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Effect of Foliar Application of Naphthalene Acetic Acid (NAA) and Calcium Chloride on Vegetative Growth, Yield, Berry Cracking and Firmness in Grape (*Vitis vnifera* L.) "Autumn Royal"

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Abstract: A field experiment was performed during the seasons 2019 and 2020, to evaluate the effect of the foliar application of Naphthalene acetic acid (NAA) at three concentrations (25, 50, 75 ppm) and Calcium chloride ($CaCl_2$) at 1000 ppm, alone or in combination with each other on the vegetative growth, yield and its attributes along with the berry cracking and shattering phenomena of 'Autumn Royal' grape cultivar. Results showed that average yield and its attributes were higher in the combined treatment of Naphthalene acetic acid (NAA) at 75 ppm and Calcium chloride ($CaCl_2$) at 1000 ppm than the other combined or single treatments and the control whereas $CaCl_2$ at 1000 ppm treatment gave the highest significant values in term of TSS%, TSS/acid ratio and anthocyanin. In addition, values of berry cracking were less than 15% using (NAA) at 75 ppm and Calcium chloride ($CaCl_2$) at 1000 ppm followed by Naphthalene acetic acid (NAA) at 50 ppm and Calcium chloride ($CaCl_2$) at 1000 ppm treatment. However, after eliminating the cracked berry, the net yield was significantly higher than the control. This result suggests that treated the vines by Naphthalene acetic acid (NAA) at 75 ppm and Calcium chloride ($CaCl_2$) at 1000 ppm could have a beneficial effect on berry cracking due to the change in vine canopy microclimate through increasing vegetative growth that decrease berry temperature resulting in lowering the pressure inside the berries as higher temperatures and pressures caused permanent berry plastic deformation and cracking.

Key words: Naphthalene Acetic Acid · Calcium Chloride · Berry cracking · 'Autumn Royal' Grape Cultivar

INTRODUCTION

Autumn Royal is a medium-late ripening seedless table grape cultivar, with a large size bunch and berry that is oval-shaped with purple-black to dark blue skin color. This cultivar has a susceptible to berry cracking, which is a great problem as it causes bunch cluster rot. Another problem in this cultivar is the weak attachment of the berries to the rachis causing berry shattering so clusters must be very carefully handled in order to avoid the berry loosening [1].

NAA application affects fruit formation through cell division and elongation [2]. Also, Iqbal *et al.* [3] and Rizk-Alla *et al.* [4] reported that NAA significantly reduced fruit drop, increased yield and improved fruit quality. Moreover, Hifny *et al.* [5] reported that NAA at 25 ppm applied one week after full bloom on Washington navel orange trees increased significantly vegetative growth parameters such as shoot length, leaves number per shoot at the three growth cycles, as well as leaf area compared the other treatments and control.

Furthermore, Calcium (Ca^{++}) is an important constituent of plant tissues and has a vital role in maintaining and modulating various cell functions and in addition to cell walls, Ca2+ also stabilizes cell membranes through the interaction with phospholipids [6]. Calcium is a necessary nutrient element for plant growth and development and plays an important role in many physiological processes of plants [7]. Ca^{2+} can maintain the cell wall structure which makes the fruit keep firmness [8]. Additionally, studies have shown that when calcium is deficient, the amount of sugar accumulation of photosynthesis in plants is significantly reduced, compared with the condition of sufficient calcium,

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enhancing rapidly sugar output after increasing calcium in a certain range [9]. However, calcium is considered as an immobile element so, foliar absorption is considered the most efficient method to supply secondary macronutrients such as calcium nutrients [10]. Efficiency of foliar application with Ca depends on the source of Ca and applied dosage. In this regard, foliar application of $CaCl_2$ was more efficient than that of chelate calcium and calcium oxide [11]. Spraying the vines with calcium leads to an increase in berry firmness and adherence [12].

The objective of this trial is to ascertain the effect of foliar application of Naphthalene acetic acid (NAA) and Calcium chloride on relieving berry cracking and shuttering in grape (*Vitis vinifera* L.) "Autumn Royal" through increasing the vegetative growth in order to reduce berry temperature and pressure and increasing berry firmness and adherence as well.

