

## EFFECT OF FOLIAR SPRAY OF ZINC SULPHATE AND CALCIUM CARBONATE ON FRUIT QUALITY OF KINNOW MANDARIN (*Citrus reticulata* BLANCO)

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In citrus Kinnow mandarin is the most abundantly grown fruit in Pakistan, because of its excellent fresh intake, processing value, flavor, quality, high yield and better adaptability. Quality of Kinnow varies depending upon management practices and climatic conditions. Uniform quality and size of fruits is obligatory for export prospect. As Pakistan targets to increase export up to 15-20% of produce, present study was carried out to study the effects of preharvest foliar spray of zinc sulphate (ZnSO<sub>4</sub>) and calcium carbonate to improve the yield and quality of Kinnow mandarin. Fruit bearing trees were sprayed with calcium carbonate (CaCO<sub>3</sub>) and zinc sulphate each @ 0%, 3% and 4%. Foliar application of ZnSO<sub>4</sub> and CaCO<sub>3</sub> significantly increased the fruit weight, fruit diameter, juice percentage, TSS, ascorbic acid, total antioxidants, total phenolics, carotenoids and flavonoids contents. ZnSO<sub>4</sub> @ 4% gave the best results for Kinnow fruit quality and yield.

**Keywords:** Rutaceae, citrus, mandarins, preharvest, fruit quality, yield.

### INTRODUCTION

Citrus belongs to family Rutaceae in kingdom Plantae. Pakistan is significant for its production in large scale and worldwide distribution. It has economically remunerative and highly valued fruits, with distinct taste and beneficial values. Juice is soothing, refreshing and delightful (Ahmed *et al.*, 2007). Nutrients are usually quickly available by plants as foliar spray than soil application. Foliar spray of micronutrients is 7-21 times extra effective than applied in soil. ZnSO<sub>4</sub> plays an important role in increasing the production by improving the fruit quality and reduce fruit drop. Foliar application of 4 to 12 kg/ha zinc (ZnSO<sub>4</sub>) gave beneficial effect on chemical and physical attributes of fruits, but the lowest application of Zn indicated the better flavor and juice contents, total sugar and vitamin-C (Altaf and Khan, 2008). Fruit weight, TSS, diameter, ascorbic acid and juice fraction are best obtained at the rate of 0.06% Zn in the form of foliar spray on Kagzi lime (Ashraf *et al.*, 2012). Basically, Zn acts as a metal activator for numerous enzymes, including RNA polymerase carbonic anhydrase, super oxidase dismutase and alcohol dehydrogenase. Zn deficiency limits RNA synthesis, resulting in reduced protein synthesis. It is involved in tryptophan biosynthesis a pre-cursor of auxin (IAA). Zn holds significance importance as imparting sustainability in production/productivity by reducing the fruit drop and granulation. Zinc sprayed at the rate of 0.3-0.8% on 13 years old mango trees at bloom stage gave great

enhancement in TSS, total sugars and fruit weight. Foliar application of zinc effect on fruit quality and yield of mango, and increase TSS in fruits and decreased the alternate bearing in Valencia orange and mandarins (Tariq *et al.*, 2007).

Calcium plays significant role in sustaining fruits quality, preserving fruit firmness, proliferation of vitamin C, decreased breakdown of storage rotting and reduction in browning of apple. It also defends from membrane disorganization and protects apparent-free space of tissue generally related with senescence and sustains the protein manufacturing capability of cell. Calcium increases the mechanical power of cell wall because it is the main component of cell wall as calcium pectate in plants which play significant role in establishment of pedicel attachment to proximal of fruit thus resulted in reduced fruit drop (Guardiola and Garcia, 2000). Commercially preharvest spray of calcium delays senescence and increase consumer acceptance with less damaging effect during fruit storage (Lester and Grusak, 2004). Smaller amount of calcium carbonate has been effectively used to decrease softening in fresh fruit. High level of calcium contents in fruit may sustain membrane permeability and decrease the process of ripening during storage and it also increase fruit retention because it stimulate the development of lignin and cellulose and stimulate translocation and formation of carbohydrates (Aguayo *et al.*, 2008). The present study was to investigate the preharvest foliar application of zinc sulphate and calcium

carbonate to improve the fruit quality characteristics (physical and chemical) of Kinnow mandarin.

