Comparative changes in quality parameters and nutrient composition in healthy and creased fruit, and the effectiveness of moringa leaf extract in reducing creasing incidence of Washington navel orange (Citrus Sinensis L. Osbeck)

By

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Comparative changes in quality parameters and nutrient composition in healthy and creased fruit, and the effectiveness of moringa leaf extract in reducing creasing incidence of Washington navel orange (Citrus Sinensis L. Osbeck)

Abstract:

The study was conducted on twelve-year-old Washington navel orange (Citrus Sinensis L. Osbeck) trees at a private orchard in Badaway, Al-Dakahlia governorate, Egypt, between 2019 and 2020. The objective of study was evaluated the changes in quality parameters and mineral composition in the healthy and creased fruit, and the effects of calcium chloride (CaCl$_2$) and moringa leaf extract (MLE) sprays on the creasing, yield and fruit quality of Washington navel orange. The experiment was laid out in randomized complete block design with three replications and five treatments: T1=Control (distilled water only), T2= CaCl$_2$ at 1%, T3= CaCl$_2$ at 2%, T4= MLE at 2%, T5= MLE at 3%. Spraying treatments were applied three times in both seasons: during the full bloom stage, the fruit setting stage, and two weeks after the fruit setting stage. The results showed a strong correlation between the quality parameters, the nutrient composition, and the fruit creasing incidence. Foliar application of moringa leaf extract reduced the incidence of fruit creasing while increasing fruit yield, fruit weight, fruit firmness, fruit peel thickness, TSS, and vitamin C in 'Washington navel' orange. Hence, moringa leaf extracts at 3% is adapted to decrease the incidence of creasing and improve the yield components and fruit quality of ‘Washington navel’ orange under experimental conditions.

Keywords: Creasing, Calcium, Moringa, Extract, Washington navel orange.
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Introduction

Citrus is an important fruit crop in tropical and subtropical countries, it is considered the first among economic fruit crops in Egypt as well as all over the world. Washington navel orange (Citrus sinensis L. Osbeck) is the main cultivated variety of citrus in Egypt and it is considered the popular fresh citrus fruits for the Egyptians due to its seedless, large sized, fruit nutritive value, and low prices compared to other fruits, it is also a major source of early-season income for citrus farmers in all citrus commercial areas in Egypt (El Sheikh et al., 2007; El-Mehy and El-Badawy 2017; Abobatta, 2018; Abobatta, 2019). Occupies an area of 30.94% of the total area of cultivated citrus,
with an area of about 152806 fed. produced about 1608806 tons per year (Bulletin of the Agricultural Statistics, 2021). The net income from Washington navel oranges production is in closed relationship to fruit qualities, which play the great important role in determining the price (Magwaza et al. 2013). Moreover, the recently distribution of creasing disorder is one of the most prevailing problems facing the Washington navel oranges producer, which certainly reflected negatively on grading and marketing value of produced fruits (Wang et al., 2021).

Creasing known as Albedo breakdown, is a serious pre-harvest physiological disorder, due to the abnormal separation of cells in the middle lamella of albedo tissue, causing puffiness of washington navel orange peel (Agusti et al., 2001; Alquezar et al., 2010; Alva et al., 2006; Pham et al., 2012; Saleem et al., 2014; Juan and Chen, 2017). Orange fruit creasing is a very complex process. Even with in-depth studies, the mechanisms and influencing factors related to fruit creasing are still uncertain. Recent studies reported that fruit creasing has been directly related to cultivar characteristics (Agusti et al., 2003), weather conditions (Gambetta et al., 2000), rootstock (Storey et al., 2002), peel thickness (Holtzhausen, 1981), peel hardness (Li et al., 2013), growth regulators (Li et al., 2016), and mineral contents of some nutrients such as calcium, potassium (Elharouny et al., 2015).

Controlling of creasing in navel orange is important for the marketing of quality fresh orange and to prolong the life of the fruit with high quality characters as long as possible after harvest. Currently, most of the studies on the prevention of creasing have been based on foliar treatments with mineral elements such as boron, potassium and calcium (Pham, 2009; Huai et al., 2022; Lopez-Zaplana et al., 2020; Michailidis et al., 2022), synthetic plant-growth regulators such as gibberellic acid
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(GA$_3$), cytokinins and auxins (Agusti et al., 2002; Kassem et al., 2012).