MATERIALS AND METHODS

This study was carried out during two successive seasons 2019 and 2020 in a vineyard located at El-Sadat City, with a coordinates 30° 22' 30" N and 30° 30' 1" E, where this area is characterized by hot to moderate climate whereas the air temperature varied between 37°C and 40°C. Four-year-old seedless table grape "Autumn Royal" spaced 2 x 3 m grown in a sandy soil were used in this investigation. Vines were cane pruned, trellised by Spanish parron system with a bud load of 96 buds/vine (12 canes x 8 buds) in addition to 12 spurs x 2 buds / spur. Pruning was carried on the last week of January and irrigated via a drip irrigation system. One hundred and twenty uniform vines were chosen for this study (8 treatments x 3 replicates x 5 vines/replicate). The experiment was carried out on the same vines for both seasons and received common horticultural practices recommended by Ministry of Agriculture. All treatments were applied as a foliar application on the leaves and clusters three times, the first was three weeks after bud burst (shoot length 50 cm), the second was after berry set and the third two weeks later for both seasons as follow:

- Control
- NAA at 25 ppm
- NAA at 50 ppm
- NAA at 75 ppm
- $CaCl_2$ at 1000 ppm
- NAA at 25 ppm + $CaCl_2$ at 1000 ppm
- NAA at 50 ppm + $CaCl_2$ at 1000 ppm
- NAA at 75 ppm + $CaCl_2$ at 1000 ppm

The following measurements were taken to evaluate the effect of the different treatments:

Yield and its Attributes: Samples of 15 clusters were collected randomly for each treatment, when clusters reached their full color and total soluble solids reached about 15-18%, according to Badr and Ramming [13].

- Yield per vine (kg)
- Average cluster weight (g)
- Average berry size (cm^3)
- The percentage of cracked berries /cluster: At harvest the number of cracked berries in each cluster were counted and divided by the total number of berries/cluster in each treatment to calculate the percentage of cracked berries.
- The net yield (Kg): it was calculated for each treatment by the following equation

Total yield – (Total yield x percentage of cracked berries)

- Berry firmness and adherence (g/cm^2) by using PHSH-PULL (Dynamometer Model DT101).

Chemical Characteristics of Berries
The Following Determinations Were Carried Out:

- Refractometric total soluble solids (TSS %) and titratable acidity % (one gram of tartaric acid for 100 ml of juice) and TSS / acid ratio were determined according to AOAC [14].
- Total anthocyanin in berry skin (mg/100 g fresh weight): the spectrocolourimeter is used at 250 nm according to Yilidz and Dikmen [15].

Vegetative Growth Parameters:

- Leaf area (cm^2): Samples of 20 leaves taken from the 6^{th} to 8^{th} leaf, were randomly collected from each treatment for leaf area determination at harvest time (using leaf area meter, Model CI 203, U.S.A.).
- Shoot length (cm): was determined using a measuring tape.
- Weight of pruning (Kg): after winter pruning the weight was determined using a spring balance.
- Total chlorophyll content of leaves (SPAD): were measured at harvest time in the mature basal leaves of the sixth and seventh nodes by using the nondestructive Minolta chlorophyll meter model SPAD 502.

Statistical Analysis: The statistical analysis of the present data was carried out according to Snedecor and Cochran [16]. Averages were compared using the new L.S.D. values at 5% level using a randomized complete block design.

RESULTS AND DISCUSSION

Yield

Average Yield per Vine (Kg), Cluster Weight (g), Berry Size (cm^3): All foliar application of NAA and $CaCl_2$ increased yield per vine, cluster weight and berry size over the control in both seasons (Table 1). It can be observed that, vines sprayed by NAA at 75 ppm + $CaCl_2$ at 1000 ppm followed by NAA at 50 ppm + $CaCl_2$ at 1000 ppm respectively produced the higher significant yield and cluster weight than the other treatment. In a previous study, Colapietra and Alexander, [17] reported that Ca as foliar applications can increase yield. On another trial, it was stated that treating the vines with NAA showed a high grape yield of very good quality as well as the best physical properties of cluster [4]. Preharvest calcium chloride ($CaCl_2$) spray significantly increased fruit Ca^{2+} content, improved clusters quality in grape [18].

In addition, our data ensures that spraying NAA at 75 ppm + $CaCl_2$ at 1000 ppm significantly increased the berry size giving the best values than the lower concentrations. This increase in berry size may be attributed to the increase in cell division and cell elongation caused by NAA addition. Our results are in harmony with Colapietra and Alexander, [17] demonstrating that berry size was the greatest when applying calcium as a foliar application.

