IMPROVEMENT IN YIELD, QUALITY AND REDUCTION IN FRUIT DROP IN KINNOW (CITRUS RETICULATA BLANCO) BY EXOGENOUS APPLICATION OF PLANT GROWTH REGULATORS, POTASSIUM AND ZINC

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Abstract

Kinnow (*Citrus reticulate* Blanco) fruit is one of the best commercial fruits of Pakistan. It is cultivated on a large area in the Punjab province due to its reasonably higher yield, quality, taste and flavor than those of the other citrus fruits. However, its average yield in Pakistan is far below than that of the other citrus growing countries of the world. Fruit dropping is one of the main reasons of low citrus fruit yield in Pakistan, which is thought to be mainly due to hormonal imbalance in the plants. This imbalance may occur as a result of nutrient deficiency in orchard soils, water shortage, and insect pest attack on the citrus trees. Therefore, experiments were conducted to assess the influence of growth regulators [2,4-D and salicylic acid (SA)] and nutrients [potassium (K) and zinc (Zn)] to improve yield and quality of Kinnow fruit and control the fruit drop at four selected sites in the citrus growing tract of Punjab, Pakistan. Foliar applications of 2,4-D, SA, K, and Zn significantly improved the fruit weight, number of fruits per plant juice percentage, total soluble solids (TSS), ascorbic acid content, acidity, and TSS/acid ratio, and reduced the fruit drop. Application of 2,4-D+K+Zn and SA+K+Zn showed beneficial effects on all the afore-mentioned parameters.

Introduction

Citrus is grown on about 170,000 ha of land in Pakistan, constituting about 30 percent of the area under all fruit orchards (Saleem *et al.*, 2008; Ashraf *et al.*, 2010). Citrus is generally grown all over Pakistan but the Punjab province has the largest area under this fruit tree. The major citrus variety grown in Punjab is Kinnow covering 80 percent of the total citrus growing area (Altaf, 2006).

In Pakistan, Kinnow is grown on a large scale in Punjab (Naz et al., 2007) where its fruit yield is 9.5 tons ha⁻¹ and 1.28 million tons per season(Ibrahim et al., 2011). The average yield in Pakistan is far below than other citrus producing countries like Brazil where it is 40 to 60 tons ha⁻¹ (Ibrahim et al., 2011). In Pakistan, Kinnow is preferably grown under natural environmental conditions so as to maintain its original flavor and quality intact. Citrus orchards in Pakistan are facing problem of fruit size, color, quality and excessive premature fruit drop which is due to the deficiencies of essential nutrients (Ibrahim et al., 2007). Nutrient deficiency disturbs the production of plant growth regulators controlling size, color and premature fruit dropping. Excessive premature fruit dropping in fruit crops is also dependent on other factors like high temperatures and water deficits, insect/pest attack, and wind velocity of the area (Ibrahim et al., 2007; Ashraf et al., 2012; Razi et al., 2011). Citrus trees produce profuse flowers which develop into fruits that are later shed off necessarily to reduce heavy fruit load, so that the fruits remaining on trees can be sustained. The abovementioned factors cause fruit drop and application of nutrients or plant growth regulators is recommended to reduce premature fruit drop (Modise et al., 2009, Ashraf et al., 2010;2012).Flower/fruit dropping was recorded at anthesis which continued up to the time of harvest (Modise et al., 2009). The initial dropping is due to

