



## Effect of Ethylene Inhibitors on Regulation of Ripening and Quality of “Fuerte” Avocado Fruits



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ETHYLENE plays a major role in promoting fruit ripening and change in its biosynthesis could be an important avenue delay this process. During the 2019 and 2020 seasons, a number of ethylene-inhibiting chemicals were tested to regulate the ethylene production of Fuerte avocados and assess their quality. As well as studying the physical, chemical, physiological properties of the fruit and the properties of the oil. Fuerte avocados were surpassed with applications of AVG (Aminoethoxyvinylglycine) at 200ppm and AgNO<sub>3</sub> at 100 μM, with slight differences between them, followed by CaCl<sub>2</sub> at 2.0%. Spraying these treatments on trees twice after 30 and 75 days from fruit set synergistically improved all tested characters as reduced fruit drop, increased yield, fruit quality and delayed ripeness over the other treatments or the control. Overall, both ethylene inhibitors were effective in reducing fruit softening and color development. On the other hand, all applied treatments that impeded the ethylene production or action delayed fruit maturation and this delayed fruit maturation paralleled with a reduction in TSS and acidity. Ethylene inhibitors treatments especially AgNO<sub>3</sub> at 100 μM, increased Fuerte avocados mineral contents of P, K, Mg and Ca along with preserve them from loss, dry matter% and fruit oil%, while slightly affected on iodine and peroxide values. With regard to fruit physiological properties the treatment of AgNO<sub>3</sub> at 100 μM and AVG at 200 ppm greatly reduced ethylene production, respiration rate, polyphenol oxidase (PPO) and pectin methylesterase activity (PME) enzymes activities.

**Keyword:** Ethylene Inhibitors, Fuerte” Avocado, Fruit maturation, Salicylic acid, Silver nitrate, Aminoethoxyvinylglycine, Calcium chloride.

### Introduction

Avocado (*Persea americana* Mill) is classified as a tropical-subtropical fruits. Avocado is unparalleled in their nutritional value due to their high content of unsaturated oils (Donetti and Terry, 2014). The fatty acids in fruit mesocarp may reach approximately 79% (Takenaga et al., 2008). Avocado is a climacteric fruit characterized by an increase in the respiration rate and ethylene production at the onset of ripening (Yang and Hoffman, 1984). Avocado is one of the fastest ripening fruits completing ripening 5 days after harvest (Brecht, 2002).

Fuerte avocado is known as the second largest commercial variety after Haas and is considered a prototype of avocado. The shape of the fruits varies from pear-necked to oval, medium in size and dark green in color, the skin is waxy thin, slightly rough and separates easily from the flesh. The pulp is attractive with excellent quality, a rich, nutty flavor and better taste. The seed is tight in the cavity and does not stick to the pulp. In warmer regions, ripens happen in mid-March with a long picking season. One of the major problems with this cultivar is its irregular bearing history in some regions (Popenoe and Zentmyer 1997).

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Ethylene is classified as a natural plant growth hormone that has many effects on plant growth and development in addition to its regulatory role in the ripening process of climacteric fruits (Attaly et al., 2000). Plant cells comprise ethylene binding receptors, has an ethylene-binding site, which is activated by ethylene and leads to ripening of fruits. Ethylene molecules bound to the receptor initiate a series of reactions within the fruit cells by propagating chemical signals (Choi and Huber, 2008), these molecular reactions lead to the ripening of the fruits. However, ethylene gas also acts as a variable antagonist rather than as a catalyst for climacteric fruits ripening, which means that ethylene must be present continuously in order to maintain copies of the genes required for fruit ripening (Theologis, 1992). To delay the ripening process of climacteric fruits, there is an urgent need to slow or inhibit down the production of ethylene (Ponce et al., 2009). By using some chemical compounds, the response to the basic level of ethylene in the fruit can be inhibited and delaying the natural ripening process (Osorio et al., 2013). The interaction between ethylene and the receptor is dynamic as the ethylene inhibitors interact with the ethylene receptor and inhibit the action of ethylene, therefore inhibitors cannot permanently bind to the receptor sites. Maturity process is restored again when all ethylene inhibitor molecules are used, as new receptor sites are formed and ethylene regains sensitivity to them (Choi and Huber, 2008).

The role of ethylene in fruit ripening can be interfered with by three actions, include manipulation of ethylene receptors (Owino et al., 2002), inhibition of ethylene biosynthesis (inhibit the conversion of S-adenosyl methionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) and the conversion of ACC to ethylene) or inhibition of ethylene action (Yang & Hoffman, 1984). There are numerous attempts to neutralize the effect of ethylene using safe compounds that interfere with the ethylene biosynthesis pathways. ACC synthase inhibitors like Aminoethoxyvinylglycine (AVG) and Aminoxyacetic Acid (AOA) or ethylene action inhibitors like calcium chloride and silver ions in the form of thiosulfate or nitrate silver (Paliyath et al., 2008).