## MATERIALS AND METHODS

**Collection of samples:** The proposed study regarding the effects of foliar application of zinc sulphate and calcium carbonate on the fruit quality of Kinnow mandarin (*Citrus reticulata* Blanco) was conducted at Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Fifteen fully mature Kinnow trees were selected under randomized complete block design. There were five treatments including the control with three replications. The combinations of treatments were as T<sub>0</sub> = Control, T<sub>1</sub> = 3% CaCO<sub>3</sub>, T<sub>2</sub> = 4% CaCO<sub>3</sub>, T<sub>3</sub> = 3% ZnSO<sub>4</sub>, T<sub>4</sub> = 4% ZnSO<sub>4</sub>. Foliar spray was applied at the end of December. After treatment uniform mature, fruits of Kinnow were harvested. Physio-chemical analysis of treated and untreated fruits were performed in the Pomology Lab. at University of Agriculture Faisalabad Pakistan.

**Physical parameters:** Fruit samples (15 fruits per sample) were weighed on digital balance and average fruit weight was calculated by equation:

$$\text{Average fruit weight} = \frac{\text{Total samples weight}}{\text{Number of fruits in sample}}$$

The diameter of twenty randomly selected fruits from each plant was measured at center of each fruit with the help of Vernier caliper and average fruit diameter was calculated. Juice of each sample was extracted and sieved to eliminate pulp and then percent juice weight of each sample was calculated using equation.

$$\text{Juice weight \%} = \frac{\text{Average juice weight}}{\text{Average fruit weight}} \times 100$$

**Biochemical parameters:** A digital refractometer ATAGO, RS-5000 (Atago, Japan) was used to measure TSS of fruit juice. The instrument was calibrated with distilled water before and during each measurement. A drop of extracted juice sample was placed on clean prism of the instrument; reading was taken directly from refractometer and was expressed as °Brix at room temperature (24-26°C). Ascorbic acid contents of juice were determined following the method described by Ruck (1961). 5 mL of saved juice was taken in 100 mL volumetric flask and volume was made by adding 0.4% oxalic acid solution. Out of this, 5 mL filtrated aliquot was taken, and titrated against 2, 6-dichlorophenolindophenol dye, to light pink color end point, persisted at least for 15 seconds.

For phenolics estimation added 100 µL of sample in a fresh eppendorf tubes and added 200µL 10% F.C. reagents and vortexed thoroughly for few seconds. Then added 800µL Na<sub>2</sub>CO<sub>3</sub> in each tube and again vortexed for few seconds and incubated the tubes at room temperature for 1 to 2 hours. Blank sample was also prepared by using 100µL of extraction mixture (M.A.A. & HCl) instead of sample. Transferred

200µL sample and blank to a clear 96 well microplate and read absorbance at 765 nm for phenolics.

For antioxidants, 50µL extract was added to 5ml 0.004% (4mg/100ml) of methanol solution of DPPH. It was left for 30 minutes in incubation period at room temperature and then absorbance was measured at 517 nm. Then same procedure was repeated while taking 50µl, 100µl and 150µl extracts and average of all three extracts was calculated for antioxidants.

Total carotenoids were determined by the method given by Lalel *et al.* (2003). One gram of fruit pulp was grounded with 0.05 g of magnesium carbonate in silica sand by glass. Extraction was made twice by using acetone: n-hexane (75:60, v/v) mixture (20 ml/sample). The samples were centrifuged at 12000 rpm. The final yellow color was appeared in tubes and extract was obtained in separating funnel and rinsed with the 40 mL of 10% NaCl and distilled water. The samples were read at 436 nm absorption on spectrophotometer.