abscission of weak fruit lets, which appear after anthesis. Abscission layer at the stem resulting in fruit drop is formed due to imbalance of auxins, cytokinins, and gibberellins (Lahey et al., 2004; Chen & Dekkers, 2006; Balal et al., 2011). As the fruit size at an early stage is very small, the dropping is minimal. However, it is very severe when fruits are of medium size and whole area under citrus trees is covered with dropped fruits (Saleem et al., 2005). Literature (Lahey et al., 2004; Saleem et al., 2005; Chen & Dekkers, 2006; Modise et al., 2009) indicates that application of plant growth regulators is effective in reducing the excessive premature fruit drop. Plant growth regulators like 2,4-dichlorophenoxyacetic acid (2,4-D) and salicylic acid (SA)have been reported to be effective in controlling fruit drop in citrus (Coggins & Lovatt, 2004; Ashraf et al., 2012). Other plant growth regulators which are being used to prevent fruit drop include 2,4,5-trichlorophenoxypropionic acid (2,4,5-TPA), naphthalene acetic acid (NAA), and gibberellic acid (GA) (Michael et al., 1999). Citrus fruit tree takes up higher amount of potassium (K) as compared to other macronutrients (Alva & Tucker, 1999: Ashraf et al., 2010; 2012) because it has a key role in many key physiological processes like water relations, opening and closing of stomata, cell division, formation of sugars and starch, neutralization of organic acids, synthesis of proteins, and activation of enzymes, etc.(Liu et al., 2000; Srivastava & Singh, 2006; Ashraf et al., 2008). Potassium improves fruit quality by enhancing fruit size, juice contents, color, size and juice flavor (Tiwari, 2005; Ashraf et al., 2010). So, limited supply of K may affect the yield and quality of citrus fruit and accelerate the fruit dropping. Similarly, deficiency of micronutrients (Zn, Cu, Fe and Mn) in the soils of citrus orchards also affects the fruit yield, quality and fruit dropping (Ibrahim et al., 2007; Ashraf et al., 2012). Severe deficiency of Zn was noted long ago in the citrus orchards of Punjab, Pakistan (Rehman et al., 1999). However, foliar application of Zn

can improve the citrus fruit yield and quality and control the premature fruit drop (Rodríguez et al., 2005; Ashraf et al., 2012). Different workers suggested that application of suitable combinations of plant growth regulators, and macro- and micro-nutrients can control the excessive fruit drop and improves the yield and quality of circus fruits (Doberman & Fairhurst, 2000; Rodríguez et al., 2005; Saleem et al., 2005). Therefore, effective supply of nutrients and plant growth regulators is necessary to produce high quality citrus fruits and control excessive citrus fruit drop which involves selection of appropriate combinations of nutrients and plant growth regulators, and their rate, time and method of application. Limited findings on control of excessive premature fruit drop in Kinnow are available. Information on the nutrient application to control fruit drop is also scanty. Therefore, the present experiments were conducted to study the control of excessive premature fruit drop and improvement in Kinnow fruit yield and quality by the application of 2,4-D, salicylic acid (SA), K+Zn, and their combinations.

Materials and Methods

Experimental sites: Studies were conducted in 4 Kinnow orchards selected in the citrus growing belt of Punjab, Pakistan [Sargodha-I (Chak # 75 Janubi), Sargodha-II (Chak # 38 Shamali), Toba Tek Singh (Chak # 297/G.B) and Jhang (Ghar Mor)]. For the determination of physico-chemical characteristics (Table 1) of orchard soils, samples up to 60cm depth of each site were analyzed for pH, electrical conductivity (ECe), organic matter (Nelson & Sommers, 1982), total N (Kjeldhal method), Na⁺, K⁺, Ca²⁺, Mg²⁺, $CO_3^{2^-}$, HCO₃⁻ and P (Anon., 1962; Jackson, 1962). Each orchard was irrigated with canal water, which was also analyzed for chemical characteristics (Table 1) following the methods of US Salinity Laboratory Staff, (1962). Plants at two sites, i.e., Sargodha-I (Chak # 75 Janubi) and Toba TekSingh (Chak # 297/G.B), were 30 year-old, while on other 2 sites [Sargodha-II (Chak # 38 Shumali & Jhang (Ghar Mor)] the plants were 15 year-old.

 Table 1a. Soil characteristics (0-60 cm) of four selected sites used to study the effect of 2,4-D, SA, K+Zn, and their combinations on Kinnow fruit yield and quality and control of excessive fruit drop.

