significantly higher than vitamin C in the control.

Concerning the changes in tannins in response to various treatments and the time of picking. The data also revealed no significant changes in the first season. However, in the second season many treatments resulted in a significant reduction of tannins such as ProTone 200ppm plus magnesium nitrate, then lisophos 400ppm plus magnesium nitrate followed by lisophos at 400 ppm alone.

The effect of the interaction between various applied treatments and the time of picking on reducing sugars of grape berries showed no significant difference in the first season. However, in the second season, many treatments such as lisophos at 400ppm plus magnesium nitrate as well as ProTone at 200ppm plus magnesium nitrate. These last mentioned treatments were more effective on increasing reducing sugars than magnesium nitrate alone. Moreover, lisophos alone caused a significant increase when applied at 400 ppm relative to the control.

3-C: Leaf Characteristics for the first picking:

Changes in chlorophyll a of the leaves in response for preharvest application in the first picking were reported in (Table 12). The data showed a significant reduction by almost all the treatments except lisophos alone at 400ppm alone or in combination with magnesium nitrate in the second season. In addition, ProTone at 200ppm plus magnesium nitrate didn't result in a significant alteration of chlorophyll a as compared with the control. Meanwhile, a consistent reduction of chlorophyll a occurred by the application of ProTone alone at 200ppm and at 100ppm or the combination of ProTone (at 100ppm) plus magnesium nitrate. Moreover, a marked chlorophyll a reduction occurred in both seasons by the sole application of magnesium nitrate. On the other hand, the assessment of chlorophyll b in the leaf at the first picking provided evidences that the most pronounced reduction was found with the application of ProTone at 100ppm or at 200ppm followed by the combination of ProTone (at 100ppm) plus magnesium nitrate. Thus, most ProTone included treatments resulted in greater

chlorophyll b breakdown than other treatments and the control (Table12).

Moreover, the trend of results of both chlorophylls a+b together proved that all ProTone treated leaves whether individually or in a combination had significantly less chlorophylls a+b than the control in the first picking in both seasons. In addition, the application of magnesium nitrate alone also resulted in lower chlorophylls a+b content than the control. Moreover, ProTone at 200ppm resulted in more break down of chlorophylls a+b than that done by lisophos at 400ppm. With regard to the changes in total carbohydrates relative to the applied treatments, the data in (Table 12) revealed that there was a reduction in total carbohydrates by ProTone containing treatments in the first season except with ProTone at 200ppm when combination with magnesium nitrate when compared with control. However, magnesium nitrate alone in the first season also reduced total carbohydrates in the leaf. Meanwhile, lisophos containing treatments eitherdidn ’tcauseasignificant reduction in total carbohydrates in the first season or slightly reduced total carbohydrates relative to the control. These trends, however, occurred in the first season while in the second season there were no significant changes in total carbohydrates.

3.2.C. Leaf characteristics for the second picking:

The effect of various used treatments on chlorophyll a in the leaf at the second picking was reported in (Table 12). The data provided avoidances that chlorophyll a changed due to some treatments in different ways for example, PoTone at 200 ppm plus magnesium nitrate resulted in increasing chlorophyll a in the leaf as compared to the control and all other used treatments. Meanwhile, ProTone alone at 200 ppm resulted in an increase in chlorophyll a relative to the control. On the other hand, lisophos at 400 ppm plus magnesium nitrate caused a significant increase in chlorophyll a. In addition, lisophos at 400 ppm also caused a significant increase in chlorophyll a content in the second picking in both seasons. Meanwhile, the application of magnesium nitrate alone had no influence on chlorophyll a as compared with the control.