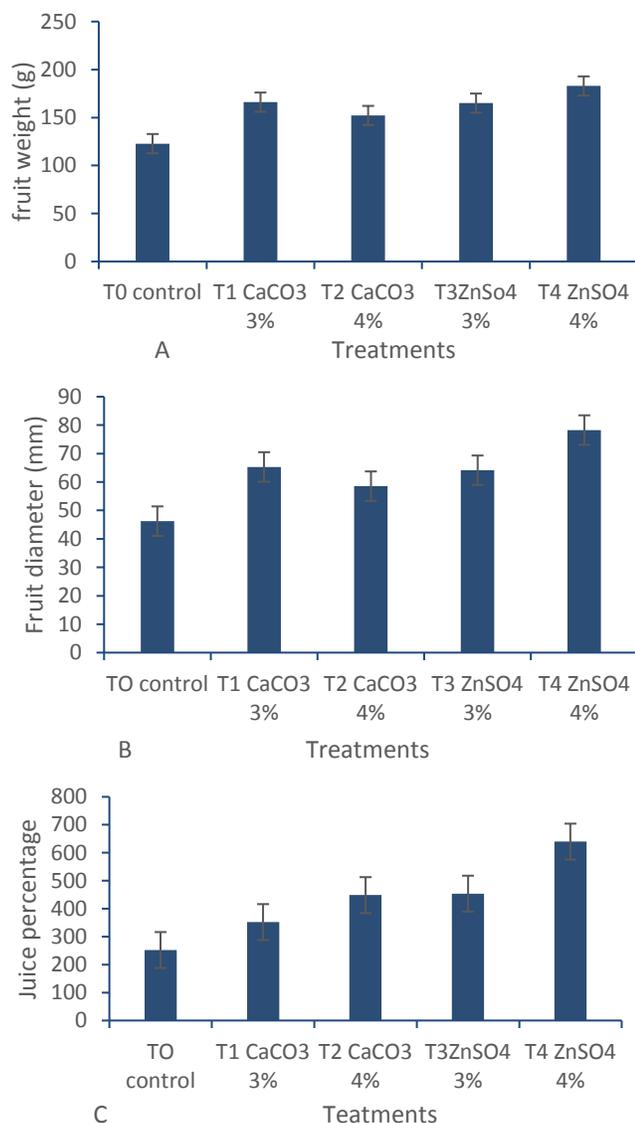
Colourimetric aluminum chloride method was used for flavonoid determination. 0.5 mL solution of each fruit extract in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water and left at room temperature for 30 minutes. The absorbance of extraction mixture was measured at 415nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). Total flavonoid contents were calculated as quercetin from a calibration curve and reading was expressed as TFC (mg of CEQ/100 gm of FW).

## RESULTS AND DISCUSSION

**Fruit weight (g):** Application of zinc sulphate @ 4% (T<sub>4</sub>) showed maximum fruit weight (182.87g). The treatment T<sub>1</sub> (CaCO<sub>3</sub> @ 3%) and T<sub>3</sub> (ZnSO<sub>4</sub> @ 3%) were better (166.03g and 165g, respectively) than CaCO<sub>3</sub> @ 4% (152.13g) but were non-significantly different with each other; however, untreated trees exhibited minimum fruit weight (122.83g) (Fig. 1A). Foliar application of Zn revealed the highest fruit weight in treated fruits as compared to untreated fruits in 'Kinnow' mandarin (Mishra *et al.*, 2003). Another scientist Bhardwaj *et al.* (2010) concluded that calcium applications have no noticeable improvement in average fruit weight of cherry.