*Moringa oleifera* L., has been used as an organic biostimulant (Jardin et al., 2015 and Elrys et al., 2022), leaves of moringa are rich in minerals (El Sohaimy et al. 2015; Gopalakrishnan et al., 2016), vitamins (Yap et al., 2021), amino acids (Abd El-Mageed et al., 2017) and phytohormones such as salicylates, auxins, cytokinins and gibberellins (Elzaawely et al. 2017a). This study aimed to assess the effects of calcium chloride and moringa leaf extract on the incidence of fruit creasing, yield, and quality of Washington navel oranges, as well as the relative changes in quality parameters and nutrient composition in the albedo and flavedo of the creased and the healthy fruit.

**Materials and Methods**

**Experimental setup and site information**

This study was carried out during 2019 and 2020 seasons on forty five trees 12 years old of “Washington navel” orange (*Citrus sinensis* L. Osbeck) budded on sour orange rootstock (*Citrus aurantium* L.) rootstock and planted at 5 × 5 m in loamy clay soil under surface irrigation system in a private orchard at Badaway, Al-Dakahlia governorate, Egypt (latitude: 31.05° N, longitude: 31.38° E and 2.89 m above the Mediterranean Sea level). Trees were normal growth, uniform in vigour, trained on open vase training system and were received uniform management practices. Soil analysis properties of the experimental site were provided in Table 1.
Table 1. Physical and chemical characteristics of the experimental soil.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Values</th>
<th>Properties</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand %</td>
<td>27.6</td>
<td>CaCO₃ %</td>
<td>17.5</td>
</tr>
<tr>
<td>Silt %</td>
<td>31.7</td>
<td>Total N mg/kg soil</td>
<td>99.8</td>
</tr>
<tr>
<td>Clay %</td>
<td>40.7</td>
<td>K⁺ mg/kg soil</td>
<td>421.2</td>
</tr>
<tr>
<td>Texture class</td>
<td>Clay- loam</td>
<td>P mg/kg soil</td>
<td>3.36</td>
</tr>
<tr>
<td>pH **</td>
<td>7.8</td>
<td>Ca²⁺ mg/kg soil</td>
<td>324.1</td>
</tr>
<tr>
<td>E.C. (dS/m)</td>
<td>1.5</td>
<td>Mg²⁺ mg/kg soil</td>
<td>1103.38</td>
</tr>
<tr>
<td>Organic matter %</td>
<td>1.09</td>
<td>Zn mg/kg soil</td>
<td>0.99</td>
</tr>
</tbody>
</table>


**Preparation of Moringa leaves extract (MLE):**

The aqueous extract of Moringa leaves was prepared using the method previously described by Yasmeen (2011). 100g of air-dried Moringa oleifera leaf powder was soaked in 1 liter of water for 24 hours and filtered out; then diluted with water in the following concentrations: 2%, 3%. Some chemical compositions of Moringa leaf extract were determined as shown in Table 2.

