Achieving Both Increasing Size and Ripening Uniformity of "Dollcy" Olives by Safe Treatments without Leaf Abscission

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ABSTRACT

This study was conducted on ten-year-old "Dollcy" olive trees at EL-Nubaria region, Beheira governorate, Egypt to achieve both increasing size and ripening uniformity of Dollcy olives without leaf abscission by using GA_3 treatment near the end of stage I of fruit growth and spraying Ethrel at 350 ppm and lisophos at 400 ppm 2- 3 weeks before normal harvest. The results proved that GA_3 at 100 ppm caused a significant increase in fruit weight, size, flesh weight, fruit length, fruit diameter, TSS and chlorophyll a, b as compared with the control. Meanwhile, they decreased carotene and anthocyanin content. On the other hand, Ethrel-treated fruits did not show a significant change in fruit size, weight, fruit length and fruit diameter. Furthermore, it decreased TSS, fruit acidity and chlorophyll a, b. Moreover, it increased carotene and anthocyanin content. Lisophos-treated Dollcy olive fruit did not cause a significant change in fruit size, weight and diameter. The incorporation of lisophos with Ethrel in one formulation reduced the percentage of leaf abscission as compared with Ethrel alone. Thus, the use of GA_3 at 100 ppm followed by the formulation containing Ethrel at 350 ppm plus lisophos at 400 ppm was optimum for achieving both increasing size and ripening uniformity of Dollcy olives while reduced leaf abscission percentage.

Key words: olive – size and ripening uniformity- GA_3- Lysophosphatidylethanolamine.

INTRODUCTION

There has been an expansion of olive tree cultivation in Egypt and in the Mediterranean region in the last decade due to the nutritional value and health promoting effects (Zahra, 2014) so that the olive tree orchards became a typical feature of the Mediterranean landscape (Spinelli and Pichi, 2010). However, olive producers and growers, especially in arid lands, faces many problems such as the cost of labor force and required energy for mechanical harvest, high percentage of leaf abscission due to Ethrel spray that releases ethylene and promotes pedicle loosening and facilitates mechanical harvest (Sessiz and Özcan, 2006) in addition to the non-uniformity of fruit size and ripening. Since, maintaining the leaf area is very important for carbohydrate partitioning and allocation, excessive leaf drop adversely affects the next year's crop. Stored carbohydrates in the tree branches, as one of the main sinks, play an important role in floral induction and differentiation and the tree vigor which reflects on olive productivity. Attempts have been made to modify mechanical harvesting direction or used force (Yousefi *et al*., 2010). On the other hand, it was reported that the time of Ethrel application is very critical when desired to enhance ripening and anthocyanin formation in olives (Rugini *et al*., 1982) since Ethrel application to olives after the occurrence of the maximum respiratory ratio only reduced the fruit detachment strength, and the results confirmed the hypothesis which attributed a climacteric model to the attached fruits and a nonclimacteric model to the detached ones. While others tried to use ABA (abscissic acid) as an alternative to Ethrel spray. However, the application of ABA produced a strong defoliation of olive trees together with a marked fruit fall especially at 300 ppm and 400 ppm in addition to delaying color development at 400 ppm while oil content of the fruit did not change (Contreras and Lagos, 2012).

Olive fruit has three distinguished phases of growth and development. The fruit grows rapidly in the first phase, then the growth rate markedly slows down then in the third phase during the fall, the growth rate increases rapidly again where in this phase the fruit color changes from green to yellow, red, or black when olive oil content increases reaching to maximum by the end of December when the fruit color becomes fully black. Many fruits in the cluster during the second phase of fruit growth are not uniform in size or color development. Thus, GA_3 spray near the end of phase I of the growth curve could increase the size of small olives in that cluster since GA_3 proved to have a positive effect on olive fruit size (Ramezani and Shekafandeh, 2009) and (Ramezani *et al*., 2010).

