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Comparative Study on Organic Calcium Diffusion and Content in Apples and their Influence on "Anna" Apple Fruits Quality

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Keywords: organic calcium, calcium lignosulfonate, calcium acetate, calcium foliate, calcium content, apple quality.

I. Introduction

Calcium is very important essential element for plants as well as for living been. Its sufficiency is very crucial for the integrity of cell membranes and for the cell wall which prolong the shelf life of fruits and preserve the firmness of the plant tissue. No wonder, the deficiency of calcium results in many physiological disorders such as bitter pit of apples, internal breakdown of tissues spotted of lenticels, water core of apple fruits and superficial scorch or scald. Moreover, calcium increases the tolerance to many abiotic stresses (McAinsh and Pittman, 2009; Dodd *et al.*, 2010; Sarwat *et al.*, 2013; Liu *et al.*, 2018). There are variations in the ability of calcium compounds to diffuse across the cuticles. It was found that Ca absorption in the form of nitrate or formate was more active than its application as chloride or acetate across citrus leave (Song *et al.,* 2006). The mobility of various calcium compounds in another critical factor since calcium ions are immobile. However, the application of the organic form of calcium such as acetate, citrate, formate or lignosulfonate increase calcium mobility within the plant and enhances its efficacy and even the speed of its action (Wojcik 2013). It was reported that calcium acetate speed of action is very high and even described as immediate as compared with other form such as chloride (Borchert 1986 and Treesubsuntorn and Thiravetyan 2019). Furthermore, Ca lignosulfonate was reported as a beneficial compound that is mobile, water soluble, increases chlorophyll and sugar content. It is a nutrient based on lignin which affect its rate of break-down, slowing down the rate of release and non-volatile. Thus, it is an important component in the manufacturing of many fertilizers and pesticides especially slow release fertilizers. Even in the case calcium formate it was reported that calcium forms a complex with formate and it is completely soluble in water. Moreover, it was mentioned that calcium formate can be easily assimilated by the roots and trans-located in an acropetal sense (Hanger,1979). Aforementioned, it was reported that applying pre-harvest foliar calcium spray has been a standard practice to control fruit Ca concentrations in certain deciduous fruit trees. Conversely, the effects of foliar Ca spray on the Ca concentration in fruit and the incidence of disorder were inconsistent. Foliar Ca sprays reduced bitter pit, enhanced fruit firmness and storage time, and sometimes reduced the incidence of brown rot during storage of apples, reduced internal browning during storage and the incidence brown rot in peaches (Yamane, 2014). Three possible reasons were reported to explain the reasons for inconsistencies of foliar Ca replication. First, was the environmental conditions particularly air humidity in areas of high temperature and low relative air humidity. Second, the uneven distribution of Ca in fruit within the canopy especially fruit at the top. Third, the condition and management of a tree fruit Ca concentration is affected by fruit size. Thus, the objectives of this study were to provide some

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information about effect of spraying some organic calcium ability to diffuse and change internal Ca concentration in "Ann" apple fruit whether applied individually or along with ethanol or small concentration of urea as adjuvants and the accompanied change in main quality parameters at harvest.

II. Materials and Methods

The present study was conducted during the two successive seasons 2021 and 2022 on Anna apple cultivar (*Malus domestica*). The trees were 4-years-old spaced at 4×5 m and grown under drip irrigation system in a private orchard at Nubaria region, Beheira Governorate, Egypt. Trees had been under the standard agricultural practices throughout the season. Soil texture was sandy and drip irrigation system was adopted. Treatments were arranged in a completely randomized block design. Three replications were used for each treatment and one apple tree represented one replication, thus twenty four trees were employed to this study in each season. Twenty four standard "Anna" apple trees were randomly assigned to receive one of eight treatments and applied as spraying two times at rapid elongation and at maturity. The treatments included; tap water spray (as the control), Ca lignosulfonate at (0.2%), Ca Lignosulfonate at (0.4%) + Ca foliate at (0.2%), Ca foliate at (0.4 %) + Ca acetate at (1%), Ca acetate at (1%) + urea at (1%) and Ca acetate 1% + ethanol 2%. The non-ionic surfactant Top film at 0.05% (*V/V*) was added to all treatments to reduce the surface tension and to increase the contact angle of sprayed droplets. These treatments were arranged in Analysis of Variance (ANOVA) for Randomized Complete Block Design (RCBD).