**Fruit diameter (mm):** The maximum value of fruit diameter (78.26mm) was observed in T<sub>4</sub> application of ZnSO<sub>4</sub> @4% followed by T<sub>1</sub> (65.30mm), T<sub>3</sub> (64.13mm) and T<sub>2</sub> (58.53mm). However, the treatments T<sub>4</sub>, T<sub>3</sub>, T<sub>2</sub> and T<sub>1</sub> were statistically at par. The untreated trees produced fruits with minimum fruit diameter (46.23 mm) (Fig. 1B). Fruit size and fruit weight was significantly increased by foliar application of zinc sulphate (Tariq *et al.*, 2007). The study of Cerklewski (2005) reported a significant increase in fruit diameter of Balady mandarin and grapefruit by application of calcium carbonate.

**Juice percentage (%):** Among all treatments maximum juice weight percentage was found in T<sub>4</sub> (ZnSO<sub>4</sub> @ 4%) i.e. 639.80%. The treatments T<sub>3</sub> (ZnSO<sub>4</sub> @ 3%) and T<sub>2</sub> (CaCO<sub>3</sub> @ 4%) were better for juice percentage in fruits than T<sub>1</sub> (CaCO<sub>3</sub> @ 3%) but statistically non-significant with each other (454.71, 448.56 and 352.06 %, respectively). The unsprayed trees yielded minimum juice percentage (252.10%) (Fig. 1C). Malik *et al.* (2000) observed that maximum juice was obtained when zinc sulphate was applied on mandarin trees. Li *et al.* (2007) stated that foliar application of calcium carbonate increased the juice contents in mandarin fruits.



**Figure 1. Effect of foliar application of Zinc sulphate and Calcium carbonate on fruit physical parameters.** (A) Fruit weight (B) Fruit diameter (C) Juice percentage of Kinnow mandarin fruits treated with ZnSO<sub>4</sub> and CaCO<sub>3</sub> at different concentrations. Bars indicate difference in values resulted by altering treatments which

are written as T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. At top of each bar a line represents Error bar.

**Total soluble solids (TSS %):** Minimum level of TSS was recorded in control (T<sub>0</sub>) i.e. 107.67%. Maximum total soluble solids were analyzed in T<sub>4</sub> (ZnSO<sub>4</sub> @4%) 12.86% followed by T<sub>3</sub> (CaCO<sub>3</sub> @3%) 12.13% and T<sub>2</sub> (11.6%) (Fig. 2A). Tixier *et al.* (2010) reported that foliar application of zinc sulphate increased the TSS in the fruits of mandarins. Shah *et al.* (2002) reported that foliar spray of calcium carbonate on Kinnow trees improved the TSS in mango, peaches and citrus as compared to those trees which were not sprayed.