Similar trend of results was obtained with chlorophyll b in the leaf at the second picking since again the highest chlorophyll b was found with the combination of ProTone at 200 ppm plus magnesium nitrate followed by lisophos at 400 ppm plus magnesium nitrate then lisophos alone at 400 ppm and finally ProTone individually at 200 ppm while magnesium nitrate alone had a slight increase in chlorophyll b in the effect of various treatments in the second picking on chloropyll a+b showed a similar trend of results to that found with chlorophyll b since again the greatest concentration of chlorophyll a+b was obtained with the combination of ProTone 200ppm plus magnesium nitrate in the second picking. Moreovere, lisophos at 400ppm plus magnesium nitrate caused a significant increase in chlorophyll a+b followed by the individual use of lisophos at 400ppm then ProTone alone at 200ppm in the second picking of both seasons.

Total carbohydrates in the leaf in the second picking had significant differences only in the first season. The variations in such carbohydrates took a trend that was parallel to that with chlorophylls a and b and their summation since again the greatest total carbohydrates in the leaves were found in the leaves treated with ProTone at 200ppm plus magnesium nitrate. Meanwhile, three treatments were similar in their total carbohydrates in the leaf which were lisophos at 400ppm, ProTone at 200ppm, then the combination of lisophos at 400ppm plus magnesium nitrate (Table12). The sole application, however. of magnesium nitrate didn't significantly affect the leaf total carbohydrates.

On the contrary, starch content in the leaf took the opposite direction since the treatments with greater total carbohydrates were found to have much less starch in leaf tissues such as ProTone at 200ppm plus magnesium nitrate as well as lisophos at 400ppm plus magnesium nitrate followed by lisophos alone at 400ppm. Thus, the control leaves had the greatest starch content in the second picking. ProTone treated leaves especially at 200ppm were still much lower starch content as compared with the control.

Concerning reducing sugars in the leaves as influenced by the interaction between treatments and the second picking, the data in (Table 12) revealed that as total carbohydrates in the leaves were increased by some treatments such as lisophos at 400ppm plus magnesium nitrate, there was a consistent increase in reducing sugars in both seasons. That was the case with ProTone at 200ppm plus magnesium nitrate that proved to have a significant influence on chlorophyll a and b and increased total carbohydrates in the leaf. However, the significant increase in reducing sugars occurred in the second season only with some treatments such as lisophos at 400ppm, ProTone at 200ppm, and lisophos at 200ppm plus magnesium nitrate.

Discussion

The present study provided evidences about the possibility of accelerating the *veraison* (the onset of maturity changes) by lisophos (Lysophosphatidylethanolamine, LPE) followed by ProTone (the commercial name of Abscissic acid) to improve "Crimson" grapes quality especially berry coloration and quality at harvest.

Magnesium is an important macronutrient with a number of physiological functions in plants. It is the central atom of chlorophyll and it activates some enzymatic processes connected with photosynthesis through influencing thecarbon assimilation process (Mengel and Kirby, 2001) the deficiency of magnesium results in leaf chlorosis especially older ones and causes premature abscission. Chlorosis generally, is caused by either Mg deficiency, high content of soil Ca (calcareous soils) or the combination of both (Marschner 2002, Ksouri *et al.* 2005, Gluhic *et al.* 2009). In addition, Skinner and Matthews (1990) reported that Mg deficiency also occurs in low-soil-pH value and low- phosphorus content vineyards. Thus the application of magnesium at the proper time as a foliar spray results in increasing carbohydrate partitioning from the leaves to grape berries, as a strong sink in the form of sucrose. Then sucrose is converted to glucose and fructose (Lavee and Nir, 1989, Hrton *et al.,* 2006). Glucose and fructose usually represents more than 99% of carbohydrates in the grape juice. Fresh weight of mature berries can contain 12% to 27% glucose and fructose (Winkler *et al*., 1974). Just after *veraison*, glucose and fructose concentrations increase substantially in both the flesh and skin (Coombe and Nii, 1983).