	Values			
Soil characteristics	Sargodha-I	Sargodha-II	T. T. Singh	Jhang
	(Chak # 75 Janubi)	(Chak # 38- Shumali)	(Chak # 297/G B)	(Ghar More)
		Physical		
Soil texture	Clay loam	Clay loam	Sandy clay loam	Sandy clay loam
		Chemical		
$EC_e(dS m^{-1})$	1.92-3.28	0.754-1.68	0.96-0.98	0.98-1.89
pH _s	7.62-7.77	7.23-7.35	6.8-7.3	7.0-7.5
Organic matter (%)	0.6-0.66	0.72-0.76	0.5-0.6	0.25-0.32
$NO_3-N (mg kg^{-1})$	14.1-15.3	16.58-17.35	17.3-17.4	7.4-11.3
P (mg kg ⁻¹)	9.4-11.4	6.44-7.95	8.6-11.2	7.5-9.8
K(mg kg ⁻¹) total	82-117	56-76	80-115	78-107
Ca+Mg (meq L^{-1})	12.6-15.7	10.34-12.45	7.0-7.5	0.18-0.30
$CO_3(meq L^{-1})$	Nil	Nil	Nil	Nil
$HCO_3 (meq L^{-1})$	1.6-3.0	1.7-2.6	3.0-6.0	2.0-2.5

Table 1b. Characteristics of irrigation water.

Canal water characteristics	Values
EC ($dS m^{-1}$)	0.77
pH	7.9
SAR (Sodium adsorption ratio)	5.7
Na (meq L^{-1})	7.0
Ca+Mg (meq L^{-1})	3.0
$K(meq L^{-1})$	0.7
$CO_3 \pmod{L^{-1}}$	Nil
$HCO_3 (meq L^{-1})$	2.0

Treatments: Our previous findings (Ashraf *et al.*, 2010; 2012) showed that the soils and trees of all the selected citrus orchards were deficient in K and Zn and foliar application of K+Zn and SA+Zn+K showed encouraging results in controlling fruit drop and improving fruit yield and quality. So, the present experiments were conducted with the aim to improve the control of excessive

premature fruit drop and enhance the fruit yield and quality by exogenous application of 2,4dichlorophenoxyacetic acid (2,4-D), salicylic acid (SA), Zn+K and their combinations through foliar spray. Soil amendment with NPK fertilizers [100 kg N, 50 kg P₂O₅ and 75 kg K₂O ha⁻¹ as urea, DAP and sulfate of potash (SOP), respectively] was done just after harvesting the pervious fruits. Foliar application of 2,4-D or SA (10 mg L^{-1} each), Zn+K (0.25% Zn as ZnSO₄ + 0.25% K as K₂SO₄ solution) and their combinations containing 0.02% Tween-20 (treatment details in Table 2) was carried out at initiation of flowering, fruit formation and at color initiation on fruit stages, while control plants were sprayed with distilled water. Each orchard consisted of 100 trees (10 rows and 10 trees in each row). The 1^{st} , 10^{th} and 6^{th} trees in 5 rows and in the remaining 5 rows, 1^{st} , 10^{th} and 5^{th} trees were kept as nonexperimental. Of the remaining 70 trees, 10 randomly selected trees were sprayed foliarly with each treatment in each orchard.

Fruit drop and yield: Fruit samples were collected randomly from each side of the tree (East, West, North and South) of control and treated plants, and fruit size determined by measuring circumference/diameter. Flowers were counted at full bloom to determine the fruit set. Fruits formed after two weeks of full bloom were counted and fruit setting determined by the following formulae:

Fruit set (%) =
$$\frac{\text{Total number of fruitlets}}{\text{total number of flowers}} \times 100$$

Fruit drop was calculated by counting fruits in July and percent fruit drop determined as:

Fruit retention was determined by counting the number of fruits retained upto final harvest:

Fruit retention (%) =
$$\frac{\text{[Total number of retained } - \text{Total number of fruitlets]}}{\text{Total number of fruitlets}} \times 100$$

Fruit yield was noted at the time of harvest on an individual tree basis and expressed as total fruit number.

Juice quality and chemical analysis: Juice volume was estimated after mechanical extraction from fruits of equal size selected from all sites. The juice pH was determined with a pH meter and total brix (total solids in the juice) with a refractometer. Citric acid was estimated by titrating the juice against 0.1 N sodium hydroxide, and ascorbic acid by reducing 2,6-dichlorophenol indophenols with the juice. Data recorded for each parameter were statistically analyzed and significant means separated using the Least Significant Difference test (Steel *et al.*, 1997).