**Table 2. Chemical composition of principal component ofMoringa oleifera leaves extract.**

<table>
<thead>
<tr>
<th>Component</th>
<th>2019</th>
<th>2020</th>
<th>Component</th>
<th>2019</th>
<th>2020</th>
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</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>126.2</td>
<td>126.9</td>
<td>Zinc</td>
<td>0.466</td>
<td>0.517</td>
</tr>
<tr>
<td>Proline</td>
<td>27.29</td>
<td>28.10</td>
<td>Copper</td>
<td>0.212</td>
<td>0.230</td>
</tr>
<tr>
<td>Total soluble sugars</td>
<td>155.9</td>
<td>163.7</td>
<td>Soluble phenols</td>
<td>2.235</td>
<td>2.178</td>
</tr>
<tr>
<td>Ash</td>
<td>113.5</td>
<td>117.2</td>
<td>Total carotenoids</td>
<td>2.301</td>
<td>2.477</td>
</tr>
<tr>
<td>Calcium</td>
<td>8.789</td>
<td>9.435</td>
<td>Total chlorophyll</td>
<td>4.722</td>
<td>5.024</td>
</tr>
<tr>
<td>Magnesium</td>
<td>6.077</td>
<td>7.014</td>
<td>Ascorbic acid</td>
<td>3.321</td>
<td>3.561</td>
</tr>
<tr>
<td>Potassium</td>
<td>28.15</td>
<td>29.88</td>
<td>Phytoregulators (µg g⁻¹ DW):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td>6.233</td>
<td>6.266</td>
<td>Indole-3-acetic acid</td>
<td>0.911</td>
<td>0.988</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.512</td>
<td>0.422</td>
<td>Gibberellins</td>
<td>0.817</td>
<td>0.833</td>
</tr>
<tr>
<td>Iron</td>
<td>1.922</td>
<td>1.930</td>
<td>Zeatin</td>
<td>0.945</td>
<td>0.988</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.978</td>
<td>1.055</td>
<td>Abscissic acid</td>
<td>0.302</td>
<td>0.344</td>
</tr>
</tbody>
</table>
Treatments and experimental design
The experiment followed complete randomized blocks design with three replicates for each treatment (5 treatments × 3 replicates = 45 trees), as follows:
Control (distilled water only)
Calcium chloride at 1%
Calcium chloride 2%
Moringa leaf extract at 2%
Moringa leaf extract at 3%
These treatments were sprayed to runoff at full flower stage, at fruit set stage and two weeks after fruit set with the indicated solutions. Tween-20 was added at 0.01% as a surfactant.
All the trees received the specific fertilizer doses recommended by the Ministry of Agriculture, which were 140, 40, and 100 units/fed. of NPK, respectively.

Data collection and analysis
Mineral content and concentration: nitrogen (N), potassium (K), calcium (Ca) and magnesium (Mg).
Total nitrogen, potassium, calcium, and magnesium content in the leaves and fruits of Washington Navel oranges were determined using the ICP (Inductively Coupled Plasma) technique (McQuaker et al., 1979).

Thickness and firmness of fruit peel
Indicators of fruit strength include the thickness and firmness of the fruit peel were determined, fruit peel thickness (mm) was determined using an electronic digital caliper. The peel firmness was determined as (kg/m²) by using a handheld fruit firmness tester ("Penetrometer" (Model FT 327, QA Supplies, Norfolk, VA, USA) (Chawla et al., 2018).

Creasing incidence (%)
The incidence of creasing was examined on the fruit from whole tree, based on the appearance of fruit surface (Storey et
al., 2002). The creasing incidence percentages were calculated according to the following equation:

\[
\text{Creasing } \% = \left( \frac{\text{Creased fruits}}{\text{Total fruit number per tree}} \right) \times 100
\]

**Fruit yield and quality**

**Determination of yield:** All fruits were harvested at commercial maturity stage (the first week of January) from each tree of various replicates and yield was recorded as a number of fruits/tree and weight in Kilograms.

**Quality assessments:** Tow fruit samples were taken from each experimental unit. The first sample of 10 randomly fruits was used for measuring the physical and chemical quality parameters. Fruits of the second sample were selected according to visual symptoms, 10 fruit with creasing and 10 fruit without creasing symptoms were harvested. In the laboratory, fruits of each category were observed with a scanning Electronic microscope (SEM). The same fruits were used to determine the fruit quality parameters.

**Fruit weight (g), length and diameters:** Fruit weight was determined using a sensitive scale. Fruit dimensions (length and diameter “cm”) were determined using an electronic digital caliper.

**Total Soluble Solids (TSS %):** Carl Zeiss hand refractometer was used to determine the TSS in fruit juice according to Chen and Mellenthin (1981).

**Titratable acidity % (TA):** Titratable acidity% was determined by titrating the juice against 0.1 N sodium hydroxide using phenolphthalein as an indicator. Results were expressed as percentage of citric acid in fresh pulp weight (AOAC, 1990).

**Ascorbic acid contents (Vitamin C):** Ascorbic acid (mg/100 g FW) in fruit juice was determined by using the dye 2, 6- dichlorophenyl indophenols method described in A.O.A.C (1970).
Statistical analysis
The obtained data were subjected to the analysis of variance using Statistix 9.0 software program (Analytical Software, Tallahassee, FL. USA). The least significant differences test (LSD) at a 5% level of probability was used to compare the mean between treatments (Steel et al. 1960).