Aminoethoxyvinylglycine (AVG) is defined as an ethylene synthesis inhibitor by blocking the conversion of S-adenosyl methionine into 1-aminocyclopropane-1-carboxylic acid (Yang and Hoffman, 1984), thus reduces the ripening

of climacteric fruits (Mir et al., 1999). AVG appears readily at elevated indoor ethylene levels. The mode of action of AVG is evident during inhibition of self-ethylene synthesis stimulating for fruit ripening (Pereira Neto, 2001). Applying AVG not only reduces ethylene production to control levels but also participates in stress-induced ethylene production and also through its effect on the synthesis of functional proteins (Hong et al., 2008). It is a human and environment friendly material for delaying the ripening of apples (Greene and Schupp, 2004). Using AVG four weeks before harvesting inhibited ethylene production, reduced pre-harvest drop and delayed fruit ripening (Ozturk et al., 2015)

Silver ions are unique among heavy metals as an inhibitor of ethylene action due to their ability to generate sensitivity to ethylene in plants, thus delaying fruit ripening (Zhao et al., 2002). Silver ions are believed to disturb the ethylene binding sites (Rodriguez et al., 1999), where it is proposed that a single copper ion (Cu) mediate one of the ethylene receptor sites (the copper present at the ethylene binding site) and the copper agent is replaced by silver, which leads to the locking of the receptor in such a way that it continuously suppresses the ethylene responses (Zhao et al., 2002). Another hypothesis is that  $\text{AgNO}_3$  inhibits the action of ethylene in plants by potent antagonists such as silver compounds. This is possibly due to the oxidation of ethylene by a metallic ion enzyme system (Yang and Hoffman 1984). The sensitivity to ethylene is conferred by the predominant mutation of the receptor and the reduced ability of the receptor to bind ethylene (Kumar et al., 2009). External application of silver ion is able to inhibit the action of ethylene in classic responses for example, abscission, growth retardation and senescence. (Wu et al., 2006). Also,  $\text{AgNO}_3$  has been used in studies to inhibit the action of ethylene due to its water solubility and lack of phytotoxicity at effective concentrations. (Dar and Tahir 2018)

Salicylic acid (SA) or ortho-hydroxybenzoic acid and its compounds are classified into a variety of plant phenols, these play a fundamental role in regulating physiological processes, such as plant growth and development, ion uptake and photosynthesis (Beckers and Spoel, 2006). The positive effects of salicylic acid are that it is a plant hormone that inhibits ethylene biosynthesis and delays senescence (Horváth et al., 2007). Besides, Leslie and Romani, 1988 demonstrated that it

prevents the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene by reducing the production and activity of ACC oxidase. In this respect Ganesan and Thomas (2001) showed that, the SA inhibitory effect depends on the pH. However, Tirani et al., (2013) reported that, SA has double effects on ethylene metabolism which may be concentration dependent and changeable depending on the stage of fruit growth. The low doses have the potential to regulate ethylene biosynthesis by increasing the expression of transcripts of ACC. whereas, high doses inhibit the ACC and oxidase genes and thus prevent the timely ethylene production during ripening.

Calcium plays a major role in preventing physiological disturbances in fruit and plant tissues during the growth and development stages (Kirkby and Pilbeam, 1984). Calcium is known to act as senescence retardant, the beneficial effects of calcium are attached to preserving membranes (White and Broadley, 2003). Calcium at low concentrations acts as a second messenger that mediates several metabolic events in plant systems, being one of the most diverse signaling molecules in plants (Ranty et al., 2016). Calcium application can effectively delay fruit ripening while maintaining fruit quality (Zhang and Wang, 2019). In general, calcium can effectively reduce the metabolic activities of endogenous substances and inhibit or delay physiological metabolic disturbance within a certain concentration range (Shafiee et al., 2010). The processing effects of calcium extend to ethylene synthesis and signal transduction (Aghdam et al., 2012). Cheverry et al., (2010) cleared the responses to calcium-dependent protein phosphorylation as safeguard to the cell membrane structure and inhibiting the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase.

In this present work, the performance and applicability of a number of chemicals that inhibiting ethylene biosynthesis and/or action to improve "Fuerte" Avocado fruits quality with regulate or delay the ripe well tested, thus increasing the period of fruit marketing.

### **Materials and Methods**

This work was performed during the seasons of 2019 and 2020 on 27 Fuerte avocado trees

(*Persea americana* Mill) budded on seedy rootstock, which were seven years old and growing in a private orchard in Al Khatatba, Menoufia Governorate, Egypt. The trees were healthy, uniform, free from defects and cultivated with well-drained sandy soil, a planting distance of 6 x 5 m<sup>2</sup> under the drip irrigation system. All trees for this trial received the same regularly recommended horticultural practices. This experiment was carried out in order to study the effect of spraying with some ethylene inhibitors on ripening behavior of Fuerte avocado fruits as well as on their physical-chemical properties. The tested nine treatments were as follows:

(T1) Control (only spraying water); (T2) Aminoethoxyvinylglycine (AVG) at 100ppm; (T3) Aminoethoxyvinylglycine (AVG) at 200ppm; (T4) Silver nitrate (Ag NO<sub>3</sub>) at 100 μM; (T5) Silver nitrate (Ag NO<sub>3</sub>) at 200 μM; (T6) salicylic acid (SA) at 1.0 mM (T7) salicylic acid (SA) at 2.0 mM; (T8) Calcium chloride (CaCl<sub>2</sub>) at 1.0% and (T9) Calcium chloride (CaCl<sub>2</sub>) at 2.0%.

All treatments were applied by hand spraying, the trees were sprayed twice: Firstly, after 30 days of fruit set (diameter of 5-8 mm, early May). Secondly, after 75 days of fruit set (diameter of 15 - 18 mm, mid-July). The non-ionic surfactant Tween 20 at 0.05% (v / v) was added to all treatments to reduce surface tension and increase the contact angle of the sprayed droplets.

#### *Yield attributes*

##### *Maturity delay in days than control*

Harvest time is determined for each treatment according to Carvalho et al., (2015) who summarized that, the texture and appearance of the skin is an indicator of harvest maturity as the skin surface becomes smoother, with loss of glossiness. The area of the stem closest to the fruit changing from green to brown or black and the seed coat typically turns brown when the fruit is sufficiently mature for harvest. Then, compare the harvest time of all treatments with the harvest time of control (first week of September for control avocados)

##### *Pre-harvest fruit drop%*

*Pre-harvest fruit drop% was calculated using the following equation:*

$$\text{Fruit drop\%} = \frac{\text{Total fruit number (after fruit set)} - \text{final fruit number (at harvest)}}{\text{Total fruits number (after fruit set)}} \times 100$$

Number of fruit/tree, Fruit weight and yield: Total of fruit number per tree was collected at harvest time (first week of September for control avocados) then yield was determined as

Yield (Kg/tree) = (Total number of fruits/tree X Average fruit weight (g.)/1000.