a) *Physical Characteristics*

Samples of five fruits per tree were collected randomly and the fruit weight was measured using an electronic balance and fruits diameters. Fruits diameter were measured by vernier caliper.

b) *Chemical Characteristics*

Acidity in the fruits juice was assayed 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 soluble solids (TSS %) was estimated using Galli 110 Refractometer according to *(A. O. A. C.; 1975).*

Determination of Anthocyanins:

Extracts were prepared by the method described by (Onayemi *et al.* 2006). 1g fruit skin samples were pulverized with 20 ml of 85 Ethanol and 1.5 M HCL (by volume) solution. The samples were covered and kept overnight in the deep freeze. The extracts were completed to 50 ml of the solvent and then absorbance of the solution was measured at a wave length of 530 nm, using spectrophotometer (Unico 1200-USA). Result is expressed as mg/100 g of fresh fruit.

Total anthocyanin was calculated using the succeeding equation developed by (Lees and Franci 1971).

$$\text{Total anthocyanins (mg/100g)} = A_{530} \times V / 98.2 \times W$$

Where: A530 is the rate of absorption of the sample at the wavelength of the subtitle A. for example, A530 is the absorption at a wavelength of 530 nm, V= total volume of extract (ml), W= weight of fresh sample (g).

Ca content (skin) and (flesh) was determined photometrically using flame photometer as described by (peterburgski 1968).

Determination of vitamin C content is determined using UV spectroscopy. In this method according to (Desai and Desai, 2019). Bromine water is added which oxidizes the ascorbic acid into dehydroascorbic acid. 2, 4 dinitrophenyl hydrazine gives coupling reaction at 37°C temperature for 3 hours. After 3 hours solution is treated with 85% H2SO4 which gives coloured complex and the absorbance was measured at 491nm.

c) *Statistical Analysis*

Data of the first experiment was analyzed as a Randomized Complete Block Design (RCBD) with three replications. Comparisons among means were made *via* the least significant difference (LSD) at 0.05 level according to Snedecor and Cochran (1980). The data was analyzed using SAS (2009).

III. Results

a) *Calcium Content in the Apple Skins*

The influence of various calcium compounds on the skin and flesh contents of apples cv "Anna" at harvest was shown in table 1. The data indicated that the highest calcium content in the skin was found with the treatment of Ca Acetate at (1%) plus ethanol at 2% (v/v) as compared with the control and all other treatments. Meanwhile, the application of Ca Acetate (at 1%) alone resulted in lower calcium content than Ca Acetate plus urea (at 1%) which in turn gave less calcium in the apple skin when compared with Ca Acetate in the presence of ethanol (at 2%). These trends were consistent for both seasons. Moreover, Ca foliate at 0.2% or at 0.4% applications resulted in greater calcium content in apple skins than that obtained in the control in both seasons. In addition, calcium lignosulfonate resulted in greater calcium content than that found in the comparable concentration of Ca foliate in both seasons.

b) *Calcium Content in the Apple Flesh*

Changes in calcium content in the flesh of "Anna" apple inresponse to various used treatments were reported in table 1. The data indicated that using the same compound, namely Ca Acetate at the same concentration but with different enhancers of diffusion across the fruit cuticle showed that ethanol at 2 % (v/v) was more effective than urea at 1% on enhancing the diffusion of calcium, while both compound had greater Ca acetate diffusion than the individual use of Ca acetate in the absence of any included enhancers. Moreover, Ca foliate at 0.4 % had higher Ca diffusion in the flesh as compared with Ca foliate at 0.2%. Meanwhile, both compound concentrations (0.4 or 0.2 %) resulted in higher calcium content in the flesh as compared with the control. Moreover, that was the data trend when we compare calcium content in the apple flesh caused by Ca lignosulfate at 0.4 or at 0.2% compared with the control. Higher the calcium compound concentration, high the diffused amount of calcium in both seasons.