Results and Discussion

Kinnow fruit yield and dropping: Foliar application of plant growth regulators and nutrients (SA, 2,4-D, K+Zn) or their combinations significantly improved the fruit yield and fruit setting per tree in all selected orchards. The trees treated with 2,4-D+Zn+K exhibited the highest fruit setting which was at par with that recorded at SA+Zn+K and minimum setting was recorded in trees sprayed with distilled water. Site to site variations in fruit setting indicated that it was maximum at Sargodha-I closely followed by T.T. Singh and minimum in the orchard of Jhang (Fig. 1A). The findings of the present study proved that foliar application of plant growth regulators and nutrients or their combinations was effective in enhancing the fruit setting (Fig. 1A), which ultimately resulted to increase the Kinnow fruit yield. The above results are well supported by some other reports published earlier (El-Saida, 2001; Saleem et al., 2005; Omaima & Metwally, 2007; Ashraf et al., 2012) which clearly indicated that foliar-applied plant growth regulators and/or nutrients were effective in enhancing citrus fruit setting.

Table 2. Treatments details.				
Treatment	Description /Sources	Rate		
С	Control	Distilled water		
2,4-D	2,4-dichlorophenoxyacetic acid	10 mg L ⁻¹		
SA	Salicylic acid	10 mg L^{-1}		
Zn+K	Zn+K (Zn as ZnSO ₄ + K as K ₂ SO ₄)	(0.25 % Zn as ZnSO ₄ +0.25 % K as K ₂ SO ₄ solution)		
2,4-D+SA	2,4-dichlorophenoxyacetic acid + Salicylic acid	$5 \text{ mg L}^{-1} 2,4\text{-}D\text{+}5 \text{mg L}^{-1} \text{ SA}$		
2,4- D+Zn+K	2,4-dichlorophenoxyacetic acid + $ZnSO_4$ + K_2SO_4	10 mg L ⁻¹ 2,4-D+ 0.25 % Zn +0.25 % K		
SA+Zn+K	Salicylic acid+ ZnSO ₄ + K ₂ SO ₄	10 mg L ⁻¹ Salicylic acid + 0.25 % Zn +0.25 % K		

Fruit retention was significantly higher in trees sprayed with 2,4-D+Zn+K closely followed by that observed at SA+Zn+K, while the least retention was recorded in trees under control conditions in all selected orchards (Fig. 1B). However, fruit retention differed from site to site; it was significantly higher at T.T. Singh and Sargodha-I than that recorded at the other sites, Sargodha-II and Jhang. The results clearly indicated that foliar application of 2,4-D+Zn+K and SA+Zn+K was effective in promoting fruit retention upto final harvest, which ultimately increased the fruit yield. Modise et al., (2009) found that foliar application of 2,4-D increased the fruit retention in citrus, while El-Baz (2003), Omaima & Metwally (2007) and Ashraf et al., (2012) suggested that application of SA+Zn+K could improve fruit retention per tree. Application of combinations of plant growth regulators and nutrients is suggested by different workers

for achieving enhanced fruit retention, which includes 2,4-D (Agustí *et al.*, 2006; Modise *et al.*, 2009), lowbiuret urea and GA₃ (Saleem*et al.*, 2008), Zn+K (Ashraf *et al.*, 2010), urea+GA₃ (Ibrahim *et al.*, 2011), and SA+Zn+K (Ashraf *et al.*, 2012).

Fruit drop decreased significantly due to foliar spray of 2,4-D, SA, Zn+K and their combinations in all selected citrus orchards (Fig. 1C). A maximal fruit drop was recorded in the trees sprayed with distilled water (control), while minimal noted in those sprayed with 2,4-D+Zn+K followed by SA+Zn+K, 2,4-D, K+Zn, SA, or control. Site to site variations were also recorded and the highest fruit drop was found in the Jhang orchard followed by Sargodha-II, T.T. Singh and Sargodha-I. Kinnow fruit drop is a serious problem in citrus orchards, which could be due to various factors, such as limited water availability during