#### *Physical fruit properties*

Samples of five fruits from each treated tree/replicate and untreated (control) were collected at similar maturity stage to estimate some properties i.e. fruit length (L) and diameter (D) in cm. as well as fruit shape (L/ D ratio) and fruit flesh % were calculated.

Fruit firmness: In addition, fruit firmness was determined in 5 fruits from each replicate, on two sides of each fruit by the penetrometer, Firmness is presented in Kg./ cm<sup>2</sup> required to penetrate unpeeled fruit, using a 6-mm (diameter) conical probe. Values were expressed in Kg./ cm<sup>2</sup> according to Meir et al., (1995).

Dry matter %: The percentage of dry matter (DM) was determined according to Lee (1981).

#### *Chemical fruit properties*

Total acidity (expressed as g. Citric acid per 100g. flesh) total soluble solids was measured by hand refractometer. Moreover, L- ascorbic acid content (mg/100g. flesh) was evaluated according to A.O.A.C.2000). In addition total chlorophyll of peel content (mg/100g. fresh weight) and total carotenoids of pulp content (mg/100g. fresh weight) were determined according to Wellburn (1994).

#### *Fruit mineral contents*

Dried pulp samples were used to detect the fruit mineral contents P, K, Ca and Mg %.

Potassium content was determined by Flame photometer as percentage according to method of Jackson, (1967).

Phosphorus: was estimated as the method described by Bringham (1982).

Calcium and Magnesium, were determined according to Chapman and Pratt (1982) using an atomic absorption spectrophotometer.

#### *Fruit physiological attributes*

Respiration rate (mg CO<sub>2</sub>/kg. fresh fruits/hr.): The producer of carbon dioxide from the Fuerte avocado was evaluated immediately after application of the treatments as an initial sample and after finished of storage periods (the end of *Egypt. J. Hort. Vol. 51, No. 1 (2024)*

experiment and fruits came out of ripening room). The air-flow was passed through concentrated NaOH to ensure that the air-flow was free of carbon dioxide, before passing into 1liter container 1Kg fruits/ jar was considered as one replicate. The out -flow of air was then introduced into 100 mL of 0.1N NaOH for 1hour. This solution was then titrated against 0.1N HCl and levels of carbon dioxide produced by the fruits were measured as (mg. CO<sub>2</sub>/kg. fresh fruit /hour, according to A.O.A.C. (2000)

Ethylene production (µl C<sub>2</sub>H<sub>4</sub>/Kg fruit/hr.) : The rate of ethylene production was determined by incubating 1Kg. fruits in 1liter glass containers. After two hours of incubation, one ml. gas sample was drawn from each jar vacuum and injected into a gas chromatography model (Hewlett Packard 5890 Series II, USA). The ethylene production rate was expressed in µl C<sub>2</sub>H<sub>4</sub>/Kg fruit/hr.

PolyPhenolOxidase (PPO) activity OD at 420nm min<sup>-1</sup> mg protein<sup>-1</sup>): The enzyme was purified following Erzengin (2009).

PectinMethylEsterase activity (PME, Unit mg-1 protein): Pectin methyl esterase was extracted and assayed by the method of Hagerman and Austin (1986).

#### *Oil properties*

Avocado pulp was ground the and extended it in the rotating plate of a domestic microwave oven, heated to the maximum for 11 min. take 5g. of the resulting mass and extracted oil by with hexane

Oil percentage: The oil percentage of the dry samples was determined by the method of Lee (1981).

Iodine value mg I<sub>2</sub>/100 g.: Iodine value (mg I<sub>2</sub>/100 g) was assessed using Wijs method of Anon (1988).

Peroxide value meq O<sub>2</sub>/kg oil: The oil percentage in the dry fruit flesh samples was determined by the method of (Lee, 1981) and Iodine value (mg I<sub>2</sub>/100g.) was assessed using Wijs method (Anon, 1988). Also, Peroxide values (meq O<sub>2</sub>/kg oil) were measured according to the method of Garcia et al. (1996). Peroxide values were measured according to the method of Garcia et al. (1996)

#### *Statistical analysis*

Data were analyzed for statistical significant differences using MSTAT-C software (MSTAT,

Michigan University East Lansing). Duncan multiple rang test (LSR) at 5% level was completed to define any significant difference among various treatments, according to Snedecor and Cochran (1990)

## Results and Discussion

### Yield attributes

Date in Table (1) shows the effect of some ethylene inhibitors (either for ethylene production or ethylene action) on yield attributes of Fuerte avocado fruits, during 2019 and 2020 seasons. All applied treatments induced an evident delay in fruit maturity over control by 7-21 days in the first season and by 6-19 days in the second season. However AgNO<sub>3</sub> at 100 µM treatment was more effective than other treatments in recording the highest days in fruit maturity delaying (21 and 19 days in the first and the second seasons, respectively). Additionally, CaCl<sub>2</sub> at 2.0% came next in delaying Fuerte fruit maturity than control (19 and 18 days in the first and the second seasons, respectively).

