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FOLIAR APPLICATION OF 3,5,6-TRICHLORO-2-PYRIDYLOXYACETIC ACID FOR IMPROVING FRUIT SIZE AND QUALITY OF KINNOW MANDARIN (*CITRUS RETICULATA* BLANCO)

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ABSTRACT

Background The marketing and export of citrus fruit from Pakistan is not competitive with other citrus growing countries of the world mainly because of alternate bearing and smaller size fruits. Fruit thinning is a viable option to control alternate bearing and improve fruit size.

Methodology The study was conducted with the objective to assess the effect of foliar application of 3,5,6-trichloro-2-pyridyloxyacetic acid (3,5,6-TPA) on fruit thinning, fruit size and quality characteristics of Kinnow mandarin (*Citrus reticulata* Blanco). Twelve full mature seedless Kinnow plants (10 year old) were selected, and sprayed with 3,5,6-TPA at 2,000 5,000 and 7,000 mg L^{-1} just during early fruit development stage, while water spray in case of control treatment. The experiment was laid out according to randomized complete block design (RCBD) with three replicates. Fruit quality parameters including fruit weight, juice percentage, total soluble solids (TSS), titratable acidity (TA), ascorbic acid, reducing sugars, non-reducing sugars and total sugars were recorded.

Results The application of 3,5,6-TPA reduced the crop load in a dose-dependent manner, and improved the fruit size. Application of 3,5,6-TPA at 7,000 mg L^{-1} during early fruit development stage caused abscission of fruit up to 85%, enhanced fruit size, fruit weight and sugar content compared with control. However, no apparent effect was observed for juice percentage, TSS, TA and ascorbic acid contents.

Conclusion Foliar application of 3, 5, 6-TPA at 7,000 mg L^{-1} during early fruit development stage can be utilized as a thinning agent for Kinnow mandarin to improve fruit size and some other fruit quality characteristics.

INTRODUCTION

Citrus is one of the commercially produced crops grown in fifty three countries of the world. China is the leading producer of citrus fruits, and Pakistan is at 14th position with the share of 1.65% in the world's citrus production (AMIS 2015). In Pakistan, the annual production of citrus fruits exceeds 2.18 million tons (AMIS 2017); and it's the leading fruit crop (Ahmed et al. 2006, 2007a, b; Khan et al. 2010, 2016; Hayat et al. 2017). Growers face the problem of alternate bearing, heavy crop with smaller sized fruits during the "on" year and less crop during the "off" year. Smaller fruit size is a limiting factor for marketing of some citrus cultivars such as Kinnow. Kinnow mandarin is a high yielding cultivar of citrus with excessive fruit production that causes the carbohydrate depletion, leading towards small size and low quality fruits (Goldschmidt et al. 1996). An inverse relationship exists in fruit size and number of fruits per tree. Consumer's choice for large-sized fruits creates a huge difference in prices as they are ready to pay the premium prices for large sized fruits. Beside the fruit size, excessive fruit load on the tree also reduces the shoot growth and adversely affects quality related attributes such as fruit color, sugar

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content and flavoring compounds (Crane and Iwakiri 1987). Several techniques are used to overcome this problem, however fruit thinning has proved effective.