c) Fruit Weight and Diameter

With regard to the changes in some physical characteristics in response to various applications in both seasons. The data in table 2 showed that fruit weight at harvest showed similar trends to that obtained with calcium since the greatest fruit weight was found with calcium acetate treatment plus ethanol follow by Ca acetate treatment in the presence of urea. The same concentration of calcium acetate. However, when used alone without the incorporation of any enhancer resulted in lower fruitweight than that in the presence of ethanol or urea. In a similar manner ca foliate at 0.4% resulted in greater fruit weight as compared with Ca foliate treatment at 0.2% that was also the case with Ca lignosulfonate at 0.4% and 0.2% when their influence on fruit weight was compared with each other and with the control. However, each of the lignosulfonate compound was able to cause a greater fruit weight than that found was calcium foliate concentration at 0.2 % in a consistent manner in both seasons. Regarding the changes in fruit diameter at harvest in response to various applications. The data in table 2 showed that both Ca acetate treatments alone with ethanol or urea were equally effective on increasing fruit diameter in both seasons. However, the sole application of calcium acetate caused a significant increase in such diameter only in the first season only when compared with the control. On the other hand, calcium lignosulfonate at 0.2 and 0.4 and calcium foliate at 0.2 and at 0.4, all had similar influence on fruit diameter at harvest. Meanwhile, the diameter of the control fruit did not significantly vary from that of some treatments such as Ca foliate (0.2%), Caacetate (1%).

d) Chemical Characteristics

i. Total Soluble Solids (TSS)

The response of total soluble solids to various pre-harvest treatments was reported in table 3. The data revealed that Ca acetate either plus ethanol at (2%, v/v) or plus urea at (1% w/v) resulted in the highest TSS when compared with the control or with the rest of the treatments. Meanwhile, the control fruits had the lowest TSS as compared with all other treatments in both seasons. Moreover, calcium acetate at the same concentration but used alone resulted in significant change in TSS when compared with the control in both seasons. Meanwhile, Ca foliate at both applied concentrations resulted in a significant increase in the TSS as compared with the control. Furthermore, Ca lignisulfonate at 0.4% resulted in greater TSS than that obtained with Ca lignosulfonate at 0.2% in the two seasons.

ii. Juice acidity

The effect of pre-harvest treatments of "Anna" apples with various calcium compounds on juice acidity was reported in table 3. The data indicated that the least acidity in both seasons was found with calcium acetate treatments whether along with ethanol at (2%) or plus urea at (1% w/v) as compared with the control or with all other treatments. Meanwhile, the two mentioned calcium acetate was used alone, juice acidity was significantly higher the combinations in both seasons. Calcium foliate, on the other hand, either at 0.4% or at 0.2% was able to cause a significant reduction in fruit acidity as compared with the control. Similar trend was obtained with calcium lignosulfonate at the two used concentrations at (0.4% and 0.2%) since they resulted in a significant reduction in juice acidity when compared with the control.

iii. TSS/Acidity Ratio

The response of the TSS to acidity ratio to various used applications before harvest was shown in table 3. The data revealed that many treatments were able to increase TSS/ Acidity of "anna" apples when compared with the control such as calcium lignosulfonate at both used concentrations at (0.2% and 0.4%). In fact, the highest ratio was obtained with Ca actate plus ethanol at 2%. In a similar manner, Ca acetate at (1%) plus urea at (1%) did not vary significantly from the above lignosulfonate at (0.4%).Even Ca foliate at (0.4%) was able to cause a significant increase in the TSS to acidity ratio as compared with the control in both seasons but not at 0.2%. On the other hands, Ca acetate plus urea resulted in a significant increase in TSS/Acidity ratio as compared with Ca acetate at (1%) alone. Such individual treatment of Ca acetate did not vary from the control. Thus, the increase in the applied concentration resulted in a significant change in the TSS to acidity ratio especially with the treatment of lignosulfonate while with Ca acetate, the addition of the effectiveness of applied Ca acetate.

iv. Vitamin C Content

Changes in vitamin C content in "anna" apple fruits in response to various applications were shown in table3. The data revealed that Ca acetate at (1%) plus ethanol at (2%) resulted in the highest vitamin C content followed by Ca acetate at (1%) plus urea at (1%). In general, most treatments had higher vitamin C content when compared with the control except with Ca acetate at (1%) in the second season. The increase in lignosulfonate concentration from 0.2% to 0.4% resulted in a parallel increase in vitamin C content but this was not the case with Ca foliate since the magnitude of the increase was slight. It could be concluded that the ability to diffuse across the fruit cuticle was not the critical factor since lignosulfonate molecule has a much greater value as compared with Ca foliate.

v. *Anthocyanin Content In Fruit Skin*

The effect of pre-harvest applications of various calcium organic compounds on anthocyanin content in the skin of "Anna" apples was reported in table 3. The data indicated that some treatments were effective on increasing anthocyanin in apples skin such as lignosulfonate at (0.4%) in both seasons as compared with the control. In a similar manner, the application of Ca acetate at (1%) whether plus urea or ethanol at (2%) resulted in higher anthocyanin content than that obtained with the control in both season. The application of Ca foliate resulted in a non-significant increase in anthocyanin except with Ca foliate at (0.4%)in the second season. The individual application of Ca acetate at (1%) did not result in a significant change in anthocyanin as compared with the control in both seasons.