Results and Discussion
Effects of calcium chloride and moringa leaf extract on the incidence of fruit creasing, yield, and quality of Washington navel oranges.

Leaf mineral (N, P, K and Ca) content
The mineral content of plant leaves is a very important tool to determine the status of nutrients (Ayoub et al., 2014). MLE sprayed had the highest mineral content, followed by calcium chloride treatments and control (Figure 1 A, B, C and D). The highest concentrations of MLE (3%) significantly (P≤0.05) resulted in the highest mineral content of ‘Washington navel’ orange leaves compared to the other treatments: 2.91 and 2.93 % for N, 0.36 and 0.38 % for P, 1.85 and 1.87 for K, 4.55 and 4.58 for Ca in the first and second season, respectively (Figure 1 A, B, C and D). These findings are consistent with the report by Jain et al. (2020), who noted an increase in Stevia rebaudiana Betoni's N, P, K, and Ca contents as a result of MLE foliar spraying. Additionally, a different study found that the foliar application of 3% MLE at the fruit set stage increased the concentration of macro- and micronutrients (N, P, K, Ca, Mn, and Zn) in "Kinnow" mandarin leaves (Nasir et al., 2016).
Figure 1. Effect of moringa leaf extract and calcium chloride treatments on leaf mineral content; (a) Nitrogen (N), (b) Phosphorus (P), (c) potassium (K) and (D) calcium (Ca) of ‘Washington navel’ orange during 2019 and 2020 seasons. Values in the bar followed by the same letter(s) are not significantly different at a 5% level of probability.

Thickness and firmness of ‘Washington navel’ orange fruit peel

The thickness and firmness of the "Washington navel" orange fruit peel were also examined in this study in relation to treatments with calcium chloride and moringa leaf extract (Figure 2 A and B). The fruits treated with MLE had
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significantly (P≤0.05) thicker peels than the calcium chloride treatments and control, as shown in Figure 2 A. The biggest fruit peel thickness was observed when navel orange trees sprayed with MLE at 3% (5.8 mm and 6.0 mm during the 1st and 2nd seasons, respectively). The maximum increase in fruit peel thickness compared to control was 61.76% and 70.58% during 2019 and 2020, respectively. This was followed by treatment of MLE at 2%, which were 5.5 mm and 5.8 mm in the first and second seasons, respectively. The result obtained is in agreement with those reported by Abo El-Enin (2015). They reported that foliar application of moringa leaf extract at 3% increased the fruit peel thickness of Navel orange. The high content of zeatin in moringa leaf aqueous extract is attributed to the increase in fruit peel thickness. During fruit development, zeatin increases cell division and cell enlargement. The increase in fruit peel thickness is attributed to the high level of zeatin in moringa leaf aqueous extract. Zeatin increases cell division and cell enlargement during fruit development (Teribia et al., 2016).

Results presented in Figure 2 B show that MLE and calcium chloride treatments gradually increased the firmness of fruit peels. Fruits treated with MLE had significantly (P ≤ 0.05) harder peels than that of the calcium chloride treatments and control in the first and second season. Highest firmness was observed in fruit harvested from trees treated with 3% MLE (22.54 and 22.66 kg/m²) in first and second seasons, respectively. Meanwhile, lowest firmness was recorded in fruit harvested form control (13.83 and 14.1 kg/m²) during 2019 and 2020 seasons respectively. These results are consistent with those of ShM et al. (2017), who found that a 6% aqueous extract of moringa enhanced the fruit peel hardness of the "Hollywood" plum cultivar (Prunus salicina Lindl.). The increased fruit firmness may be due to the high calcium content of moringa leaf extract (Table 2). Since calcium is crucial for the structure of cell
walls, it contributes to the firmness of fruit tissue (Ismail and Ganzour et al., 2021 and Martins et al., 2020), prevents physiological disorders, and improves fruit quality (Picchioni et al., 1995).

![Figure 2](attachment:image.png)

Figure 2. Effect of moringa leaf extract and calcium chloride treatments on (A) peel thickness (mm) and (B) fruit peel firmness of ‘Washington navel’ orange during 2018-2019 and 2019-2020 seasons. Values in the bar followed by the same letter(s) are not significantly different at a 5% level of probability.