flowering/fruiting season, temperature fluctuations, and nutrient deficiency etc.; all these factors are reported to cause plant hormonal imbalances (Modise *et al.*, 2009). Different types of treatments have been suggested by different workers. For example, Sattar (1999) recommended foliar application of urea. Anthony & Coggins (2001) advocated NAA and 3,5,6-TPA as potential chemicals for controlling citrus fruit drop. Stover (2000) showed that application of GA₃ and NAA is beneficial in controlling the pre-harvest fruit drop in Californian citrus crops. Exogenous application of GA₃ not only reduces early fruit abscission in citrus but it also delays senescence (Hershely, 2001). Different workers suggested different chemicals like foliar spray of combination of 2,4-D and GA₃ (El-Otmani1992),combined fertilizers [(NH₄)₂SO₄+SSP+K₂SO₄] (Saleem *et al.*, 2005), 2,4-D (Modise *et al.*, 2009; Agustí *et al.*, 2006), low-biuret urea (Saleem *et al.*, 2008), urea andGA₃ (Ibrahim *et al.*, 2011) and SA+Zn+K (Ashraf *et al.*, 2010; 2012) to effectively control citrus fruit dropping.

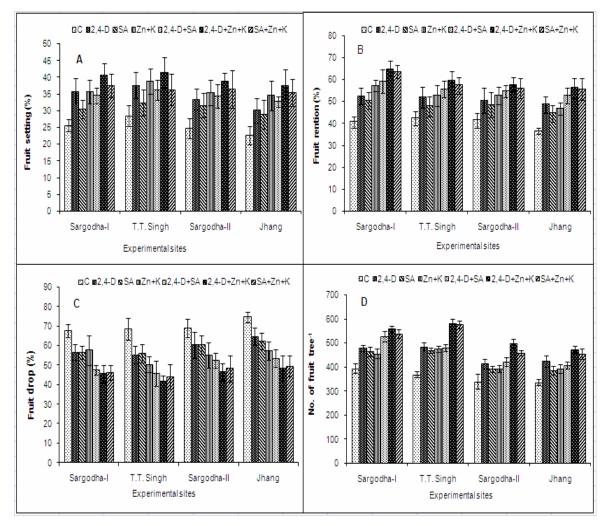


Fig. 1. Effect of 2,4-D, SA, Zn+K, and their combinations on fruit setting (A), fruit retention (B), fruit drop (C) and number of fruits per tree (A), of Kinnow plants growing in four orchards.

In the present studies, trees sprayed with 2,4-D, SA, Zn+K or their combinations produced significantly higher fruit yield than the control in all selected orchards (Fig. 1D). The highest fruit yield (numbers) was in trees treated with 2,4-D+Zn+K followed by those with SA+Zn+K. Number of fruits per tree varied from site to site and the highest number of fruits per tree was recorded in T.T. Singh orchard followed by Sargodha-I. Minimum fruit yield was recorded for Jhang orchard followed by Sargodha-II. Our results indicated that, foliar spray of 2,4-D+Zn+K or

SA+Zn+K is necessary for obtaining optimal Kinnow fruit yield. Literature also confirmed that application of plant growth regulators and nutrients supply is necessary for controlling the premature fruit drop and fruit yield (Srivastava & Singh, 2006; Saleem *et al.*, 2008; Ashraf *et al.*, 2010; 2012). The results of the present study were also supported by some earlier published reports (Tariq *et al.*, 2007; Ibrahim *et al.* 2011) which clearly depict that foliar application of macro- and micro-nutrients, and plant growth regulators enhances citrus fruit yield. **Quality parameters:** The data for skin color of harvested fruit showed the highest number of green fruits on trees treated with 2,4-D followed by those with SA or control, whereas minimum on those treated with Zn+K followed by SA+Zn+K and 2,4-D+Zn+K (Fig. 2A). The pattern obtained for skin coloring may

not be only attributed to the treatment of 2,4-D while there could be the contribution of some other factors also such as partitioning of assimilates which resulted in varying response in case of fruit skin color (Modise *et al.*, 2009) or it may be due to growth retarding action of 2,4-D.