Fruit drop% greatly decreased with application of AgNO<sub>3</sub> at 100 µM and CaCl<sub>2</sub> at 2.0%, such treatments were more effective than the others and recorded the least fruit drop%. However, a great increase in the number of fruits/tree were recorded by all treatments compared to the control and the highest number of fruits / tree in the first season (420 and 406.7) were obtained with AgNO<sub>3</sub> at 100 µM and CaCl<sub>2</sub> at 2.0% respectively. An opposite trend was found in fruit weights as affected by the applied treatments where the control trees produced higher fruit weight than all applied treatments. This finding could be attributed to the

great effect of applied treatments in increasing the number of fruit/tree and consequently producing small fruits. Finally, total yield/tree increased significantly with all applied treatments than control in both studied seasons, untreated trees produced 96.7 Kg/tree in the first season compared to 123.7 Kg with AgNO<sub>3</sub> at 100 µM.

Concerning fruit ripening Zhang et al. (2013) and Liu et al. (2015) showed that ethylene signals count on specific receptor that in turn regulate the genes underlying ripening after harvest. Ethylene is an effective auxin transport inhibitor and motivates the activity and synthesis of hydrolysis-enzymes like polygalacturonase, cellulase and pectin methyl-esterase that degrade cell walls in the area of stem detachment, leaving the fruit attached to the tree only during vascular sutures, which can be easily broken. Therefore, it is a factor in controlling fruit drop and abscission (Bonghi et al., 1992). The use of ethylene inhibitors that decrease ethylene biosynthesis would be salutary to minimize fruit drop (Jobling et al., 2003).

### Physical fruit properties

Data presented in Table (2) show the effect of some ethylene inhibitors on some physical fruit properties of Fuerte avocado fruits during 2019 and 2020 seasons.

No clear trend was obtained for fruit length except the untreated trees produced fruits with higher length than all applied treatments. However, the treatments of AVG at 100 ppm, AgNO<sub>3</sub> at 200 µM and SA at 1.0 mM were similar to control, whereas the other treatments recorded less fruit length values than control. Data on fruit diameter showed a similar trend to those found

**TABLE 1. Effect of ethylene inhibitors on the yield attributes of «Fuerte» avocado fruits during 2019 and 2020 seasons**

Treatments	Maturity delay in days than control		Fruit drop		Number of fruit/tree		Fruit weight (g)		Total yield Kg/tree	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
			%	%			(g)	(g)		
Control	--	--	60.3 a	62.6 a	294.0 f	304.0 f	328.8 a	324.4 a	96.7 e	98.6 e
AVG 100 ppm	8	11	55.0 b	57.2 bc	333.7 d	348.0 d	314.2 b	321.6 b	104.8 d	111.9 c
AVG 200 ppm	17	19	46.7 de	49.2 e	395.0 b	413.0 b	279.7 d	274.3 de	110.5 c	113.3 c
Ag NO <sub>3</sub> 100 µM	21	19	43.3 f	45.0 f	420.0 a	447.0 a	294.5 c	269.4 e	123.7 a	120.4ab
Ag NO <sub>3</sub> 200 µM	11	14	54.6 b	58.3 b	336.4 d	339.0 de	306.0 b	313.7 b	102.9 d	106.3 d
SA 1.0 mM	7	6	57.5 ab	60.1 ab	315.0 e	324.3 e	311.4 b	318.5 b	98.1 e	103.3 d
SA 2.0 mM	14	12	51.4 c	52.3 d	360.0 c	387.7 c	283.6 d	303.5 c	101.9de	117.7 b
CaCl <sub>2</sub> 1.0%	13	15	49.3 cd	54.1 cd	375.7 c	373.3 c	295.3 c	298.6 c	111.2 c	111.5 c
CaCl <sub>2</sub> 2.0%	19	18	45.1 ef	47.3 ef	406.7ab	428.3ab	288.2cd	291.3 d	117.2 b	124.8 a

Values followed by the same letter (s) of each column are not significantly different at 5% level  
AVG: Aminoethoxyvinylglycine & SA: Salicylic acid

in fruit length. However, the treatments of AVG at 100ppm and SA at 1.0mM produced fruits similar in diameter to the control, whereas other treatments produced fruits with less diameter than the control.

Regarding fruit shape, all applied treatments were superior to the control, except the treatment of SA at 1.0mM was less effective compared to the control. It is well known that the increase in fruit shape is favorite for Fuerte avocado fruits. Flesh% data showed an evident increase with all applied treatments than control and the great effect in this respect was obtained with the treatments of CaCl<sub>2</sub> at 2.0% (87.2%) and AVG at 200 ppm (86.3%) in the first season of study. However, the other treatments exhibited flesh% values similar to or slightly high than the control. Additionally, fruit firmness of Fuerte avocado fruits greatly increased with all applied treatments compared to control except treatments of SA at 1.0mM which was similar to control. However, the highest values of fruit firmness (16.7&17.4 and 16.3&17.7 Kg/force) were obtained with AgNO<sub>3</sub> at 100 µM and CaCl<sub>2</sub> at 2.0% in both seasons, respectively.

Fruit softening is a main factor in determining fruit quality and post-harvest period, ethylene plays a necessary role in softening. Payasi et al., (2009) explained that the suppression of ethylene production is a potent instrument in delaying the softening events by acting on either ACC oxidase or ACC synthase, consequently inhibition of cell wall degradation events. They also explained that, the modified proteins of the cell wall that

participate in the dismantling of the various elements depend on the ethylene receptors.