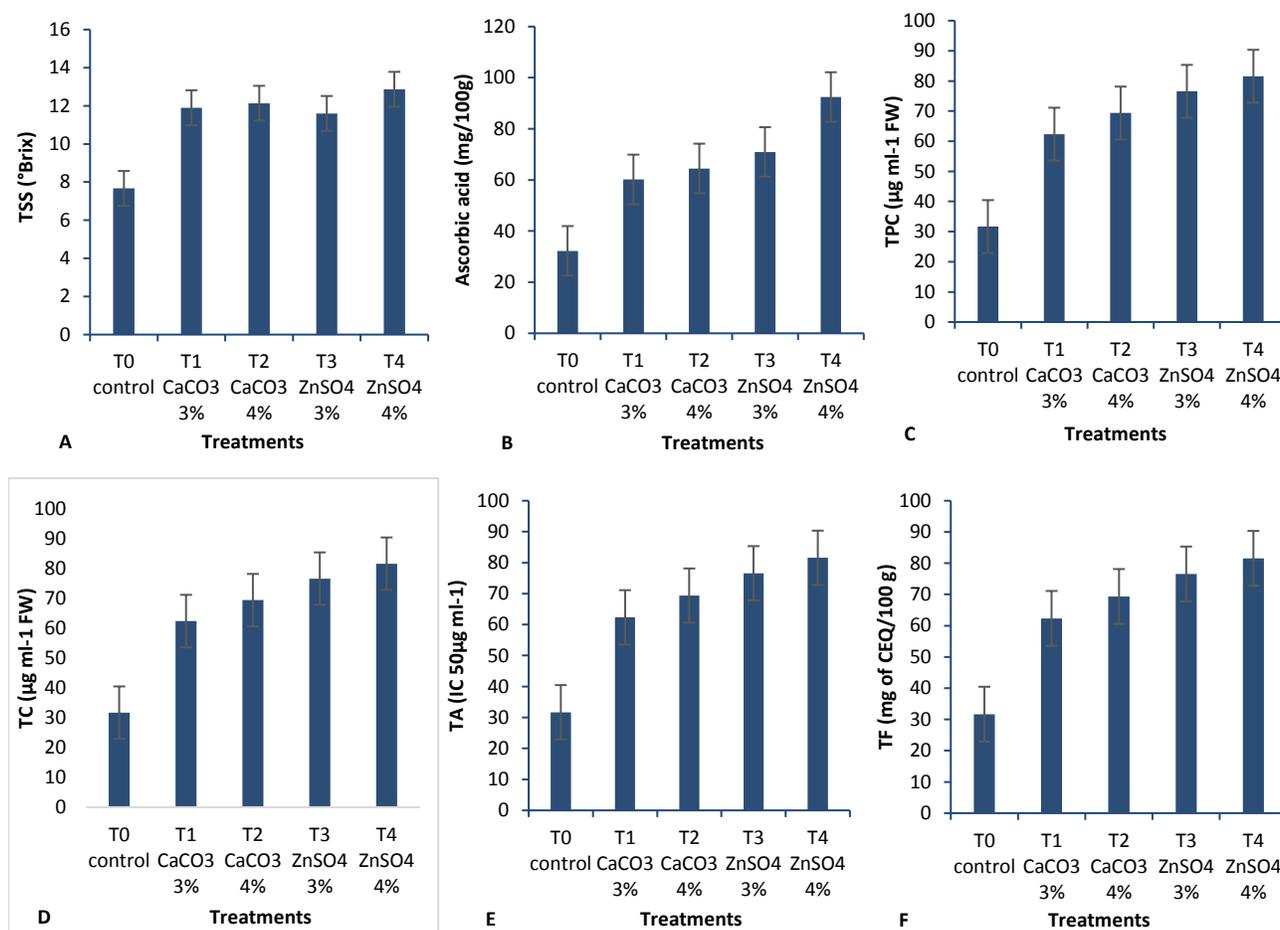
**Ascorbic acid (mg 100 g<sup>-1</sup>):** Maximum level (92.464 mg 100 g<sup>-1</sup>) of ascorbic acid contents were measured in T<sub>4</sub> treatment followed by T<sub>3</sub> (70.46 mg 100 g<sup>-1</sup>), T<sub>2</sub> (64.51 mg 100 g<sup>-1</sup>) and T<sub>1</sub> (60.20 mg 100 g<sup>-1</sup>). Minimum ascorbic acid was recorded in fruits of untreated trees (32.25 mg 100 g<sup>-1</sup>) (Fig. 2B). El-Mwshawi *et al.* (1997) concluded that Zn sprays increased ascorbic acid in Balady mandarin trees. Elmar *et al.* (2007) also reported that calcium increased the ascorbic acid contents thus retained fruit firmness and reduced storage breakdown in apple.

**Total phenolic contents (µg ml<sup>-1</sup> FW):** The results revealed that T<sub>4</sub> showed maximum (85.56 µg ml<sup>-1</sup> FW) total phenolic contents followed by T<sub>3</sub> and T<sub>2</sub> (78.65 and 64.10 µg ml<sup>-1</sup> FW, respectively). Whereas T<sub>0</sub> showed the minimum TPC 31.69 µg ml<sup>-1</sup> FW (Fig. 2C). Phenolics present in fruits have been received considerable attention because of their potential antioxidant activity. Therefore, it can be concluded that the application of Zn and Ca increased the phenolics in Kinnow mandarin as compared to control. Cicco *et al.* (2007) found that application of Zinc sulphate increased the phenolic contents in Kinnow mandarin, lemons and grapefruits.