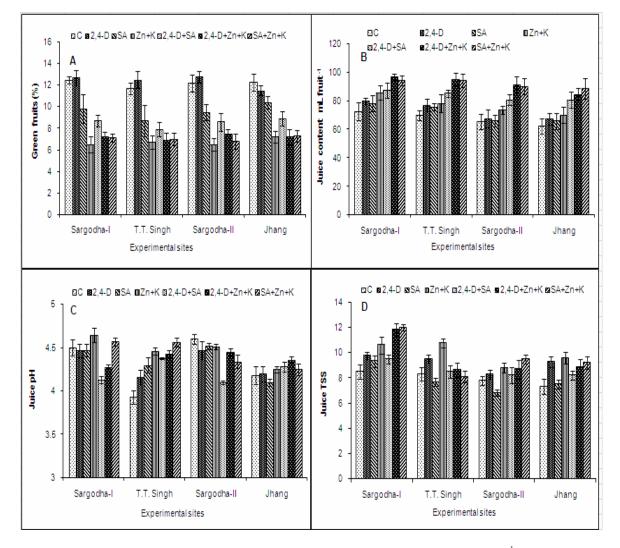


Fig. 2. Influence of foliar application of 2,4-D, SA, Zn+K, and their combinations on number of green fruits tree⁻¹ (A) juice volume (B), juice pH (C), and juice TSS (D) of Kinnow plantsgrowing in four orchards.

Plant growth regulators (2,4-D and SA), nutrients (Zn+K) or their combinations significantly enhanced the juice contents per fruit in all orchards (Fig. 2B). The highest juice volume was obtained from the fruits of plants which were sprayed with 2,4-D+Zn+K followed by SA+Zn+K. The juice volume was also influenced due to the site to site variations. The highest juice contents were noted in fruits of Sargodha-Iorchard which was closely followed by T.T. Singh, while lowest from the fruits of Jhang orchard. Earlier reports showed higher juice percentage in fruits of plants treated with SA (Lahey *et al.*, 2004), 2,4-D (Modise *et al.*, 2009), Zn+K (Omaima & Metwally, 2007; Ashraf *et al.*, 2010), and SA+Zn+K

(Ashraf *et al.*, 2012). Similarly, Rehman *et al.*, (1999) reported that foliar application of only Zn improved the juice contents in orange, while Saleem *et al.*, (2008) reported that application of low-biuret urea increased yield and quality of sweet orange (*Citrus sinensis*) resulting in higher number of marketable fruits. Juice contents are very important parameter for juice industry because fruits with higher juice contents are preferred and fetch good price.

Foliar application of plant growth regulators (2,4-D and SA) and nutrients (Zn+K) increased the juice pH, but was not so significant (Fig. 2C). Variations in juice pH with respect to different sites were also nonsignificant. In contrast, total soluble solids (TSS) of juice increased significantly with foliar application of plant growth regulators and nutrients as well as their combinations in all the selected orchards (Fig. 2D). Juice with the highest TSS was obtained from the fruits treated with SA+Zn+K closely followed by those treated with 2,4-D+Zn+K. Fruit juice of trees obtained from Sargodha-I had the maximum TSS followed by the fruits from T.T. Singh, Sargodha-II and Jhang. These results clearly indicate that foliar application of 2,4-D+Zn+K or SA+Zn+Kis effective in improving fruit yield and juice quality. Modise et al., (2009) found that foliar spray of 2,4-D improved the TSS of the citrus juice. Ashraf et al., (2010) reported that foliar supply of Zn+K enhanced the TSS of Kinnow juice. Similarly, El-Khawag (2007) noted an improvement in TSS of pomegranate juice after the foliar application of Zn. Ashraf et al., (2012) suggested application of SA+Zn+K to obtain juice with higher TSS and low TSS/acid ratio.