**Incidence of fruit creasing (%)**

Fruit creasing decreased significantly (P < 0.05) in all treatments compared to the control (Figure 3). In the first season, 70.34% of untreated fruits showed creasing, whereas, in the second season, control fruits registered values of 68.45%. The application of moringa leaf extract at 3% reduced the number of creasing fruits to 7.6% and 7.37% in the first and second seasons respectively. Spraying this extract at 1% also produced lower values than the control in both seasons (9.19% and 9.28% for the first and second seasons, respectively). While values for fruits treated with calcium chloride showed lower effectiveness in both seasons (39.32% and 39.73%, respectively).
The occurrence of creasing is known to be controlled by multiple factors, including external factors such as agronomic (mineral nutrition) and fruit characteristics (mechanical properties of the peel (peel thickness and hardness) (Gambetta et al., 2000; Treeby and Storey, 2002; Bower, 2004; Li et al., 2009; Pham et al., 2012; Khadivi-Khub, 2014; Li and Chen, 2017 and Correia et al., 2018). The beneficial effects of MLE on fruit creasing incidence may be attributed to increased leaf mineral content, thickness, and firmness of the fruit peel of 'Washington navel' orange.

Figure 3. Effect of moringa leaf extract and calcium chloride treatments on incidence of creasing in ‘Washington navel’ orange during 2019 and 2020 seasons. Values in the bar followed by the same letter(s) are not significantly different at a 5% level of probability.

Fruit yield and quality

Figure 4 A illustrates the effect of Moringa leaf extract and calcium chloride foliar application on fruit yield per tree during the 2019 and 2020 seasons. The analysis variance of navel orange fruit yield per tree (kg/tree) confirmed that the effect of moringa leaf extract and calcium chloride treatments was significant (P<0.05) for fruit yield during the two seasons.
(Figure 4 A). In general, all MLE treatments resulted in the highest fruit yield per tree compared to the control trees. During the two seasons, spraying orange trees with MLE at 3% produced the highest (116.3 and 124.9 kg/tree) fruit yield of navel oranges per tree. On the other hand, the results showed that control trees recorded a 40.75 and 43.07% reduction in navel oranges fruit yield per tree compared to MLE at the 3% treatment. This increase in yield can be attributed to the Moringa leaf extract content of high minerals and hormones, which are directly or indirectly involved in fruit growth and development processes and consequently increase the number of fruits/trees (Abdalla, 2013). Our results are in agreement with those obtained by Abd El-Hamied and El-Amary (2015) and Nasira et al. (2016). They reported that foliar application of Moringa leaf extract increased the yield of ‘Le Conte’ pear and ‘Kinnow’ mandarin. Moreover, Fuglie (1999) reported a yield enhancement of 25–30 % in onions, bell peppers, soya, maize, sorghum, coffee, tea, chilli, and melon by moringa leaf extract application. They suggested that this yield enhancement was due to the presence of growth hormones, antioxidants, and minerals in moringa leaves.

The fresh weight of navel orange fruit in response to moringa leaf extract and calcium chloride treatment was investigated. There was a significant difference (P<0.05) between the treatments (Figure 4 B). Treated navel orange trees with MLE had a significantly greater average fruit weight (332.5 and 338.6 g during 1st and 2nd seasons, respectively) compared to other treatments. The lowest fruit weight was recorded in the control, which was 255.3 and 260.0 g in the first and second season, respectively. Moreover, moringa leaf extract and calcium chloride treatments significantly affected fruit diameter and
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length during the two seasons (Figure 4 C and D). Compared to the control, all MLE treatments recorded the greatest values for fruit length and diameter. MLE at 3% treatment recorded the biggest fruit diameter in both seasons (8.16 and 8.11 cm, respectively). Also, navel orange trees subjected to treatment of MLE at 3% showed the highest value of fruit length (8.25 and 8.22 cm in the 1st and 2nd seasons, respectively). In contrast, the control trees had the lowest fruit length and diameter during the two experimental seasons. These results are similar to those reported by Abd El–Hamied and El-Amary (2015), who showed that application of MLE caused a significant increase in the fruit weight and size of the ‘Le Conte’ pear. Similarly, Nasira et al (2016) reported that treated ‘Kinnow’ mandarin plants with moringa leaf extract at the fruit set stage caused a significant increase in fruit weights and size.