Chemical sprays (thinning agents) during bloom or post-bloom period removes the extra crop load up to 20-30%, leading towards improved fruit size, fruit quality, and reduced postharvest losses because of improved fruit texture (Rahemi and Remezanian 2007; Stern et al. 2007; Cruz and Moreira 2012). Several plants growth regulators are utilized as thinning agent. 3,5,6-trichloro-2-pyrydil-oxyacetic acid is a synthetic auxin, and is also used as a chemical thinning agent. Aapplication of synthetic auxin during early cell division and developmental stage may reduce the number of fruits by creating the abscission layer and reducing the competition of carbohydrates depending upon cultivar, chemical concentration and environmental conditions (El-Otmani et al. 2000; Davis et al. 2004). In sweet cherry (*Prunus avium* L.) and lychee (*Litchi chinensis* L.), application of 3,5,6-TPA at pit hardening stage (13 mm diameter) apparently improved the yield of large-sized fruit without negatively affecting fruit quality (Stern et al. 2000, 2007). In Clementine mandarin cv. Clemenules, application of 3,5,6-TPA has no significant effect on fruit yield but improved average fruit weight (John et al. 2000). Similarly, in Clausellina Satsuma mandarin the 3,5,6-TPA treatment reduced the number of fruits in a dose-dependent manner with a maximum abscission of 70% at 25 mg L^{-1} that significantly enhanced the fruit size (Agusti et al. 2007). Fruit abscission is closely related with ethylene production stimulated by indoleacetic acid (IAA) accumulation in the abscission zone (Agusti et al. 2007).

However, in Clementine mandarin (*Citrus clementina*), reduction in cell growth during initial cell division stage, and enhancement in growth rate with improved size at fruit enlargement stage suggested photosynthetic disorder as a reason for abscission at initial stage (Mesejo et al. 2012). In Navealate' orange 3,5,6-TPA application improved the juice content of fruits with reduced acidity and pH (Muller 1995).

Some previous studies suggested the role of synthetic auxin 3,5,6-TPA in fruit abscission and improved fruit size for horticultural crops but limited information are available for Kinnow mandarin under the climatic condition of Pakistan. Thus, the present study was aimed to investigate the effect of 3,5,6-TPA on fruit thinning and fruit quality attributes of Kinnow mandarin.

MATERIAL AND METHODS

The study was conducted at experimental area square number 32, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan (longitude 73°0776′ E, latitude 31°4181′ N, and altitude of 175 m above the sea level). Ten years old, 12 seedless plants of Kinnow mandarin (*Citrus reticulata* Blanco) grafted onto rough lemon (*Citrus jambhiri* Lush) were selected for this study. The experiment was laid out according to randomized complete block design with three replicates. Single plant was considered as a treatment unit. Plants were sprayed with different concentration of 3,5,6-TPA (2000 mg L^{-1} , 5000 mg L^{-1} , and 7000 mg L^{-1}) during early fruit development stage (after June drop), while water sprayed plants were considered as control. All the experimental plants were maintained under similar agro-climatic conditions and crop husbandry practices. Uniform mature fruits were selected for physical and biochemical analysis. The numbers of dropped fruits before and after the spray were counted and fruit drop percentage (FDP) was calculated by using the following formula.

$$FDP = \frac{\text{Total dropped fruits after spray}}{\text{Total number of fruits before spray}} \times 100$$

To measure the fruit size, ten fruits per plant were used at the time of harvest. Fruit size was measured using digital Vernier caliper (KINCROME, Part No. 2313, Victoria, Australia) and expressed in mm. Average fruit weight was calculated by weighing ten fruits from each experimental plant on electrical weighing balance. The juice of harvested fruit was extracted by juice squeezer and weighed. The average juice percentage (JP) was calculated by using the following formula.

$$Juice\ percentage = \frac{\text{Juice weight per fruit}}{\text{Average fruit weight}} \times 100$$

Total soluble solids were measured with a digital refractometer (ATAGO, RX5000, USA) by placing 1-2 drops of juice on the prism of refractometer. The acidity of juice was determined by taking 10 mL of juice from each sample, diluted with distilled water in a 100 mL beaker and 2-3 drops of phenolphthalein were added as an indicator. The samples were titrated against N/10 NaOH and the results were expressed as percent citric acid (Hortwitz 1960).

$$TTA = \frac{0.1\text{ N NaOH used } \times 0.0064}{\text{Volume of juice used}} \times 100$$

TSS/TA was calculated for each sample by dividing the TSS with the corresponding percentage of the titratable acidity (TTA).