Average of initial value of Calcium before treatments was 12.23 mg/100g and 3.00 mg/100g for the skin and the flesh, respectively in 2021 season, while in the 2022 seasons, these value were 11.86 mg/100g, 3.13 mg/100g for the skin and the flesh, respectively.

Table 1: The Effect of Various Organic Calcium Solution on Calcium Content of the Skin and the Flesh of "Anna" Apples at Harvest During the Two Seasons 2021 and 2022

Treatments	Ca (skin) (mg.100g^{-1})		Ca (flesh) (mg.100g^{-1})	
	2021	2022	2021	2022
Control	16.47^{h}	14.82^{h}	3.15^{h}	2.80^{h}
Ca lignosulfonate 0.2%	20.24^{e}	18.86^{e}	4.90^{e}	4.26^{e}
CaLignosulfonate 0.4%	23.36^{c}	21.56^{c}	6.12^{c}	5.32^{c}
Ca foliate 0.2%	18.71^{f}	17.49^{f}	4.38^{f}	3.78^{f}
Ca foliate 0.4 %	21.71^{d}	20.17^{d}	5.51^{d}	4.79^{d}
Ca acetate 1%	17.15^{g}	16.10^{g}	3.73^{g}	3.24^{g}
Ca acetate 1% + urea 1%	24.75^{b}	22.80^{b}	6.65^{b}	5.81^{b}
Ca acetate 1% + ethanol 2%	26.30^{a}	24.15^{a}	7.22^{a}	6.36^{a}
LSD $_{at\ 0.05}$	0.51	0.64	0.24	0.30

Values in each column when accompanied with similar letters, were not significantly different by using the least Significant Difference at 0.05 for comparing the means

Table 2: The Effect of Various Organic Calcium Solution on Some Physical Characteristics of "Anna" Apples at Harvest During the Two Seasons 2021 and 2022

Treatments	Weight of Fruit (g)		Fruit Diameter (cm)	
	2021	2022	2021	2022
Control	119.74^{g}	105.21^{g}	5.67^{e}	5.23^{c}
Calignosulfonate 0.2%	139.09^{e}	123.52^{e}	6.47^{bcd}	5.73^{abc}
Ca Lignosulfonate 0.4%	152.98^{c}	137.22^{c}	6.83^{abc}	6.10^{ab}
Ca folic 0.2%	131.94^{f}	117.52^{f}	6.23^{cde}	5.60^{abc}
Ca folic 0.4 %	145.75^{d}	130.69^{d}	6.53^{ad}	5.93^{abc}
Ca acetate 1%	126.91^{f}	113.72^{f}	6.03^{de}	5.37^{bc}
Ca acetate 1% + urea 1%	159.11^{b}	143.59^{b}	7.03^{ab}	6.10^{ab}
Ca acetate 1% + ethanol 2%	166.21^{a}	151.27^{a}	7.17^{a}	6.33^{a}
LSD $_{at\ 0.05}$	5.35	4.90	0.68	0.77

Values in each column when accompanied with similar letters, were not significantly different by using the least Significant Difference at 0.05 for comparing the means

Table 3: The Effect of Various Organic Calcium Solution on Some Chemical Characteristics of "Anna" Apples at Harvest During the Two Seasons 2021 and 2022