The increase in fruit weight, fruit length and fruit diameter are ascribed to the high level of zeatin and minerals such as potassium and zinc in moringa leaf aqueous extract. Potassium regulates the accumulation of starch and sugar in fruits (Ramezani et al., 2011; Marschner, 2011), while Zn, a tryptophan precursor, is involved in indole acetic acid synthesis, which is required for the growth and development of fruit (Zekri and Obreza, 2003; Nasir et al., 2016). Zeatin accelerates cell division and cell enlargement during fruit development (Teribia et al., 2016) and also improves sink capacity and photosynthate assimilation as a result of more extended green areas in the leaf (Zwack and Rashotte, 2013).
Figure 4. Effect of moringa leaf extract and calcium chloride treatments on (A) yield, (B) fruit weight, (C) fruit diameter and (D) fruit length of ‘Washington navel’ orange during 2018-2019 and 2019-2020 seasons. Values in the bar followed by the same letter(s) are not significantly different at a 5% level of probability.

Among treatments, foliar application with MLE showed a significantly (P ≤ 0.05) higher percentage of TSS in the navel orange fruit than those in the CaCl₂ and control treatments, as shown in Figure 5 A. Spray application with MLE at 3% produced a higher percentage of TSS, which was 12.9 in the first season and 13.6 in the second season, respectively.

Data illustrated in Figure 5 A, B clarified that obtained results of titratable acidity % (TA) were in contrast with soluble solids content in 2019 and 2020. The control had the highest TA.
content, which was 0.77 and 0.79 % during the first and second seasons, respectively. Meanwhile, the MLE treatment recorded a reduction percentage (29.8 and 27.8 %, respectively, in the two seasons) compared with the control. These results are in agreement with Abd El–Hamied and El-Amary, (2015) on ‘Le Conte’ pear, who found that spraying MLE at 4% caused a significant increase in the total soluble solids. It also caused a significant decrease in total acidity. In addition, Alsalhy and Aljabary (2020) they studied spraying MLE at 45 g L\(^{-1}\) on the Halawani grape vines, which caused a significant increase in the total soluble solids. In another study, Khan et al. (2020) found that spraying five cultivars of grape vines with moringa leaf extract at 3% significantly affected total soluble solids and total acidity in fruits compared to the control treatment.

The effect of the treatments on the ascorbic acid (vitamin C) content of navel orange fruit is presented in Figure 5 C. The greatest value of ascorbic acid was obtained from spraying navel orange tress with MLE at 3% (51.5 and 52.9 mg/100 ml juice during 1\(^{st}\) and 2\(^{nd}\) seasons, respectively). This was followed by treatment of MLE at 2 %, which were 50.9 and 51.3 mg/100 ml juice in the first and second seasons, respectively. On the other hand, the ascorbic acid content in control trees was 39.3 and 40.9 mg/100 ml juice in the 2019 and 2020 seasons, respectively. These results are consistent with those of Khan et al. (2020), who reported that the aqueous extract of moringa increased the ascorbic acid (VC) content of grape vines. Similarly, Nasir et al. (2020) reported that MLE application before flowering and at fruit set stages exhibited maximum vitamin C content, about 1.16-fold higher than the control in ‘Kinnow’ mandarin fruit. Considering that MLE is rich in protein and vitamin C its application via foliar spraying enhanced the endogenous vitamin C and facilitated ascorbic acid formation (Ullah et al., 2019; Ismail and Ganzour, 2021).
Figure 5. Effect of moringa leaf extract and calcium chloride treatments on (A) total soluble solids (TSS), (B) titratable acidity % and (C) ascorbic acid (VC) contents of ‘Washington navel’ orange during 2018-2019 and 2019-2020 seasons. Values in the bar followed by the same letter(s) are not significantly different at a 5% level of probability.

Comparative changes in quality parameters and nutrient composition in the creased and the healthy fruit.