Regarding silver nitrate and its effect to inhibit the action of ethylene Burkhead et al., (2009) explained that, copper is present in cells as a cofactor for many proteins especially proteins involved in electron transport and enzymes involved in redox reactions. When adding silver ions, there is an increased sensitivity to copper chelates. It replaces copper ions with silver and in turn inhibits by blocking the signals because silver ions are 70% larger than copper ions. Also, Ma et al., (2010) showed that the density of silver ions prevents the necessary harmonic change in response to ethylene. Davood (2015) indicated that, the other hypothesis is that the silver receptor complex is not stable like the copper receptor complex which leads to a faster degradation of ethylene

#### *Chemical fruit properties*

As it shown in Table (3) chemical fruits prosperities of Fuerte avocado greatly affected with ethylene inhibitors treatments during 2019 and 2020 seasons .TSS values showed that all applied treatments recorded less values than control this could be attributed to the fact that ethylene inhibitor treatments caused delays in fruit maturation which affect TSS contents. However, the treatment of AgNO<sub>3</sub> at 100 µM and CaCl<sub>2</sub> at 2.0% recorded the less TSS% and this finding was correlated with maturity delaying by about 19-21 days than control in both seasons. Total acidity% was decreased with untreated trees and exhibited

**TABLE 2. Effect of ethylene inhibitors on the physical fruit properties of “Fuerte” avocado fruits during 2019 and 2020 seasons**

Treatments	Fruit length		Fruit diameter		Fruit shape		Flesh %		Firmness	
	(cm)		(cm)		(L /D ratio)				Kg/force	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Control	14.7 a	14.5 a	8.9 a	8.6 a	1.65 d	1.76 bc	79.8 d	80.7 c	9.7 e	11.1 e
AVG 100 ppm	14.5 a	14.3 ab	7.4 d	8.1 ab	1.73 c	1.77 bc	81.9 cd	81.5 c	12.5 d	13.6 d
AVG 200 ppm	13.2 c	13.2 cd	7.2 d	7.3 cd	1.89 a	1.81a-c	86.3 a	85.1 ab	15.8 a	16.3 b
Ag NO <sub>3</sub> 100 µM	13.9 bc	13.0 d	7.3 d	6.8 d	1.90 a	1.88 a	85.5 ab	86.1 a	16.7 a	17.4 a
Ag NO <sub>3</sub> 200 µM	14.1 ab	14.5 a	8.1 bc	7.9 bc	1.74 c	1.84 ab	81.3 cd	81.4 c	13.2 cd	13.8 cd
SA 1.0 mM	14.2 ab	14.6 a	8.7 a	8.4 ab	1.63 d	1.74 c	80.4 d	80.9 c	10.8 e	12.3 e
SA 2.0 mM	13.2 c	14.0 bc	7.3 d	7.6 c	1.81 bc	1.84 ab	83.2 bc	83.0 bc	14.4 b	15.9 d
CaCl <sub>2</sub> 1.0%	13.6 bc	13.7 b	7.7 cd	7.6 c	1.77 bc	1.80 bc	82.4 cd	82.6 bc	14.1 bc	14.7 c
CaCl <sub>2</sub> 2.0%	13.5 bc	13.4 cd	7.4 d	7.1 d	1.82 ab	1.89 a	87.2 a	84.6 ab	16.3 a	17.7 a

Values followed by the same letter (s) of each column are not significantly different at 5% level  
AVG: Aminoethoxyvinylglycine & SA: Salicylic acid

an evident trend to those found in TSS%. Both TSS and total acidity are considered an indicator for maturity indices, so all applied treatments which inhibited ethylene production or ethylene action delayed Fuerte avocado fruit maturation more than control and this delayed parallel with a reduction in TSS and acidity contents.

L-ascorbic acid ranged from 13.56 - 15.11 mg/100 g fruits in control Fuerte avocado fruits increased to 14.12 - 20.42 mg. / 100g fruits due to the applied treatments. However, the treatments of AgNO<sub>3</sub> at 100 µM was superior than other treatments in recording the highest values of L-ascorbic acid in both studied seasons.

Total chlorophyll in fruit peel positively increased with all applied treatments than control due to the effect of the applied treatments on delaying fruit maturation and preserving chlorophyll molecular from deterioration. The great effect in this respect was recorded by AgNO<sub>3</sub> at 100 µM which recorded the highest values of total chlorophyll in Fuerte avocado fruits. However, total carotenoids in Fuerte avocado pulp fruits exhibited an opposite trend to those found in total chlorophyll of fruit peel. The great effect in this respect was recorded by CaCl<sub>2</sub> 2.0% in first season and AgNO<sub>3</sub> at 100 µM in second season of study.

With respect to the reduction of TSS and total sugars when applying AgNO<sub>3</sub> Yuan and Carbaugh (2007) illustrated that, it may be due dawdling down to the conversion of starch to sugar by inhibiting ethylene biosynthesis. Meanwhile, Sigal-Escalada (2006) reported that the increase of acidity can be attributed to delaying the ripening process and reducing fruit respiration. In another study, the results by Ishaq et al., (2009) indicated that the non-decreased of ascorbic acid is due to delaying or inhibition the conversion of dehydrascorbic to diketogulonic acid by using ethylene inhibitors. Thus preserving the content of ascorbic acid (Davarynejad et al., 2013). Additionally, Cox et al., (2003) mentioned that, the changes in chlorophyll and carotene content are involved in the color changes that occur in avocados during fruit growth and after harvest as treatment with ethylene inhibitors led to green color retention for a longer period compared to control. Also Hershkovitz et al., (2005) attributed that decreasing activity of the chlorophyllase enzyme regulates the loss of green color in many varieties of avocado.