The Percentage of Cracked Berries: In this trial, 'Autumn Royal' cultivar showed higher berry cracking levels in the untreated vines compared to all the other treatments, whereas the level of damaged cracked berries when spraying NAA at 75 ppm + $CaCl_2$ at 1000 ppm, was negligible in both seasons (Table 1). This result was due to the Ca effect in increasing the vegetative growth in term of leaf area and shoot length illustrated in Table (3), which in turn resulted in more shading reducing the berry temperature and thus the pressure which cause the cracking phenomena. Similar finding was stated by Duan *et al.* [19] who mentioned that the Ca^{2+}-sufficient vines exhibited significant increases in leaf area shoot length and shoot fresh weights. This results were explained by Lang and During, [20] who revealed that the rising in temperature corresponds to a berry volume increase and thus increasing the pressure. The strong rise in internal pressure of berries with temperature is interesting, for it implies a negative value for the area coefficient of thermal expansion of the skin, means that skin area reduces with rising temperature. This strong rise in internal pressure of berries is accompanied with a decrease in berry resistance to cracking phenomenon, besides, at higher temperatures there is also a significant decrease in skin elasticity and strength which makes the berries likely to crack.

In a trial done by Yu *et al.* [18], taken together, the results indicated that dipping grape berries in calcium solution is effective in preventing fruit cracking by stimulating calcium uptake, inhibiting cell wall disassembly and promoting cell wall strengthening. the fruit-cracking incidence in the calcium treatment was significantly lower than that of the control.

The Net Yield (Kg): A well, it could be observed that, vines treated by NAA at 75 ppm + $CaCl_2$ at 1000 ppm produced a higher significant yield than all other treatments in both seasons (Table, 1). By calculating the overall loss of yield after eliminating the cracked berries (5.4 and 5.5 %) compared with the control (21.1 and 21.3 %) with a reduction of about 15 %, we can notice that the net yield gave the least values of the mentioned parameter (29.4 and 29.1 Kg) and (17.7 and 17.3 Kg) in both seasons respectively, as berry cracking effect leads to significant commercial losses in the grape production by decreasing yield [21].

Berry Firmness (g/cm^2) and Adherence (g/cm^2): As shown in figure (1 & 2) the significantly beneficial effect of spraying NAA at 75 ppm + $CaCl_2$ at 1000 ppm on berry firmness and berry adherence strength is more obvious than the lower concentrations and the control as low Ca concentrations in fruits have generally been found to accelerate the ripening process by stimulating the production of ethylene and increasing the activity of enzymes, which are responsible for the tissues softening [22]. Thus, calcium is considered as an important factor for stabilizing cell wall and pre-harvest calcium chloride sprays are used to increase Ca content of the berry cell wall to delay senescence, resulting in increased berries firmness and berry adherence strength [23]. As mentioned by Bassiony [24], vines treated by Calcium sprays, enhanced berry firmness and berry adherence force and thus reducing berries shattering at harvest date. Likewise, pectins play an important role in cell-to-cell adhesion which positively affect berry firmness and since elevated

Table 1: Effect of foliar application of Naphthalene acetic acid (NAA) and Calcium chloride ($CaCl_2$) on the yield and its attributes of Autumn Royal grape cultivar

	Yield/vine (Kg)		Cluster weight (g)		Berries size (cm^3)		Cracked berries/cluster (%)		The net yield /vine (Kg)	
Treatments	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Control	22.5	22.0	642.8	628.7	5.4	5.3	21.1	21.3	17.7	17.3
NAA at 25 ppm	24.7	23.8	695.3	680.4	6.0	5.8	14.6	14.2	21.2	20.4
NAA at 50 ppm	25.2	25.1	728.7	717.0	6.3	6.0	13.5	13.7	21.7	21.6
NAA at 75 ppm	26.5	26.2	768.3	748.5	6.6	6.4	12.1	13.0	23.3	22.8
$CaCl_2$ at 1000 ppm	27.4	27.5	782.8	785.9	6.9	6.7	11.4	11.8	24.2	24.1
NAA 25 ppm + $CaCl_2$ 1000 ppm	28.7	28.4	839.4	828.6	7.1	6.9	8.7	8.9	26.2	25.8
NAA 50 ppm + $CaCl_2$ 1000 ppm	29.9	29.5	854.2	850.4	7.3	7.2	6.9	7.3	27.9	27.3
NAA 75 ppm + $CaCl_2$ 1000 ppm	31.1	30.8	887.1	880.0	7.6	7.5	5.4	5.5	29.4	29.1
New L.S.D at 5%	0.5	0.4	19.0	16.1	0.1	0.1	0.5	0.5	1.9	1.6