Lysophosphatidylethanolamine (Lisophos) is a novel plant growth regulator proved to delay leaf senescence, inhibiting polygalacturonase activity while enhancing fruit ripening (Farag and Palta, 1993a and Hong, 2008) and mitigated the defoliation action of Ethrel, and delay leaf and fruit senescence in cranberry and plum (Ozgen *et al*.

2005: Farag and Attia, 2016). Thus, the objectives of this study were:

1- To enhance size and color uniformity of "Dollcy" olives before harvest while reducing or alleviating leaf drop.
2- To utilize Ethrel application on olive tree without an associated-significant leaf abscission.
3- To provide olive growers and producers with a novel approach to enhance coloration and uniformity by some growth regulators while alleviating adverse effect on leaf drop.

MATERIALS AND METHODS

1. Plant materials and treatments:

The present study was carried out during 2014 and 2015 seasons at EL-Nubaria region, Beheira governorate, Egypt on ten years old "Dollcy" olive trees (*olea europea* L.) grown in sandy soil under drip irrigation system. Trees were planted at 6 × 6 m spacing. The experiment was designed as a completely randomized block design (RCBD) and the following seven foliar spray treatments were carried out with three replicates for each treatment (1 replicate = 1 tree):

1- Water only (control)
2- 100 mg/ L Gibberellic acid (GA_3)
3- 350 mg/ L Ethrel
4- 400 mg/ L Lisophos
5- Gibberellic acid followed by Ethrel
6- Gibberellic acid followed by Lisophos
7- Gibberellic acid followed by Ethrel plus lisophos.

The trees received the treatment at one application time. GA_3 treatment was sprayed near the end of stage I of fruit growth (28/5, 3/6) during 2014 and 2015, respectively, while Ethrel and lisophos were sprayed 2- 3 weeks before commercial harvest (25/9, 1/10) during 2014 and 2015, respectively. The surfactant Top film was added (at the rate of 0.05 % v/v) to all sprayed chemicals to reduce the surface tension. The chemicals were applied directly to the olive trees with a hand sprayer to the runoff point in the morning.

At harvest time, when fruits became fully colored (13, 17/10, during 2014 and 2015, respectively), 100 fruits were taken from each replication and transported immediately to the laboratory to determine the physio-chemical quality characteristics of olive fruits.

I- Physical characteristics:

1- Olive fruit size (cm^3)
2- Olive fruit weight (g)
3- Flesh weight (g)
4- Stone weight (g)
5- Flesh/ stone ratio
6- Fruit length
7- Fruit diameter (cm)

II- Chemical characteristics:

In the juice, the percentage of TSS was measured by using a hand refractometer, acidity as malic acid (MA) was determined by titration with 0.1 NaOH according to AOAC (1985) and maturity index (MI) defined as the TSS/ MA ratio was estimated. Moreover, anthocyanin pigment in the peel of olives was assessed according to the method of Fuleki and Francis (1968). Furthermore, Chlorophylls a, b and Beta-Carotene were determined according to (Lichtenthaler and Wellburn, 1985), aforementioned by Manuela *et al*. (2012). as follow: half gram of fresh peel was extracted by about 15 ml of 85% acetone and 0.5 g calcium carbonate, the mixture was filtered through a glass funnel and the residue was washed with a small volume of acetone and completed to 25 ml. The optical density of a constant volume of filtrate was measured at a wave-length of 662 nm, for chlorophyll A, 645 nm, for chlorophyll B and 470 nm, for carotene using spectrophotometer.

The following equations were used:-

Chlorophyll a = 11.75 A662 – 2.350 A645= mg/ 100 g.
Chlorophyll b = 18.61 A645 – 3.960 A662= mg/ 100 g.
Carotene = 1000 A470 – 2.270 Chl a – 81.4 Chl b/ 227= mg/ 100 g.

Where, A =Optical density at the wavelength indicated.

2. Leaf drop %:

Four shoots on each tree were tagged and their initial number of leaves was recorded, then the dropped leaves were counted.