Vitamin C content in the juice were measured according to the method described by Ruck (1961). Sugars (reducing sugars, non-reducing sugars, and total sugars) in the juice were estimated using the method described by Hortwitz (1960). Briefly, 10 mL juice of each sample was taken in 250 ml volumetric flask having 100 mL distilled water, 25 mL lead acetate solution (25%), and 10 mL potassium oxalate (20%) solution and the volume was made up to 250 ml with distilled water and filtered. The filtrate was used for the measurement of sugars.

Statistical Analysis

Treatments response on fruit quality was determined by Statistical analysis of data using IBM-SPSS software. The data were analyzed using analysis of variance (ANOVA) and the differences among the treatment means were compared according to Duncan's multiple range test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Fruit thinning (%)

Chemical thinning is a commercial horticultural practice used to enhance the cropping potential and get maximum number of large-sized fruits (Etienne 2000). Limited supply of carbohydrates during fruit development may reduce the fruit size, particularly during the heavy crop load (Bangerth 2000). The application of 3,5,6-TPA during early fruit development stage (after June drop) effectively enhanced the fruit abscission depending on the concentration. Increase in chemical concentration enhanced the abscission intensity, and application of 3,5,6-TPA at 7,000 mg L^{-1} provoked abscission of 85% fruits, whereas in control fruit thinning percentage was only 37% (Table 1). During the bloom period, competition for carbohydrates might be increased. It was evident from our results that 3,5,6-TPA application at early fruit development stage caused fruit thinning of Kinnow mandarin. The higher rate of abscission was due to the direct involvement of auxin that enhanced the ethylene levels and cellulose activity in the abscission zone by inhibiting the IAA resultantly fruit thinning occurred (Burns et al. 2003).

Physical characteristics

Currently, the quality of citrus fruit is estimated by its size which consequently led to good yield (Ahmad et al. 2009). The 3,5,6-TPA application has a positive effect on Kinnow mandarin towards enhancing the fruit size. At harvest, an increase in fruit size was observed (103.79 mm) with an increasing 3,5,6-TPA concentration up to 7000 mg L^{-1}. Although, 2000 mg L^{-1} and 5000 mg L^{-1} 3,5,6-TPA treatment also performed well in term of fruit size improvement but the effect was non-significant among each other. Whereas, minimum fruit size of 72.64 mm was observed for control plants (Table 1). The increase in fruit size possibly indicated the ability of auxin to mobilize carbohydrates towards remaining fruits, and increase the elasticity of cell wall that enlarged the fruit size (Arteca 1996; Stern et al. 2007). On the contrary, Reig et al. (2016) suggested reduce turgor pressure as a possible reason for increased fruit size in 3,5,6-TPA-treated fruits. In loquat (*Eriobotrya japonica*), the growth rate of the 3,5,6-TPA treated-fruits was 9-11% higher compared with non-treated fruits resulting a 10% increased fruit diameter (Reig et al. 2016). The fruit distribution according to fruit weight showed a considerable shifting of fruit towards maximum weight by 3,5,6-TPA application compared with control. The maximal per fruit weight of 168.24 g and 146.56 g was observed in 3,5,6-TPA treated-plants at a concentration of 7,000 mg L^{-1} and 2,000 mg L^{-1}, respectively (Table 1). Both these treatments were statistically significant ($P \leq 0.05$) among each other. On the contrary, 5,000 mg L^{-1} 3,5,6-TPA treatment and control plants both showed almost similar fruit weight of 133.31 g and 129.12 g, respectively. The synthetic auxin application significantly enhanced the fruit weight of Kinnow and Clementine mandarin (John et al. 2000; Nawaz et al. 2008, 2011). This might be because of sugars accumulation and auxin involvement in sink and source strengthening that improved fruit growth rate and transport of carbohydrates, water and ion from leaves to fruit that ultimately enhanced per fruit weight (Guardiola 2002). Moreover, during early fruit development stage (after June drop), 3,5,6-TPA application caused thinning of fruits and increased the cell division leading to improved fruit size and per fruit weight (Gavila et al. 2004). An obvious effect of 3,5,6-TPA treatment was observed for fruit juice content. The perusal of Table 1 indicated that improved juice percentage of 63.67% was achieved by 2,000 mg L^{-1} 3,5,6-TPA application whereas difference was observed for other treatments and control. Application of 3,5,6-TPA positively affected the juice percentage of Kinnow mandarin fruits compared with control. By comparing the fruit size and juice content, it was observed that an increase in fruit size was not associated with an increase in juice content. Maximum juice percentage was observed for 2,000 mg L^{-1} 3,5,6-TPA treated fruits compared with 7,000 mg L^{-1}. It suggested that higher auxin concentration generally affected fruit size and endocarp growth.