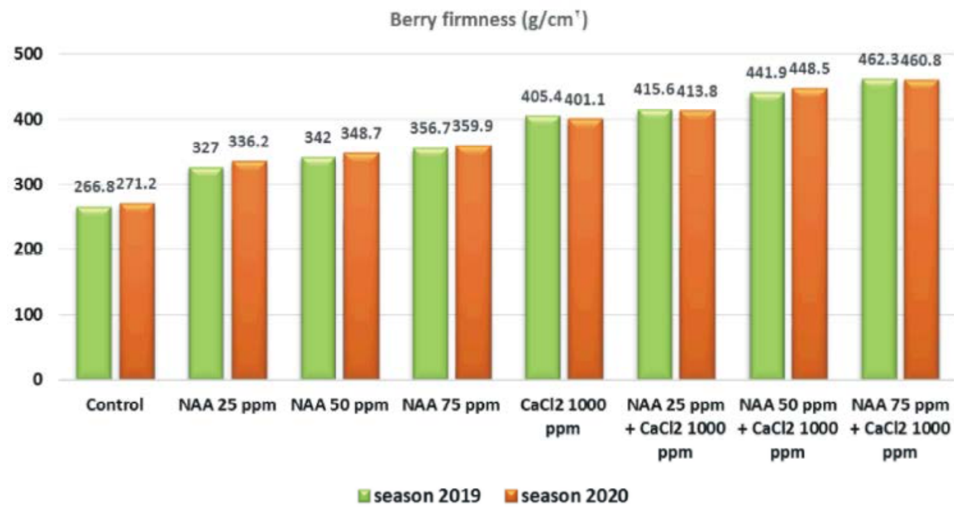

Fig. 1: Effect of foliar application of Naphthalene acetic acid (NAA) and Calcium chloride ($CaCl_2$) on berry firmness (g/cm^2) of Autumn Royal grape cultivar

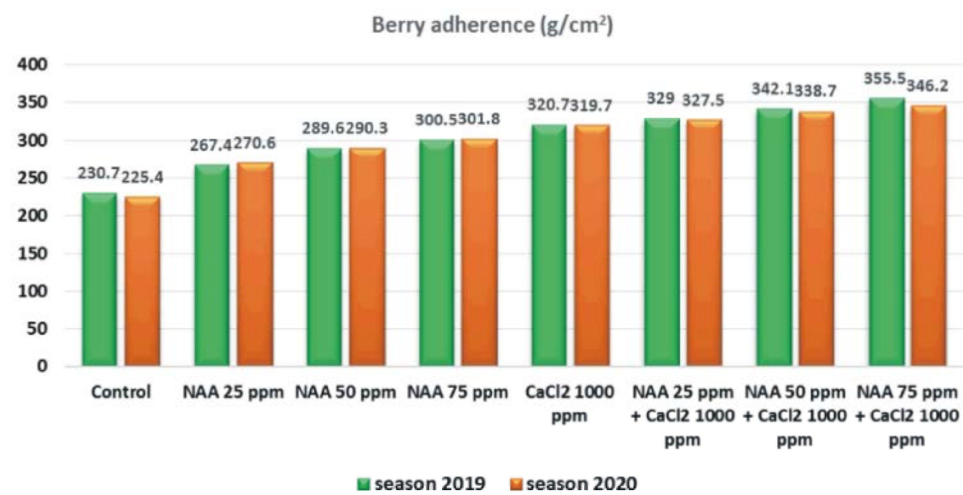

Fig. 2: Effect of foliar application of Naphthalene acetic acid (NAA) and Calcium chloride ($CaCl_2$) on berry adherence (g/cm^2) of Autumn Royal grape cultivar

Ca levels in grape cells were reported to induce a strong repression of polygalacturonase, the enzymes responsible for degradation of pectin molecules, thus increasing Ca levels increases berry firmness [25].

In addition, the obtained results revealed the role NAA in increasing berry adherence strength and decreasing berry shattering percentage which can be attributed to the beneficial effect NAA in minimize berry shattering by inhibiting the generation of ABA, inactivating the activities of cellulase and polygalacturonase enzymes and delaying the development of abscission layer [26].