3. Statistical analysis

A randomized complete block design (RCBD), with seven treatments and three replicates were used. Treatments means were separated and compared using the least significant differences (LSD) test at 0.05 level of significance according to Snedecor and Cochran (1980). The statistical analysis was performed using statistical analysis system (SAS, 2000).

RESULTS AND DISCUSSIONS

1. Leaf drop %:

The effect of the different treatments on leaf drop percentage at harvest date was presented in Table 1. Data indicated that the differences among all treatments were highly significant in both seasons. In both seasons, the lowest percentage of leaf drop was obtained with lisophos at 400 ppm, GA_3 at 100 ppm and control followed by spraying with (GA_3 at 100 ppm followed by the combination of Ethrel plus lisophos). On the other hand, Ethrel treatment at 350 ppm resulted in the highest percentage of leaf drop whether applied alone or preceded by GA_3 application. Meanwhile, the combination of Ethrel plus lisophos preceded by GA_3 treatment reduced the percentage of leaf drop to the half as compared by Ethrel treatment alone.

Table 1: Effect of the preharvest sprayed substances on leaf drop percentage of Dollcy olive trees during 2014 and 2015 seasons.

	Leaf drop (%)	
Treatments	**2014**	**2015**
Control	0 c	0.72 d
GA_3 at 100 mg/ L	0 c	0 d
Ethrel at 350 mg/ L	26.25 a	24.19 a
Lisophos at 400 mg/ L	0 c	0 d
GA_3 followed by Ethrel	23.83 a	21.6 b
GA_3 followed by Lisophos	0 c	0 d
GA_3 followed by Ethrel plus Lisophos	10.33 b	10.62 c

Values, within a column, with same letter (s) are not significantly different according to LSD ($P \leq 0.05$).

Thus, the incorporation of lisophos with Ethrel in one formulation was able to mitigate the adverse effect of Ethrel alone on leaf abscission. The role of exogenous applied Ethrel in increasing leaf drop was previously reported (Touss *et al*., 1995; Martin, 1986). Moreover, Zahra (2014) working on olives cv, Nabali found that there was a positive correlation between the percent of leaf drop and Ethrel concentrations. Ethrel applications on grapevine caused a significant leaf abscission and accelerated leaf senescence by the reduction of chlorophyll, photosynthesis and increased electrolyte leakage (Roberto *et al*., 2012). The incorporation of lisophos with Ethrel in one formulation reduced leaf abscission percentage (Farag and Palta, 1991: Farag and Attia, 2016) and mitigated the defoliation action of Ethrel. Furthermore, lisophos reduced activity of phospholipase D and membrane leakiness (Hong *et al*., 2009: Ozgen *et al*. 2005) and inhibited the cell wall hydrolyzing enzyme called polygalacturonase (Hong *et al*., 2008).

2. Physical characteristics of "Dollcy" olive fruit:

Effect of various preharvest treatments on fruit size of Dollcy olives at harvest was reported in Table 2. The data revealed that all GA_3 treatments applied near end of stage I of fruit growth of olive fruit whether alone or followed by either lisophos, Ethrel or both significantly increased fruit size of Dollcy olives as compared with the control. The individual application of either Ethrel at 350 ppm or lisophos at 400 ppm had no significant change in fruit size as compared with the control.

With regard to fruit weight at harvest as influenced by various preharvest applications either near end of stage one or two-three weeks before harvest, the data in Table 2 showed that GA_3 treatment caused a significant increase in fruit weight as compared with the control in both seasons. In general, the trend of results was similar to that obtained with fruit size. Fruit weight did not significantly change by the application of Ethrel or lisophos in both seasons relative to the control. Thus, fruits received GA_3 near end stage I of fruit growth were superior in their weight relative to Ethrel and lisophos treatments.

The changes in the flesh weight in response to various treatments at harvest time were reported in Table 2. The data provided evidence that such flesh weight was considerably increased by GA_3 application whether applied alone or followed by either Ethrel or lisophos treatments as compared with the control. On the other hand, no significant change was found in flesh weight as a result of applying Ethrel alone or lisophos relative to the control in both seasons.