#### *Fruit mineral contents*

A tested effect of the applied treatments on P, K, Ca and Mg contents of Fuerte avocado fruits was recorded in Table (4). An evident increase in the four studied elements by all applied treatments

**TABLE 3. Effect of ethylene inhibitors on the Chemical fruit properties of “Fuerte” avocado fruit during 2019 and 2020 seasons**

Treatments	TSS		Total Acidity		L-ascorbic Acid		Total Chlorophyll		Total carotenoids	
	%		%		mg/100 ml juice		(mg/100 g fruit peel)		(mg/100 g fruit pulp)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> Season	2 <sup>nd</sup> season
Control	8.22 a	7.97 a	0.64 d	0.69 d	13.56 e	15.11 e	1.55 e	1.68 d	0.671 a	0.624 a
AVG 100 ppm	6.74 b	6.53 bc	0.69 cd	0.72 cd	14.37 ef	17.16 cd	1.71 cd	1.75 cd	0.608 b	0.563 bc
AVG 200 ppm	5.23 de	5.10 de	0.80 ab	0.83 ab	17.86 ab	20.06 ab	1.97 a	1.94 b	0.484 d	0.453 ef
Ag NO <sub>3</sub> 100 µM	4.88 e	4.74 e	0.83 a	0.88 a	18.54 a	20.42 a	2.02 a	2.16 a	0.463 de	0.396 f
Ag NO <sub>3</sub> 200 µM	5.84 c	6.25 c	0.74 bc	0.77 bc	15.20 de	17.52 cd	1.78 cd	1.81 c	0.513 cd	0.526 cd
SA 1.0 mM	7.66 a	7.11 b	0.66 cd	0.73 cd	14.12 ef	16.24 de	1.67 d	1.73 cd	0.622 ab	0.614 ab
SA 2.0 mM	5.56 c	5.21 de	0.77 ab	0.81 ab	16.80 bc	19.11 ab	1.84 bc	1.97 b	0.557 c	0.491 de
CaCl <sub>2</sub> 1.0%	6.12 bc	5.72 cd	0.75a-c	0.79 bc	16.23 cd	18.68 bc	1.77 cd	1.86 bc	0.534 c	0.552 bc
CaCl <sub>2</sub> 2.0%	4.76 e	4.48 e	0.81 ab	0.86 a	18.22 ab	19.67 ab	1.94 ab	2.10 a	0.422 e	0.415 f

Values followed by the same letter (s) of each column are not significantly different at 5% level

AVG: Aminoethoxyvinylglycine & SA: Salicylic acid

than control in both studied seasons was obtained. However, the treatment of AgNO<sub>3</sub> at 100 µM was superior to other treatments in recording the highest values of P, K and Mg contents, whereas AVG at 200 ppm treatment was superior in Ca content.

The increase in mineral contents of Fuerte avocado fruit due to the applied treatments of ethylene inhibitors treatments which delay fruit maturation and consequently preserve fruit mineral contents from loss.

Regarding health and nutritional benefits, Bergh, (1992) described avocado as a functional food due to its additional health benefits from some phytochemicals. It contains high amounts of vitamins A, B, C, E and other nutrients such as folacin, niacin, iron (Fe), and magnesium (Mg) and contains 60% more potassium than bananas.

#### *Fruit Physiological attributes*

It is clear from the data in Table (5) that ethylene production, respiration rate, PPO and PME enzymes activates greatly reduced with all applied treatments than control in both seasons. However, ethylene production decreased from 46.12 – 49.57 µL/ Kg/ hr. in control fruits to 24.38-44.38 57 µL/ Kg/ hr., as an average for applied fruits. The least values of ethylene production were recorded by AgNO<sub>3</sub> at 100 µM (27.59 µL/ Kg/ hr.) in the first season and AVG at 200 ppm (24.84 µL/ Kg/ hr.) in the second season. Additionally, the respiration rate decreased from 85.42 – 91.80

mg CO<sub>2</sub>/Kg/ hr. in control fruits to 51.33- 45.71 mg CO<sub>2</sub>/Kg/ hr. for applied treatments. The least respiration rate values were recorded by AgNO<sub>3</sub> at 100 µM treatment (51.33 mg CO<sub>2</sub> / Kg/ hr.) in the first season and AVG at 200 ppm treatment (45.71 mg CO<sub>2</sub> / Kg/ hr.) in the second season. PPO and PME enzymes activates showed that all applied treatments decreased them than control in significant effects in both studied seasons, The treatments of AgNO<sub>3</sub> at 100 µM recorded the least values of PPO and PME enzymes activates in the first season whereas the treatment of AVG at 200 ppm exhibited the least values of both enzymes activities in the second season.

It is evident from previous studies by Galvis-Sánchez et al. (2006) and Wang and Wang (2011) that fruit browning disorder is influenced by pre-harvest factors like ripeness, harvest date and fruit size. Also, Kou et al. (2015) believed that browning the fruit is associated with damage to the membrane integrity. The antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD) protect the safety of the membrane from damage by scavenging H<sub>2</sub>O<sub>2</sub> superoxide and more free radicals. However, Camp et al. (1997) obtained that appropriate concentrations of AgNO<sub>3</sub> prevent brown discoloration by reducing polyphenol oxidase (PPO) activity and enhancing antioxidant activities in CAT and POD.

A great effect of calcium elemental studied by Gao et al. (2019) they explain that external

**TABLE 4. Effect of ethylene inhibitors on Fruit mineral contents P, K, Ca and Mg of “Fuerte”avocado fruit during 2019 and 2020 seasons**