Chemical Characteristics of Berries

Total Soluble Solids (TSS %), Titratable Acidity % and TSS / Acid Ratio: Significant differences among treatments in TSS %, titratable acidity and TSS/acid ratio could be observed in Table (2). All tested treatments scored statistically higher values of TSS as compared with the control in both seasons of study. Results showed that spraying the vines with the single treatment $CaCl_2$ at 1000 ppm, was better than the other treatments and the control followed by the combined treatment NAA at 25 ppm + $CaCl_2$ at 1000 ppm which could be ascribed to the application of NAA causing TSS% repression. Similar results were obtained by Salama and Elsherbeny, [27] who stated that the lower concentration of NAA proved to be the most efficient treatment in this concern than the higher treatment and the control. Furthermore, He *et al.* [28] demonstrated that sugar accumulation suppressed by NAA application as it when applied delays ripening through the repression of sugar.

Regarding to Ca effect on the mentioned parameters, Colapietra and Alexander, [17] report that Calcium as foliar applications can increase sugar berry accumulation. Besides the finding of Marzouk and Kassem, [23] who reported that spraying Thompson Seedless with $CaCl_2$ resulted in increasing total soluble solids and as mentioned by Huang *et al.* [29] decreased titratable acid content.

Total Anthocyanin in Berry Skin (mg/100g): It is widely accepted that foliar sprays of calcium chloride have a positive effect on the anthocyanin in berry skin as shown in Table (2). The best results were obtained from vines treated by the single treatment of $CaCl_2$ at 1000 ppm followed by NAA at 25 ppm + $CaCl_2$ at 1000 ppm and the least values where obtained from treating the vines with NAA at 75 ppm. Similarly, Amiri *et al.* [30], stated that the quality components including berry color and appearance were significantly improved by Ca sprays. Calcium sprayed in adequate amount at 1000 ppm gave the best results, as increasing calcium levels in grape cells to 2 % $CaCl_2$ in a trial done by Martins *et al.* [25] were shown to reduce total anthocyanin content. Moreover, the application of 2% $CaCl_2$ caused some damage to the leaves [31].

Contrariwise, the accumulation of anthocyanins was suppressed by NAA application as Naphthalene acetic acid is a synthetic auxin that when applied delays ripening through the repression of sugar and anthocyanin accumulation [28].

Vegetative Growth Parameters

Leaf Area (cm^2): Leaf area development is an important characteristic affecting yield and berry quality of grapevines. Table (3) illustrates the effect of the single and combined treatments on the average leaf area and Shoot length of “Autumn Royal” grapevines. It is obvious from the recorded data that there are significant differences among different concentrations of NAA along with $CaCl_2$ in both seasons. The highest values were obtained when spraying the vines with NAA at 75 ppm + $CaCl_2$ at 1000 ppm followed by NAA at 50 ppm + $CaCl_2$ at 1000 ppm. These results are in conformity with those obtained by El Sayed, [32] who observed that supplementary addition of foliar NAA was associated with higher leaf area of lime trees. Concerning this effect, it was deduced that NAA plays an important in preventing berry cracking through increasing the leaf area which in turn provide more shade and less temperature for the clusters limiting this phenomenon.

Shoot Length (cm): Likewise, regarding the effect of different concentrations of NAA along with $CaCl_2$ in both seasons on shoot length, data in Table (3) showed that there are significant differences among treatments. The highest values were obtained when spraying the vines with NAA at 75 ppm + $CaCl_2$ at 1000 ppm followed by NAA at 50 ppm + $CaCl_2$ at 1000 ppm as NAA plays an important role in increasing the cell division. As well, Duan *et al.* [19] mentioned that since leaves are the source of photosynthates and Ca^{2+} promotes cell elongation and cell division, the increased capacity of Ca^{2+} in leaves resulted in the increment of leaf area which eventually led to an obvious increase in shoot length.

Weight of Pruning (Kg): Pruning weights are the best way to monitor vine growth and vine size changes caused by vineyard management practices. Concerning the effect of the different materials involved in this study, data obtained in Tables (3) clearly show that the combine

Table 2: Effect of foliar application of Naphthalene acetic acid (NAA) and Calcium chloride ($CaCl_2$) on the chemical characteristics of berries of Autumn Royal grape cultivar