Effect of various treatments on stone weight of Dollcy olive cultivar was also reported in Table 2. The data showed a significant increase in stone weight in both seasons by GA_3 treatment either alone or with Ethrel and lisophos in comparison with the control fruits at harvest. However, the application of either Ethrel or lisophos did not result in a considerable change in stone weight relative to the control. The trend of flesh/ stone ratio results was similar to that obtained with fruit stone weight (Table 2).

The data revealed that the control fruits of Dollcy olive did not significantly vary from that treated with either Ethrel or lisophos (Table 2). However, the treated fruits with GA_3 at 100 ppm alone or followed by Ethrel plus lisophos had significantly larger fruit length than other treatments and the control in both seasons.

Table 2 showed no significant change in fruit diameter as a result of applying lisophos as compared with the control in the first season. Moreover, the application of Ethrtel increased fruit diameter as compared with control. On the other hand, GA_3 treatment tended to increase olive fruit diameter as compared with the control and other individual treatments except lisophos in the fiorst season.

The role of GA_3 in improving the physical characteristics of Dollcy olive fruit such as fruit size, weight, fruit length and fruit diameter were in agreement with that of (AbdElnaby *et al*., 2012; Abdrabboh 2009 and 2013; Hagagg *et al*., 2014; Ramezani and Shekafandeh, 2009).

They reported that spraying olive trees with GA_3 ranged from 25 to 100 ppm increased the physical fruit parameters compared to the control. The direct effect of gibberellic acid on stimulating cell division and cell enlargement, and increasing fruit size was previously indicated (Davies, 1995; Zhang *et al.,* 2007; Eman *et al.,* 2007). Gibberellic acid was also reported to promote growth by increasing plasticity of the cell wall followed by the hydrolysis of starch into sugars which reduces the cell water potential, resulting in the entry of water into the cell and causing elongation (Richard, 2006). On the other hand, lisophos and Ethrel caused no effect on fruit size when compared with the control. The non-significant effect of either lisophos or Ethrel on fruit size could be due to the time of application of two treatments before harvest with about 3 weeks when the fruit had a depressed growth rate.

3. Chemical characteristics of "Dollcy" olive fruit:

Changes in total soluble solids (TSS) in response to various applied treatments were reported in Table 3. Data showed that GA_3-treated olives fruit had higher TSS content than that of Ethrel and lisophos but similar to control. On the other hand, when GA_3 treatment was followed by Ethrel, lisophos or Ethrel plus liophos, there were a significant decrease in TSS as compared with the control. Furthermore, the application of lisophos at 400 ppm significantly resulted in higher TSS as compared with Ethrel but lower than GA_3 and control treatments.

Changes in fruit acidity in response to preharvest applications of various used treatments were reported in Table 3. Data showed that there was a significant reduction in fruit acidity by Ethrel treatment relative to the control in both seasons. Moreover, the application of either lisophos alone or Ethrel preceded by GA_3 resulted in a reduction in acidity compared to control or GA_3-treated fruits. However, there was a significant increase in fruit acidity when olive fruits were treated with GA_3 as compared by Ethrel or lisophos treatments.

With regard to TSS/ acidity ratio of olive fruits, as influenced by preharvest treatments, it was also reported in Table 3. The data illustrated that there was a significant increase in the ratio of TSS/ acidity as a result of using lisophos treatments when compared with other individual treatments and control. Moreover, there were no differences between GA_3, lisophos preceded by GA_3 and control treatments. From the mentioned results, GA_3 treatment increased TSS of Dollcy olive fruit as compared with Ethrel treatment. The increase in TSS of fruit at harvest by GA_3 application might be attributed to the intensive photosynthesis in trees previously treated with growth regulators. These results were in agreement with (Abdrabboh (2009) and Hifny *et al*. (2009). On the other hand, Ethrel-treated olive fruit reduced TSS of fruit and increased TSS/ acidity ratio, the significant decrease in total fruit acidity could be attributed to the promotion occurred in fruit maturity, whereas the fruit ripened earlier than those of control trees (Hifny *et al*., 2009).