Treatments	TSS (%)		Acidity (%)		TSS/ Acidity (ratio)		Anthocyanin (mg.100g-1)		V.C (mg.100g-1)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
control	10.64^{g}	9.83^{e}	0.81^{a}	0.65^{a}	13.15^{f}	15.19^{e}	4.95^{c}	3.32^{e}	4.95^{f}	3.77^{g}
Ca lignosulfonate 0.2%	11.56^{e}	10.54^{c}	0.67^{bcd}	0.52^{bc}	17.38^{de}	20.35^{cde}	5.08^{abc}	3.53^{b-e}	5.57^{cd}	4.39^{de}
Ca Lignosulfonate	12.18^{c}	10.95^{b}	0.58^{de}	0.43^{de}	21.24^{bc}	25.55^{bc}	5.21^{ab}	3.67^{abc}	6.05^{b}	4.74^{bc}
Ca folic 0.2%	11.21^{f}	10.28^{d}	0.72^{abc}	0.57^{b}	15.70^{ef}	18.20^{de}	4.98^{bc}	3.46^{cde}	5.39^{de}	4.18^{ef}
Ca folic 0.4 %	11.84^{d}	10.73^{bc}	0.64^{cd}	0.48^{cd}	18.63^{cd}	22.45^{cd}	5.15^{abc}	3.63^{a-d}	5.80^{c}	4.54^{cd}
Ca acetate 1%	10.89^{g}	10.06^{de}	0.76^{ab}	0.60^{ab}	14.31^{f}	16.96^{e}	5.01^{bc}	3.43^{de}	5.19^{e}	3.97^{fg}
Ca acetate 1% + urea 1%	12.47^{b}	11.26^{a}	0.54^{e}	0.39^{e}	23.33^{b}	29.23^{ab}	5.25^{a}	3.76^{ab}	6.25^{ab}	4.95^{ab}
Ca acetate 1% + ethanol 2%	12.79^{a}	11.51^{a}	0.48^{e}	0.35^{e}	26.72^{a}	33.53^{a}	5.30^{a}	3.81^{a}	6.48^{a}	5.15^{a}
$LSD_{at\ 0.05}$	0.28	0.26	0.10	0.08	2.87	5.42	0.24	0.24	0.23	0.26

Values in each column when accompanied with similar letters, were not significantly different by using the least Significant Difference at 0.05 for comparing the means

IV. Discussion

Since calcium is one of the most important plant nutrients as it contributes to the integrity of plasma membrane and the firm structure of the cell wall. Providing fruits such as apple with calcium through foliar spray is an agricultural practice that is gaining more interest as field application that is more target oriented application especially in arid lands with relatively high soil PH and environmentally friendly fertilization method. It means directly delivering calcium to the target (the fruit) and reducing the needed amount. That means helping to reduce the environmental impact associated with soil fertilization. In spite of using organic calcium such as lignosulfonate, acetate or foliate that are highly water soluble. However, the response to foliar spray is variable that is because there are many factors that are involved in the diffusion process through the fruit cuticle of apples. The apple cuticle was found to contain many lenticels and many have some ultra-nutural cracks in addition to the stomata (Glenn *et al.,* 1985). Furthermore, it was proposed that there are "aqeous pores" (Schönherr, 2000), (Schönherr, and Schreiber, 2004) that are generated by the adsorption of water molecules to polar moieties located in the cuticlar membrane (Schreiber, 2005) such as an esterified carboxyl groups, ester and hydroxylic groups (chamel *et al .,* 1991) in the cutin network and carboxylic groups of pectic cell wall material (Schönherr and Huber, 1977). This hypothesis was supported by others on cuticular water sorption (luque *et al.,* 1995b). However, using adjuvants such as low concentration of urea or ethanol, in this study, helped increasing the penetration of calcium application in the form of calcium acetate. Thus, the individual application of calcium acetate was not as effective as its combinations. Enhancing the diffusion sprayed growth regulators such as ethephon was reported by other studies on the stomata's cuticle of the cranberry fruits and crimson seedless grapes (*farag et al.,*1985, 2012a and 2012b). Furthermore, the ability of lignosulfonate to function as a bio-stimulant dispersion material or bonding to fruit surface (Yang *et al.,* 2008, Elsawy*et al.,* 2022). On the other hand, calcium actate has the advantage of the high speed of action in plant tissues (Frizzell *et al.,* 2017). These organic salts of calcium are highly water soluble which enable calcium to translocate with in tissues and due to their safety, their use is allowed in organic agriculture. These organic calcium compounds have a high point of deliquescence (POD) which represent an obstacle against diffusion (Schönherr 2002). However, the mentioned pathways in the apple cuticle structure represent a major help to sprayed calcium solutions. In addition, sprayed droplets can be easily diffuse through the shoots and pedicles, then dissolved calcium in lignosulfonate, acetate or foliate move to various parts. Aforementioned, there has been an emphasis on the practical significance of foliar nutrition especially when applied at the right time as at the peak of nutrient demand times and to minimize the costs.

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