**Total antioxidants (IC 50µg ml<sup>-1</sup>):** It is clear from the data that maximum total antioxidants (30.16 IC 50µg ml<sup>-1</sup>) was observed in T<sub>4</sub> (ZnSO<sub>4</sub> @4%) fruits followed by T<sub>1</sub> (CaCO<sub>3</sub> @3%), T<sub>3</sub> (ZnSO<sub>4</sub> @3%) and T<sub>2</sub> (CaCO<sub>3</sub> @4%) (18.67, 26.52 and 20.45 IC 50µg ml<sup>-1</sup>, respectively) (Fig. 2E). Berhow (2000) said that zinc sulphate increased the total antioxidants in Kinnow, grapefruit and lemons.

**Total carotenoid contents (µg ml<sup>-1</sup>FW):** Statistical data regarding Total carotenoid contents of Kinnow fruits showed that maximum contents of total carotenoid (16.14 µg ml<sup>-1</sup>FW) were observed by T<sub>4</sub> (ZnSO<sub>4</sub> @4%) while untreated fruits showed minimum contents (11.89 µg ml<sup>-1</sup> FW) of total carotenoids. Fruits of T<sub>2</sub> (CaCO<sub>3</sub> @4%) and T<sub>3</sub> (ZnSO<sub>4</sub> @3%) approximately showed the same value statistically (14.12 and 15.76 µg ml<sup>-1</sup>FW, respectively) (Fig. 2D). Rodrigo and Zacarias (2010) reported that zinc sulphate @4-6% concentration somewhat increased the contents of carotenoid in *C. sinensis* and *C. reticulata*. Babu and Yadav (2002) proved that application of calcium carbonate appeared to increase carotenoids in peel of apple.

**Total flavonoid contents (mg of CEQ/100 g):** It is obvious from the results that application of T<sub>4</sub> (ZnSO<sub>4</sub> @4%) and T<sub>3</sub>



**Figure 2. Effect of foliar application of Zinc Sulphate and Calcium Carbonate on fruit biochemical parameters.** (A) Total soluble solids (TSS) (B) Ascorbic acid (C) Total phenolic contents (D) Total carotenoid contents (E) Total antioxidants (F) Total flavonoid contents of kinnow mandarin fruits treated with ZnSO<sub>4</sub> and CaCO<sub>3</sub> at different concentrations. Bars indicate difference in values resulted by altering treatments which are written as T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. At top of each bar a line represents Error bar.

(ZnSO<sub>4</sub> @4%) showed maximum contents (46.43 and 44.97 mg of CEQ/100 g, respectively) of total flavonoid in comparison to fruits of control (T<sub>0</sub>) with 40.25 mg of CEQ/100 g total flavonoid. Fruits of both T<sub>1</sub> (CaCO<sub>3</sub> @3%) and T<sub>2</sub> (CaCO<sub>3</sub> @4%) received 42.43 and 43.06 mg of CEQ/100 g, respectively, of total flavonoid contents (Fig. 2F). Awad and Jager (2002) reported that zinc sulphate treatments considerably increased the total flavonoid contents in apple, plum, peaches and citrus.

**Conclusion:** The variation in quality attributes studied concluded that preharvest foliar spray of zinc sulphate and calcium carbonate induced a reasonable change in Kinnow fruits. All physical and chemical characteristics were improved. Inclusion of these two nutrients into nutritional cycle can boost the productivity of Kinnow mandarin in

Pakistan, consequently producing more exportable quality fruits, resulting in more income by farmers.

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