Ascorbic acid is another important characteristic determining juice quality, which was significantly influenced by the foliar application of 2,4-D, SA, Zn+K or their combinations (Fig. 3A). The highest ascorbic acid content was recorded in the juice obtained from the fruits of trees treated with 2,4-D+Zn+K or SA+Zn+K; it was minimum in the fruits of control plants. The variations among orchards for ascorbic acid contents were nonsignificant. However, fruits obtained from Sargodha-I contained higher ascorbic acid content than those from all other orchards. Foliar application of 2,4-D (Modise et al., 2009; Agustí et al., 2006), nutrients (Nakhlla, 1998; Abd El-Moneim et al., 2007; Saleem et al., 2008; Ashraf et al., 2010), and plant growth regulators as well as nutrients (Ibrahim et al., 2011; Ashraf et al., 2012) was effective in enhancing the ascorbic acid contents in citrus fruits. The results of the present study clearly show that foliar application of 2,4-D+ Zn+K and SA+Zn+K was very effective in improving ascorbic acid contents in citrus fruit.

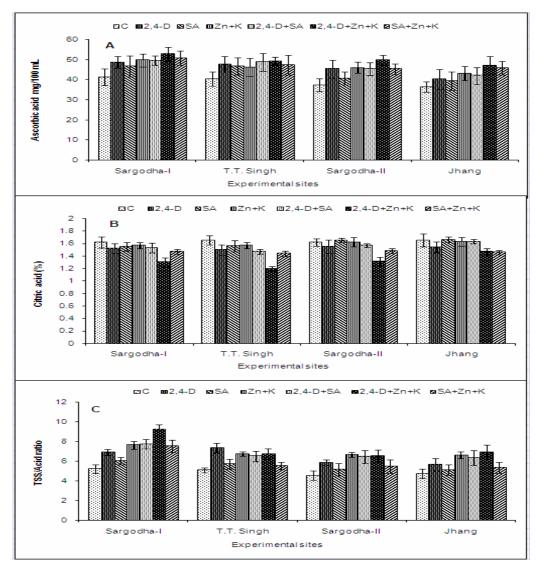


Fig. 3. Effect of foliar-applied 2,4-D, SA, Zn+K, and their combinations on juiceascorbic acid (A), citric acid (B) and TSS/acids ratio (C) of Kinnow plants growing in four orchards.

The citric acid in fruit juice was reduced due to the application of 2,4-D, SA, Zn+K or their combinations in all selected orchards. An appreciable decrease in juice citric acid content was noted in trees treated with SA+Zn+K, which was at par with that obtained by the application of Zn+K(Fig. 3B). Juice citric acid content varied non-significantly from site to site. Juice TSS/acid ratio improved with foliar spray of plant growth regulators and Zn+K as well as their combinations (Fig. 3C). The highest TSS/acid ratio was recorded in fruit juice of trees sprayed with SA+Zn+K and 2,4-D. This parameter was also influenced by environmental conditions of the study sites. The highest TSS/acid ratio was recorded for fruits from Sargodha-I followed by T.T.Singh, Sargodha-II, and Jhang. The results of the present study clearly indicate that foliar application of 2,4-D+Zn+K and SA+Zn+K could effectively control the excessive fruit drop and improve the yield and quality of kinnow. The TSS/acid ratio is generally considered important by the juice manufacturing factories because fruits with high juice content, ascorbic acid and TSS/acid ratio are preferred and fetch reasonably high price. Therefore, foliar applications of 2,4-D+Zn+K or SA+Zn+K seem beneficial. Modise et al., (2009) suggested application of 2,4-D to improve TSS/acid ratio, other quality parameters and control of excessive fruit drop. Abd-Allah (2006) applied K foliarly to improve all the above- mentioned fruit quality parameters, Ashraf et al., (2010) suggested foliar spray of Zn+K to enhance TSS/acid ratio, yield and quality of citrus fruit, while Ashraf et al., (2012) further supported the application of plant growth regulators and nutrients to achieve maximum control on excessive fruit drop and improved yield and quality parameters.

Conclusions

The present findings clearly indicate that foliar application of 2,4-D+Zn+K or SA+Zn+K is effective in improving fruit yield and juice quality by increasing juice volume/contents, pH, TSS, ascorbic acid, and TSS/acid ratio of juice and providing the maximum control on excessive drop of premature Kinnow fruit. Therefore, foliar spray of 2,4-D+Zn+K or SA+Zn+K at the onset of flowers, fruit formation and at the stage of color initiation on fruit is suggested to maximize the production of Kinnow as well as other citrus fruits.

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