4. Peel pigments characteristics of Dollcy olive fruit:

Changes in anthocyanin content of Dollcy olives in response to preharvest application of various used treatments were reported in Table 4. Data revealed that the highest anthocyanin content was found in Ethrel-treated fruits. However, GA_3-treated fruits did not significantly affect anthocyanin content compared to the control treatments. Moreover, lisophos-treated fruits had anthocyanin similar to GA_3 followed by lisophos treatment but higher than control and GA_3 treatments. Meanwhile, the incorporation of Ethrel with lisophos in one formulation preceded by GA_3 resulted in a significant increase in anthocyanin content relative to Ethrel alone or individually added to Ethrel. Moreover, there was further added advantage from the incorporation of lisophos along with Ethrel as compared with Ethrel alone in terms of increasing anthocyanin content.

Changes in carotene content in Dollcy olives in response to preharvest application of various used treatments were reported in Table 4. The data proved that carotenes were increased by Ethrel, GA_3 followed by Ethrel, GA_3 followed by Ethrel plus lisophos and lisophos as compared with the control in both seasons.

Table 3: Effect of the preharvest sprayed substances on chemical characteristics of Dollcy olive fruits at harvest during 2014 and 2015 seasons.

Treatment	TSS (%)		Acidity (%)		TSS/ acidity (Ratio)	
	2014	2015	2014	2015	2014	2015
Control	13a	13.03a	0.465a	0.469a	27.99de	27.79d
GA_3 at 100 mg/ L	12.69a	13.17a	0.465a	0.482a	27.92de	27.3d
Ethrel at 350 mg/ L	8.9d	9.03d	0.277d	0.286d	32.15b	31.61b
Lisophos at 400 mg/ L	11.33b	11.37b	0.335c	0.331c	33.83a	34.42a
GA_3 followed by Ethrel	9.83c	10c	0.331c	0.344b	29.77c	29.1cd
GA_3 followed by Lisophos	9.67c	9.83c	0.357b	0.362b	27.09e	27.3d
GA_3 followed by Ethrel plus Lisophos	9.6c	9.7c	0.331c	0.326c	29.06cd	29.77bc

Values, within a column, with same letter (s) are not significantly different according to LSD ($P \leq 0.05$).

Table 4: Effect of the preharvest sprayed substances on peel pigment characteristics of Dollcy olive fruits at harvest during 2014 and 2015 seasons.

Treatment	Anthocyanin (mg/ 100 g)		Carotene (mg/100 g)		Chlorophyll a (mg/100 g)		Chlorophyll b (mg/100 g)	
	2014	2015	2014	2015	2014	2015	2014	2015
Control	11.31e	11.07e	0.425d	0.395e	2.4b	2.37b	0.904b	0.979b
GA_3 at 100 mg/ L	11.53e	11.5e	0.362e	0.334f	2.54a	2.53a	0.955a	1.04a
Ethrel at 350 mg/ L	26.1b	28.51b	0.788a	0.785a	1.94d	1.93de	0.73d	0.797de
Lisophos at 400 mg/ L	15.86d	15.68d	0.609c	0.585c	2.13c	2.11c	0.802c	0.869c
GA_3 followed by Ethrel	24.79c	27.5c	0.752b	0.729b	1.98d	1.98d	0.745d	0.818d
GA_3 followed by Lisophos	15.7d	15.4d	0.506c	0.541d	2.18c	2.13c	0.819c	0.878d
GA_3 followed by Ethrel plus Lisophos	28.62a	30.1a	0.817a	0.798a	1.92d	1.89e	0.721d	0.779e

Values, within a column, with same letter (s) are not significantly different according to LSD ($P \leq 0.05$).