On the other hand, the found influence of the natural compound (LPE) on fruit quality and coloration and the characteristics of grape leaves is supported by the findings of previous studies that this compound was able to retard leaf and fruit senescence (Farag and Palta, 1993 a, b) by inhibiting the enzyme phospholipase D (Ryu *et al*., 1997). It was also able to inhibit the cell wall hydrolyzing enzymes in fruits such as β- galactosidase and poly galactourenase which reflected on maintaining the structure of the cell wall and retarding the loss of firmness and increased marketable yield (Hong and Chung, 2006). Hong (2006) further reported that the influence of LPE on fruit tissue was dependent on the stage of ripening. Thus, in a mature fruit (ready to ripen), LPE stimulated ripening while in a ripened fruit, it inhibited ethylene production and maintained fruit firmness and prolonged the shelf life. Thus, the results of the present study on grapes were also supported by the findings of (Farag and Palta, 1992a,b, 1993a).

With regard to the effects of the growth regulator called ProTone, previous and present study proved its ability to enhance ripening and anthocyanin biosyathesis. It has been proposed that ABA may be associated with or induce ripening in some non- climacteric fruits including grape (Coombe, 1976). To confirm that it was reported that in grape berries, the role of ABA in ripening seems likely due to the rapid increase

in ABA levels which occurs at ripening initiation. Furthermore, earlier work has suggested that ABA concentration in grape berries declines during the first stage of growth, from a high level at anthesis to its lowest server point to ten days prior to the onset of *veraison* (Scienza *et al.,* 1978, Cawthon and Morris, 1988, Davies *et al.,* 1997). At around the time of *veraison*, ABA level increases and this increase continues until the ripening events are well established after which levels decline. To further support this conclusion, Coombe (1976) showed that this increase in ABA at *veraison* was an approximately six-fold increase in the skin and approximately eight-fold increase in the flesh over the pre-*veraison* berry ABA.

Thus, the time of exogenous application is very important to have a successful outcome on berry-sugar content and the enhancement of anthocyanin formation. To prove that, it was found that exogenous application of ABA was able to stimulate an increase in sugar levels (Kataoka *et al.,* 1982). In another study, the accumulation of anthocyanin was enhanced by ABA treatment at *verasion* and suppressed by NAA and shading (Jeong *et al.,* 2004). The ABA-treated berries ripened earlier and had a higher concentration of soluble solids and lower treatable acidity than the control berries (Jeong *et al*., 2004). Thus, it appeared that there is a "window of opportunity" just prior to *veraison* to the exogenous application time of ABA to grapes to hasten ripening if the ABA was applied at one week prior to verasion, that is, once the endogenous ABA level had reached its lowest concentration (Coombe and Hale, 1973).

It was also wondered about the origin of that ABA accumulation in the berry and the stimulus that initiates its accumulation are yet to be determined. Some researchers suggest that berry ABA is made in the leaves and is transported to the ripening berry where it accumulates (Antolin *et al.*, 2003). The increase in ABA level at *veraison* and the possible influence on sugar and anthocyanin accumulation indicated that ABA may be a crucial mediator of the ripening process in grapes and need further research. In conclusion, the outcome of this study proved that it is possible to manipulate the veraison process and recommend using the formulation containing lisophos 400 ppm plus magnesium nitrate at 1 % (w/v) in addition to ProTone at 200 ppm plus magnesium nitrate enhance "Crimson" berry quality and coloration.

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تأثير الليزوفوسفاتيديل ايثانول امين و البروتون و الماغنسيوم فى أسراع مرحلة اكتمال نمو الحبات و زيادة جودة عنب الكريمسون