Bio-chemical characteristics

Total soluble solids is an important parameter for

Table 1 Effect of 3,5,6-TPA on fruit thinning and physical characteristics of Kinnow fruits

Treatments	Fruit thinning (%)	Fruit size (mm)	Per fruit weight (g)	Juice percentage
Control	37.33d	72.64c	129.12b	48.23b
2,000 mg L^{-1} 3,5,5-TPA	52.33c	83.52b	146.56ab	63.67a
5,000 mg L^{-1} 3,5,5-TPA	72.33b	84.14b	133.31b	46.28b
7,000 mg L^{-1} 3,5,5-TPA	85.67a	103.7a	168.24a	45.85b

Means in a column followed by similar letters are non-significant at $P \leq 0.05$; Control: (0 mg L^{-1} 3,5,6-TPA)

determining the fruit quality. The mandarin fruits with higher TSS content and lower acidity are preferred because of their sweet taste. The data regarding TSS of Kinnow mandarin showed maximum TSS (12.32˚) for 5,000 mg L^{-1} followed by 2000 mg L^{-1} and 7000 mg L^{-1} 3,5,6-TPA treated fruits with TSS of 10.82˚ and 10.11˚, respectively. Minimum TSS (9.79˚) was observed for non-treated plants (Figure 1a). Aranguren et al. (2004) reported that pre-harvest foliar application of 3,5,6-TPA during early fruit development stage (after June drop) improved fruit color and reduced the acidity by increasing soluble solid content in Valencia orange. Similar results were observed for this study, 3,5,6-TPA-treated fruits showed higher TSS to acidity compared with control plants. These biochemical changes are closely associated with ethylene production and availability of total 1-aminocyclopropane-1-carboxylic acid (ACC) content in flesh during fruit development stage (Amoros et al. 2003). Application of 3,5,6-TPA reduced the TA, maximum TA was found for control plants (Figure 1 b). However, no significant difference among the chemical treatments was observed. Results regarding TSS/TA demonstrated that maximum TSS/TA (7.09) was observed by the application of 3,5,6-TPA at 5,000 mg L^{-1}. The application of 3,5,6-TPA at 2,000 mg L^{-1} and 7,000 mg L^{-1} also improved TSS/TA (6.06 and 5.86) compared with the control (4.96) (Figure 1c). Data regarding ascorbic acid content showed non-significant differences among the different 3,5,6-TPA treatments and control (Figure 1d).

Similar results were also reported by Serciloto et al. (2011), they observed no difference for ascorbic acid content among the 3,5,6-TPA treated and control plants of Murcott (*Citrus reticulata* Blanco x *C. sinensis* Osbeck). Maximum reducing sugar percentage (3.81) was obtained by the application of 3,5,6-TPA at 7,000 mg L^{-1}. Application of 2000 mg L^{-1} 3,5,6-TPA and control plants showed the reducing sugar percentage of 3.45% and 3.16%, respectively (Figure 2a). Minimum reducing sugars (2.87%) were observed for plants treated with 5,000 mg L^{-1} 3,5,6-TPA. However, for the non-reducing sugars, no significant ($P \leq 0.05$) differences were observed (Figure 2b). Maximum total sugar content (7.37%) were obtained by the application of 3,5,6-TPA at 7,000 mg L^{-1} followed by 2,000 mg L^{-1} (7.18%), whereas, minimum total sugar content (6.22%) were observed for 5,000 mg L^{-1} 3,5,6-TPA followed by control (Figure 2c). According to another report, pre-harvest application of 3,5,6-TPA enhanced the sugar content of citrus fruit compared with control because of an increased accumulation of carbohydrates and conversion of these accumulated carbohydrate to hexose and sucrose (Bangerth 2000; Agusti et al. 2002).