Data in figure 6 showed averages some physical quality properties (fresh weight, diameter, peel thickness and firmness) of Washington Navel orange fruits with and without creasing symptoms during 2019 and 2020 seasons. The obtained data showed significant increments (P≤0.05) in fruit weight (g), fruit diameter in the in fruit with creasing symptoms 340.04g and
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9.7 cm, respectively as compared with fruits without creasing symptoms (Figure 6A and B). Concerning the fruit firmness there were significant difference (P ≤ 0.05) between the fruits with and without creasing symptoms 2.06 and 2.86 kg/cm², respectively (Figure 6C). The fruit peel thickness of the fruits without creasing symptoms was significantly thicker (P ≤ 0.05) than the fruits with creasing symptoms in both seasons 5.8 mm and 3 mm, respectively (Figure 6 D). These results are similar to those obtained by Sharaf et al. (2005) and Fatma, (2003 and 2009) who reported that creased fruits of Hamlin orange, Washington navel, and Tanarif orange cultivars had significantly thinner peel as compared to non-creased ones.

Figure 6. Physical quality properties of Washington Navel orange fruits with and without creasing symptoms A) fruit fresh weight, B) fruit diameter, C) peel thickness and D) fruit firmness (Pooled data of 2019 and 2020 seasons). Values in the bar followed by the same letter(s) are not significantly (p ≤ 0.05) different.
There were significant differences (P≤0.05) between the Washington Navel orange fruits with and without creasing symptoms regarding the chemical quality properties (figure 7). Fruits with creasing symptoms had higher values of titratable acidity 88.00 % as compared with fruits without creasing symptoms (Figure 7A), but fruits with creasing symptoms had lower values of soluble solids content and ascorbic acid 10.5% and 38.16 mg/100ml juice, respectively as compared with fruits without creasing symptoms (Figure 7 B and C). The results are in contrast to those of Sallato et al. (2017) on Fukumoto oranges who reported that no significantly differences were found in quality parameters between fruits with and without creasing symptoms.
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Figure 7. Chemical quality properties of fruits of Washington Navel orange fruits with and without creasing symptoms A) soluble solids content, B) titratable acidity and C) ascorbic acid (Pooled data of 2019 and 2020 seasons). Values in the bar followed by the same letter(s) are not significantly (p≤ 0.05) different.

Figure 8 displays the average mineral element concentrations in Washington Navel orange fruits with and without creasing symptoms during the 2019 and 2020 seasons. Significantly differences (P≤0.05) were found in the mineral composition of fruit with and without creasing symptoms. Oranges with creasing had higher nitrogen (242.06, 135.06, and 377.12 mg/fruit) and potassium (160.20, 135.06, and 518.00 mg/fruit) content in the pulp, peel, and whole fruit, respectively (Figure 8A and B). On the contrary, calcium (87.20, 71.00, and 158.20 mg/fruit) and magnesium (26.71, 14.33, and 41.21mg/fruit) content in fruit components, pulp, peel, and the whole fruit were lower in fruit with creasing, respectively (Figure 8C). In this research we found that creasing oranges had significantly lower calcium and magnesium in all fruit tissues when compared with healthy fruit, which confirms its importance in relation to this disorder and agrees with other authors (Saleem et al., 2014).
Figure 8. A) Nitrogen, B) potassium, C) calcium and D) magnesium content (mg/fruit) in pulp, peel and whole of Washington Navel orange fruits with and without creasing symptoms (Pooled data of 2019 and 2020 seasons). Values in the bar with the same color followed by the same letter(s) are not significantly (p≤ 0.05) different.

In terms of the internal rind anatomy of albedo, creasing fruit displayed an irregular pattern of grooves and furrows in the rind, which was absent in fruit without external creasing symptoms (Figure. 9).
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Figure 9. Scanning electronic microscope (SEM) images of albedo of mature “Washington navel” orange with (a) and without (b) creasing symptoms.

Conclusions

A strong correlation was found between the quality parameters, the nutrient composition and the fruit creasing incidence. Foliar application of moringa leaf extract reduced the appearance of fruit creasing, increased fruit yield, fruit weight, fruit firmness, fruit peel thickness, TSS and ascorbic acid, of ‘Washington navel’ orange. It can be concluded that foliar application with moringa leaf aqueous extract 3% at full bloom stage, fruit setting stage, two weeks after fruit setting stage as a biostimulants cheap source of plant growth hormones and minerals especially with the trend of organic farming for reduced incidence of creasing and improved yield and quality of ‘Washington navel’ orange under experimental conditions.
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