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الملخص

أجرى هذا البحث خلال الموسمين المتتاليين ٢٠١٥و ٢٠١٦ على شجيرات العنب من صنف الكريمسون منزرعه فى بستان خاص بمنطقة النوباريه بمحافظة البحيره بمصر. وقد كانت الشجيرات تحت العمليات الزراعيه القياسيه ومتجانسه وسليمه وخاليه من اى أعراض أو اختلالات مرئيه. تم رش الشجيرات بأحد معاملات الدراسه والتى اشتملت على الكنترول (الماء) ليزوفوسفاتيديلايثانول امين (ليزوفوس) بتركيز ٢٠٠ او ٤٠٠ جزء فى المليون ، حمض الابسيسك (اسمه التجارى البروتون) بتركيز ١٠٠ او ٢٠٠ جزء فى المليون، الليزوفوس (٢٠٠ جزء فى المليون) + نترات الماغنسيوم بتركيز ١% (وزن/حجم) ، الليزوفوس (٤٠٠ جزء فى المليون) + نترات الماغنسيوم بتركيز ١% (وزن/حجم) ، البروتون (١٠٠ جزء فى المليون)+ نترات الماغنسيوم بتركيز ١ % (وزن/حجم) ، البروتون (٢٠٠ جزء فى المليون) + نترات الماغنسيوم بتركيز ١ % (وزن/حجم) بالاضافه إلى نترات الماغنسيوم بتركيز ١ % (وزن/حجم). وقد تم اضافة الماده الناشره غير الايونيه المسماه توين ٨٠ لكل المعاملات قبل الرش وذلك بتركيز ٠,٠٥ % (حجم/حجم). وبذلك استخدمت عشرة معاملات و أربع مكررات بكل معامله. وقد تم رش الاشجار حتى نقطة بداية الجريان السطحى بتاريخ ١ و ٥ شهر اغسطس خلال الموسمين (على الترتيب). تم جمع أول عينه للتحاليل المختلفه بعد الرش بعشرة ايام بينما العينه الثانيه بعد الاولى بعشرة ايام اخرى. وقد اثبتت النتائج امكانية اسراع الوصول لمرحلة بداية اكتمال النمو وبشكل ثابت خاصة خلال الموسم الثانى كما اظهرت نتائج بداية التلوين و زيادة صبغة الانثوسيانين و الكاروتينات و حدوث نقص فى كلوروفيل أ + ب مع العينه الاولى بعد ١٠أيام من المعامله وكذلك عينة الحصاد النهائى خاصة بشكل اكبر مع معاملات الليزوفوس (٤٠٠ جزء فى المليون) + نترات الماغنسيوم (١%) وكذلك معاملة البروتون (٢٠٠ جزء فى المليون)+ نترات الماغنسيوم (١%)، بالاضافه الى المعنويه فى نسبة المواد الصلبه الذائبه للحموضه مع العينه الاولى فى كلا الموسمين والاكثر من ذلك حدوث نقصان فى الحموضه و محتوى التانينات فى حبات عنب الكريمسون بشكل معنوى بالتركيزين الموضحين كذلك تحسنت جودة الحبات نتيجة لتأثيرات المعاملات المختلفه وعدة معاملات اخرى حيث استخدمت فرديا كما هو الحال مع استخدام الليزوفوس بمفرده بتركيز ٢٠٠ و ٤٠٠ جزء فى المليون وكذلك البروتون بتركيز ١٠٠ و ٢٠٠ جزء فى المليون. وفى نفس الوقت لم يتأثر حجم الحبات معنويا بالمعاملات خلال العينه الاولى بينما زاد خلال العينه الثانيه بواسطة العديد من المعاملات المستخدمه. ويمكن التوصيه من الاستنتاج العام للبحث بأستخدام مركب الليزوفوس بتركيز ٤٠٠ جزء فى المليون مضافا له نترات الماغنسيوم بتركيز ١% (وزن/حجم) ، وكذلك استخدام البروتون بتركيز ٢٠٠ جزء فى المليون مضافا له نترات الماغنسيوم بنفس التركيز ١% (وزن/حجم) وذلك لأسراع الوصول الى مرحلة بداية اكتمال النمو وكذلك تحسين صفات جودة حبات عنب الكريمسون تحت الظروف الحقليه.

الكلمات الداله: *الليزوفوسفاتيديل ايثانول امين، البروتون، الماغنسيوم، التلوين.*