CONCLUSION

It was found that exogenous application of 3,5,6-TPA during early fruit development stage (after June drop) regulated the crop load by thinning effect and improved fruit size and fruit quality compared with non-treated fruits. Application of 3,5,6-TPA at 7,000 mg L^{-1} proved better compared with other treatments, and it might be considered an important strategy to improve the fruits size of Kinnow fruits.

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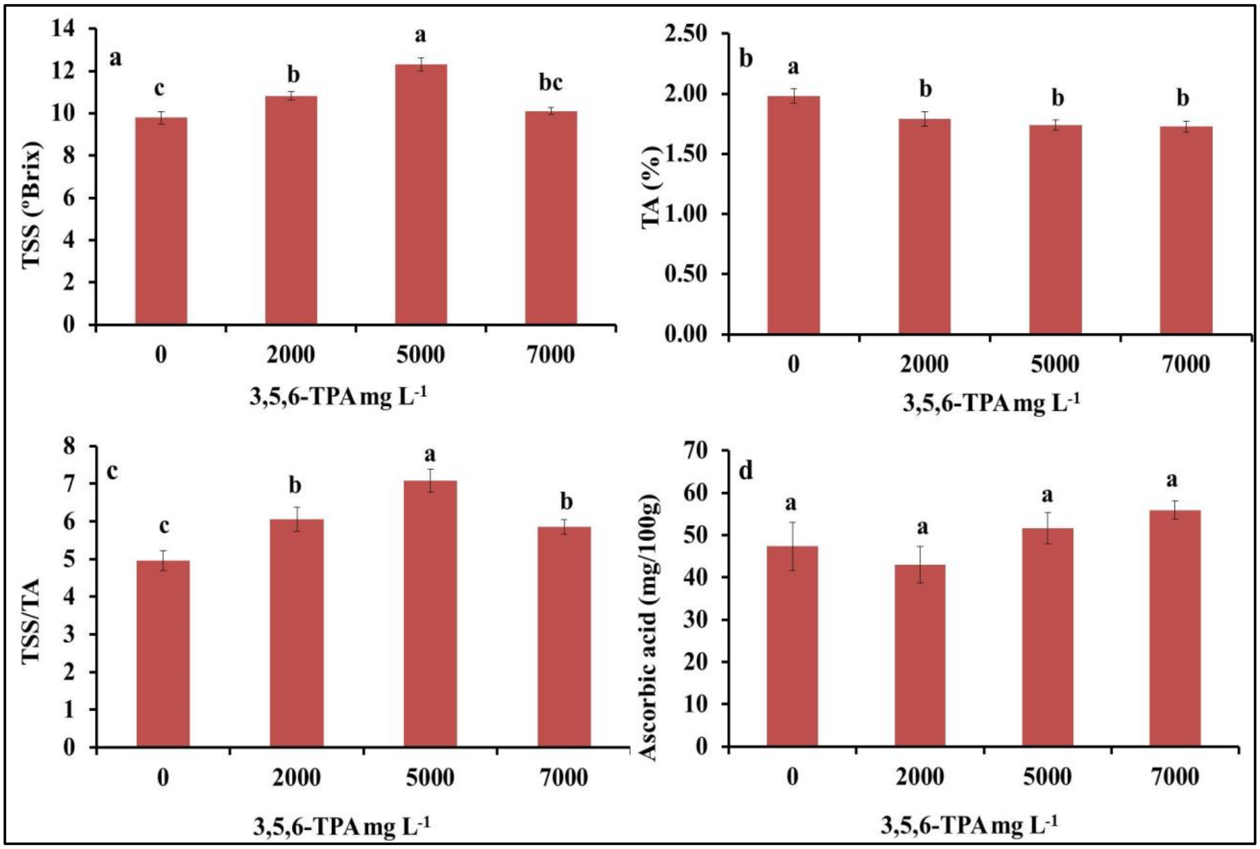