Treatments	P		K		Ca		Mg	
	g/ 100 gD.wt.		g/ 100 gD.wt.		mg/ 100 gD.wt.		mg/ 100 gD.wt.	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
	Season	season	season	season	Season	season	Season	season
Control	0.14 d	0.16 c	0.465 d	0.427 d	30 d	29 d	21 d	24 d
AVG 100 ppm	0.16 cd	0.16 c	0.516 cd	0.513 cd	37 cd	36 cd	25 cd	25 cd
AVG 200 ppm	0.22 ab	0.20 ab	0.681 ab	0.615 ab	52 a	48 a	32 ab	36 a
Ag NO <sub>3</sub> 100 µM	0.24 a	0.22 a	0.731 a	0.691 a	47 ab	44 ab	34 a	38 a
Ag NO <sub>3</sub> 200 µM	0.18 bc	0.19 ab	0.546 b-d	0.511 b-d	41 bc	38 bc	27 bc	31 b
SA 1.0 mM	0.15 cd	0.17 bc	0.502 cd	0.459 cd	32 cd	33 d	23 d	26 cd
SA 2.0 mM	0.21 ab	0.20 ab	0.642 ab	0.563 bc	48 ab	43 ab	35 a	30 bc
CaCl <sub>2</sub> 1.0%	0.19 bc	0.18 bc	0.594 bc	0.573 bc	45 b	40 bc	26 cd	28 b-d
CaCl <sub>2</sub> 2.0%	0.21 ab	0.22 a	0.663 ab	0.678 a	51 a	41 a-c	29 bc	33 ab

Values followed by the same letter (s) of each column are not significantly different at 5% level

AVG: Aminoethoxyvinylglycine & SA: Salicylic acid



TABLE 5. Effect of ethylene inhibitors on the physiological attributes of “Fuerte” avocado fruits during 2019 and 2020 seasons

Treatments	Ethylene production		Respiration rate		PPO activity		PME activity	
	$\mu\text{LC}_2\text{H}_4 \text{ Kg}^{-1}$		$\text{mg CO}_2 \text{ Kg}^{-1}$		(OD420 min <sup>-1</sup> mg protein <sup>-1</sup> )		(Unit mg <sup>-1</sup> protein)	
	1 <sup>st</sup> Season	2 <sup>nd</sup> season	1 <sup>st</sup> Season	2 <sup>nd</sup> Season	1 <sup>st</sup> Season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Control	49.57 a	46.12 a	91.80 a	85.42 a	1.11 a	0.935 a	0.308 a	0.294 a
AVG 100 ppm	42.12 bc	37.08 b	77.89 b	68.41 b	0.859 bc	0.751 b	0.278 ab	0.241 b
AVG 200 ppm	31.38 e	24.84 e	59.16 d	45.71 e	0.638 ef	0.488 d	0.207 de	0.158 d
Ag NO <sub>3</sub> 100 $\mu\text{M}$	27.59 e	25.76 e	51.33 e	47.29 e	0.528 f	0.501 d	0.175 e	0.163 d
Ag NO <sub>3</sub> 200 $\mu\text{M}$	38.59 cd	34.69 bc	70.17 c	63.52 bc	0.763 cd	0.694 b	0.247 bc	0.224 b
SA 1.0 mM	44.38 b	42.93 a	82.18 b	79.19 a	0.914 b	0.877 a	0.294 a	0.284 a
SA 2.0 Mm	35.50 d	32.94 c	64.54 cd	60.78 c	0.711 de	0.652 bc	0.231 cd	0.211 bc
CaCl <sub>2</sub> 1.0%	38.11 cd	30.11cd	68.41 c	56.94 cd	0.787 cd	0.585 cd	0.243 bc	0.189 cd
CaCl <sub>2</sub> 2.0%	30.24 e	27.07 de	57.85 de	50.13 de	0.577 f	0.532 d	0.188 e	0.171 d

Values followed by the same letter (s) of each column are not significantly different at 5% level

AVG: Aminoethoxyvinylglycine & SA: Salicylic acid

application of calcium can affect protein, cell membrane fluidity, chlorophyll content and respiration rates, which are key factors for senescence. Also, Zhang and Wang, (2019) added that calcium plays an essential role in maintaining the cell wall structure in fruits thus calcium treatment can effectively maintain the fruit and delay fruit ripening and softness. It is involved in regulating cell wall-degrading enzyme activity by influencing the activity of the membrane-bound enzyme including pectatelyase, inhibition of lipid peroxidation in the membrane and by improving tissue antioxidant capacity (Ortiz and Lara 2008). Moreover, Xu et al., (2014) extracted that calcium influences the pH level in the cell by regulating enzyme activities and by regulating the expression of genes. Accordingly, calcium treatment can enhance the cumulating of total phenols in fruits, improve and maintain fruit quality.

#### Dry matter and oil properties

As shown in Table (6), the dry matter % of Fuerte avocado fruits greatly affected with ethylene inhibitor applied treatments. All applied treatments increased dry matter percentage than control and the highest dry matter% were recorded by AgNO<sub>3</sub> at 100  $\mu\text{M}$  and AVG at 200 ppm (30.7 % in the first season and 29.4% in the second season respectively). However, an evident increase in fruit oil % was obtained with the applied treatments than control in both studied seasons. The treatment of AgNO<sub>3</sub> at 100  $\mu\text{M}$  was superior to other treatments in recording

the highest values of fruit oil % in both seasons. However, oil % in Fuerte avocado fruits ranged from 15.2- 17.6 % in control fruits against 17.6-21.8 % for other treatments. However, iodine values slightly affected with different treatments and were non-significant either between different treatments or compared with control. On the other hand, peroxide values were decreased with the applied treatments than control but the effect was clear only in the first season. The least peroxide value (2.07) was recorded by AgNO<sub>3</sub> at 100  $\mu\text{M}$  treatment in the second season.

Several studies have indicated that avocado oil's contents efficiency is due to the fact that it is oil rich in unsaturated fatty acids, approximately 70% making it suitable for direct human consumption, as well as excellent fats in diets designed to reduce cardiovascular disease (Ozdemir and Topuz 2004). In this respect, the oil content of the mesocarp increases a few weeks after the fruit set and this is related to the fruit age. With an rise in the oil content, the water content reduce by the same amount (Villa-Rodríguez et al., 2011). The content of avocado oil depends on some factors including the varieties, fruit maturity stage and climatic conditions (Donetti and Terry, 2014), the oil content and the dry matter percentage are a major harvest indicators used to assess avocado ripeness (Gamble et al., 2010).