	TSS (%)		Acidity (%)		TSS/acid Ratio		Anthocyanin (mg/100 g FW)	
Treatments	2019	2020	2019	2020	2019	2020	2019	2020
Control	14.8	14.9	0.73	0.70	20.2	21.3	37.3	37.5
NAA at 25 ppm	16.4	16.0	0.62	0.61	26.5	26.2	39.9	39.7
NAA at 50 ppm	15.6	15.6	0.67	0.66	23.3	23.6	39.2	39.1
NAA at 75 ppm	15.3	15.2	0.70	0.71	21.9	21.4	38.7	38.9
$CaCl_2$ at 1000 ppm	19.1	19.4	0.42	0.40	45.5	48.5	44.3	45.0
NAA 25 ppm + $CaCl_2$ 1000 ppm	18.0	18.5	0.51	0.49	35.3	37.8	43.2	43.8
NAA 50 ppm + $CaCl_2$ 1000 ppm	17.2	17.4	0.58	0.55	30.5	31.6	42.1	42.7
NAA 75 ppm + $CaCl_2$ 1000 ppm	16.7	16.8	0.60	0.59	27.8	28.5	40.9	41.0
New L.S.D at 5%	0.4	0.5	0.02	0.01	1.9	2.0	0.8	0.9

Table 3: Effect of foliar application of Naphthalene acetic acid (NAA) and Calcium chloride ($CaCl_2$) on the vegetative growth parameters of Autumn Royal grape cultivar

	Leaf area (cm^2)		Shoot length (cm)		Weight of pruning (Kg)		Chlorophyll content (SPAD)	
Treatments	2019	2020	2019	2020	2019	2020	2019	2020
Control	104.3	100.1	158.9	162.0	3.0	2.9	27.5	28.7
NAA at 25 ppm	127.5	122.6	166.7	168.6	3.5	3.4	29.1	29.4
NAA at 50 ppm	135.6	131.4	169.4	170.1	3.3	3.4	30.2	31.1
NAA at 75 ppm	141.5	138.0	172.1	175.8	3.7	3.7	31.5	31.9
$CaCl_2$ at 1000 ppm	148.4	145.2	177.7	182.1	4.0	4.1	35.0	35.3
NAA 25 ppm + $CaCl_2$ 1000 ppm	160.0	159.7	183.5	189.4	4.2	4.4	37.5	37.8
NAA 50 ppm + $CaCl_2$ 1000 ppm	172.8	180.9	190.1	196.5	4.6	4.7	39.6	39.8
NAA 75 ppm + $CaCl_2$ 1000 ppm	180.9	191.0	198.9	202.3	5.3	5.4	41.9	42.1
New L.S.D at 5%	6.2	5.8	0.5	0.6	0.2	0.2	0.5	0.4

treatment of with NAA at 75 ppm + $CaCl_2$ at 1000 ppm has positive effect on the weight of pruning followed by NAA at 50 ppm + $CaCl_2$ at 1000 ppm in both seasons. The weight of pruning lowest values were detected in case of lowering NAA concentrations. The pruning levels is considered one of the most important factors limiting the canopy vigor and productivity and balancing between them is the most reliable method to maintain balance between growth and production. Accordingly, it was previously demonstrated that the increases in pruning weight is directly proportional to NAA concentration [33]. Referring to Smart and Robinson [34], pruning weight is proportional to leaf area carried on the shoots in the previous growing season, as the leaf area increases the weight of pruning increases too.

Total Chlorophyll Content of Leaves (SPAD): The chlorophyll content of leaves is an indicator of the photosynthetic potency of plant tissues. It is clear that the foliar application with NAA at 75 ppm and $CaCl_2$ at 1000 achieved maximum content of total chlorophyll/ leaf above the rest of treatments (Table 3). Another interesting feature was that Calcium deficient leaves vines as in the control treatment exhibited a significant reduction in chlorophyll content. Similar result was observed by Duan *et al.* [19] who found that Calcium deficient leaves, showed lower values of chlorophyll contents which eventually led to yellowing and chlorosis of leaves.

CONCLUSION

The overall results during both years of study revealed that it is economically feasible to use NAA at 75 ppm and $CaCl_2$ at 1000, which had a beneficial effect on, firmness, adherence and berry cracking which gave a value of 15% less than the control. Also, the results showed that the higher concentration of NAA along with calcium chloride gave the highest yield and its attributes beside all vegetative growth parameters. Conversely, in respect to the chemical characteristics of berries namely TSS %, titratable acidity % and TSS / acid ratio and anthocyanin, the single treatment $CaCl_2$ at 1000 was the best over all treatments in giving the highest values as NAA tends to reduce the total soluble solids and anthocyanin but higher the control.

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