Figure 1 Effect of 3,5,6-TPA application on the biochemical attributes of Kinnow fruit **a:** total soluble solids (TSS), **b:** titratable acidity (TA)**, c:** TSS/TA, **d:** ascorbic acid content

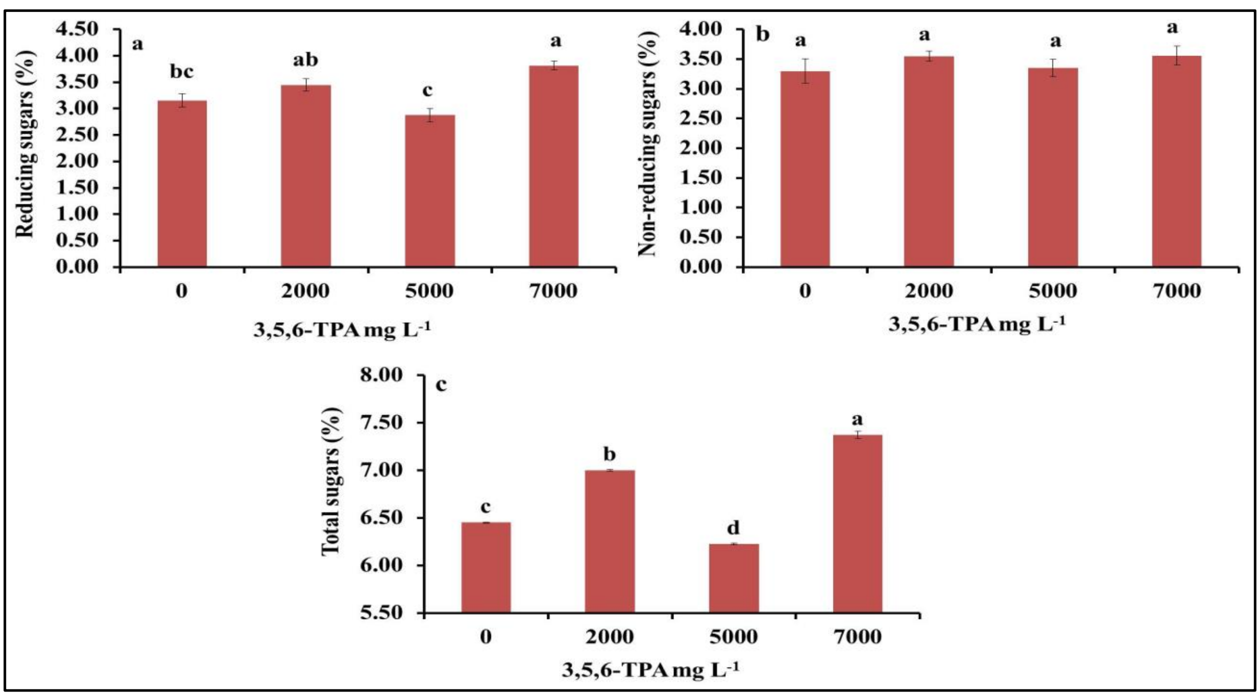

Figure 2 Effect of 3,5,6-TPA application on the biochemical characteristics of Kinnow fruits. **a:** reducing sugars, **b:** non-reducing sugars**, c:** total sugars

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