Regarding the peroxide value (PV), Perez-Camino et al. (2002) mentioned that, it is

TABLE 6. Effect of ethylene inhibitors on oil properties of “Fuerte” avocado fruit during 2019 and 2020 seasons

Treatments	Dry matter		Oil		Iodine value		Peroxide value	
	(%)		%		mg I <sub>2</sub> /100 g		meq O <sub>2</sub> /kg oil	
	1 <sup>st</sup> Season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Control	21.1 e	20.3 e	17.6 e	15.2 d	81.08 a	78.64 a	3.11 ab	3.29 a
AVG 100 ppm	23.4 d	21.5 e	19.2 d	15.9 d	77.41 a	75.77 a	3.13 ab	3.15 a
AVG 200 ppm	28.4 bc	29.4 a	20.7 b	21.4 a	81.72 a	74.52 a	2.65 b	2.65 a
Ag NO <sub>3</sub> 100 µM	30.7 a	28.7 ab	21.8 a	20.6 a	79.34 a	82.36 a	2.68 b	2.07 a
Ag NO <sub>3</sub> 200 µM	24.3 d	24.7 d	19.8 cd	18.5 bc	76.55 a	78.09 a	3.02 ab	2.91 a
SA 1.0 mM	22.6 de	20.8 e	18.3 e	16.0 d	80.16 a	75.35 a	2.97 ab	2.91 a
SA 2.0 mM	27.1 c	26.6 c	20.6 b	19.0 b	78.46 a	79.81 a	3.39 a	3.19 a
CaCl <sub>2</sub> 1.0%	26.5 c	24.1 d	20.3 bc	17.6 c	82.58 a	81.06 a	2.93 ab	2.84 a
CaCl <sub>2</sub> 2.0%	30.2 ab	27.3 bc	21.1 ab	18.8 b	76.48 a	80.84 a	2.84 b	3.23 a

Values followed by the same letter (s) of each column are not significantly different at 5% level

AVG: Aminoethoxyvinylglycine & SA: Salicylic acid

significant test for oil storage, with stability in terms of minimal hydrolysis and lipase activities. Human (1987) explained that, Peroxide serves as a useful indicator of the oxidation extent of fats, lipids and oils. Another study by Haddada et al., (2007) reported that, it is vastly used as a measure of undesirable reactions in food stuffs and oils. Similarly Otaigbe et al., (2016) describe the iodine values as the amount of unsaturation in fatty acids. It also determines the oxidation value of fatty acids and is fairly accurate for samples that do not contain a large proportion of conjugate double bonds.

### Conclusion

Spraying Fuerte avocado trees by 200ppm AVG or 100 µM Ag NO<sub>3</sub> twice after 30 and 75 days from fruit set synergistically reduce fruit drop, delay fruit ripening and enhanced fruit quality either mineral content or oil properties.

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## تأثير مثبطات الإيثيلين على تنظيم نضج وجودة ثمار الأفوكادو

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يلعب الإيثيلين دوراً رئيسياً في تعزيز نضج الثمار ، وبالتالي فإن تغيير إشارات التخليق الحيوي يمكن أن يكون وسيلة مهمة لتأخير هذه العملية. خلال موسمين ٢٠١٩، ٢٠٢٠ تم اختبار عدد من المركبات الكيميائية المثبطة للإيثيلين لتنظيم إنتاج الإيثيلين الأفوكادو صنف فيورت مع دراسة الخصائص الفيزيائية والكيميائية والفسولوجية للثمار بالإضافة إلى دراسة خصائص الزيت.

تفوقت ثمار الأفوكادو المعاملة ب ٢٠٠ جزء في المليون نترات الفضة أو ١٠٠ ميكرومول أمينو إيثوكسي فينيل جليسين مع وجود إختلافات بسيطة بينهم وتبع ذلك المعاملة بكلوريد الكالسيوم ٢,٠٪. تم رش الأشجار بالمعاملات مرتين بعد ٣٠ و ٧٥ يوم من تمام العقد فتحسنت كل الخصائص المختبرة فمثلاً قل تساقط الثمار وزاد المحصول وتحسنت الصفات الثمرية وتأخر النضج عن باقي المعاملات أو الكنترول. عموماً كانت مثبطات إنتاج الإيثيلين فعالة في تقليل ليونة الثمار وتغير اللون. على العكس من ذلك كل المعاملات المستخدمة قللت إنتاج الإيثيلين وتأثيره وأخرت نضج الثمار مع تقليل المواد الصلبة الذائبة والحموضة. معيقات إنتاج الإيثيلين خاصة نترات الفضة بتركيز ١٠٠ ميكرومول زادت من محتوى الثمار من الفوسفور والبوتاسيوم والمغنيسيوم والكالسيوم وحافظت على تلك العناصر من الفقد. بالنسبة لصفات الثمار الفسيولوجية وخصائص الزيت قللت المعاملة باستخدام ٢٠٠ جزء في المليون نترات الفضة أو ١٠٠ ميكرومول أمينو إيثوكسي فينيل جليسين من إنتاج الإيثيلين ومعدل التنفس و نشاط إنزيمي بكتين ميثيل استريزو بولى فينول اوكسيديز مع زيادة نسبة المواد الجافة ونسبة الزيت وتأثير بسيط على قيم كل من البيوركسيديز واليود.

**الكلمات الدالة:** مثبطات الإيثيلين، أفوكادو صنف فيورت، نضج الثمار، حمض الساليسيليك، نترات الفضة، أمينوإيثوكسي فينيل جليسين، كلوريد الكالسيوم.