However, lisophos alone caused a significant increase in carotenes relation to GA_3 and control treatments**.**

The data in Table 4 revealed that GA_3-treated Dollcy fruits possessed the highest chlorophyll a content among all other treatments in both seasons. However, the difference was significant between fruits treated with either GA_3, lisophos or the control. The significant reduction in chlorophyll a at harvest in Dollcy olives skin was found in Ethrel-treated ones as compared with the control. Similar trend of results was obtained with the combination of Ethrel plus lisophos when preceded by GA_3, since chlorophyll a content was significantly lower than that found in the control fruit but similar to Ethrel-treated fruits. Similar trend of results was obtained with fruit chlorophyll b (Table 4).

The above findings whether for chlorophyll a, b and carotenoids agreed with (Attia, 2009). Moreover, Hubrechts *et al*. (2003) reported that Ethrel enhanced ethylene production, stimulated progressive loss of chlorophyll and gain of more carotenoids in apple fruits. Furthermore, ethylene treatment significantly enhanced the anthocyanin accumulation in plum fruit peel and enhanced the expression levels of genes that were involved in the anthocyanin pathway (Cheng *et al*., 2015). Color improvement in lisophos-treated fruits has been attributed to enhance carotenoid and anthocyanin accumulation (Kang *et al*., 2003; Hong, 2008). The incorporation of lisophos with Ethrel in one formulation enhanced coloration and ripening uniformity in "Kelsey" plum fruit (Farag and Attia, 2016). On the other hand, GA_3-treated fruits inhibited ethylene production, delayed coloration and ripening of 'Redhaven' peach and grape fruits (Sher-Mohammad *et al*., 1996 a; Amiri *et al*., 2010).

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الملخص العربى

تحقيق كلا من زيادة الحجم وتجانس النضج لصنف الزيتون "دولسى" بواسطة معاملات أمنة بدون تساقط الورق

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أجريت هذة الدراسة على أشجار زيتون عمرها ١٠ سنوات– صنف "دولسى" مزروعة فى منطقة النوبارية بمحافظة البحيرة وذلك لتحقيق كلا من زيادة الحجم وتجانس النضج لصنف الزيتون "دولسى" بدون تساقط الورق بواسطة استخدام حامض الجبريلليك بالقرب من نهاية المرحلة الأولى من نمو ثمرة الزيتون، كما تم استخدام معاملة الايثريل بتركيز ٤٠٠ جزء فى المليون والليزوفوس بتركيز ٤٠٠ جزء فى المليون قبل ميعاد الجمع الطبيعى ب ٢– ٣ أسابيع. وقد أثبتت النتائج أن المعاملة بحامض الجبريلليك بتركيز ١٠٠ جزء فى المليون قد أدت لحدوث زيادة معنوية فى وزن الثمار وحجمها وطولها وقطرها ووزن اللحم والTSS ومحتواها من كلوروفيل أ ، ب مقارنة بالكنترول بينما أدت لحدوث نقص معنوى فى محتوى الثمار من صبغة الكاروتين والأنثوسيانين. ومن جهة اخرى، فان الثمار المعاملة بالايثريل لم يحدث تغير معنوى فى حجمها ووزنها وطولها وقطرها، علاوة على ذلك أدت لنقص محتواها من الTSS والحموضة وكذلك كلوروفيل أ، ب بالاضافة الى زيادة محتوى الثمار من من صبغة الكاروتين والانثوسيانين. علاوة على ذلك فان معاملة ثمار الزيتون بمركب الليزوفوس لم يكن له تاثير معنوى على كلا من حجم الثمار ووزنها وقطرها. وقد وجد أن اضافة مركب الليزوفوس مع الايثريل فى تركيبة واحدة أدت لتقليل نسبة تساقط الورق مقارنة بمعاملة الايثريل بمفردها. ويمكن استنتاج أن استخدام حامض الجبريلليك بتركيز ١٠٠ جزء فى المليون متبوعا بالتركيبة المحتوية على الايثريل بتركيز ٣٥٠ جزء فى المليون والليزوفوس بتركيز ٤٠٠ جزء فى المليون تعتبر المعاملة المثلى لتحقيق كلا من زيادة الحجم وتجانس النضج لصنف الزيتون "دولسى" بالاضافة الى تقليل نسبة تساقط الورق.