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Influence of Exogenous Applications of Plant Growth Regulators on Fruit Quality of Young 'Kinnow' Mandarin (*Citrus nobilis* × *C. deliciosa*) Trees

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ABSTRACT

Young 'Kinnow' mandarin (*Citrus nobilis* Lour × *C. deliciosa* Tenora) trees often produce inferior quality fruit with less juice and more rind and rag contents. The influence of plant growth regulators (PGRs) on fruit quality of mature citrus trees is well documented. However, little is known about the role of PGRs on the fruit quality of young 'Kinnow' mandarin trees. Therefore in the present investigation PGRs *viz* benzyladenine (BA) and kinetin (20 mg L^{-1}) were applied at flowering (FL) stage and, BA, kinetin (10, 20 & 30 mg L^{-1}) and gibberellic acid (GA_3) 10 mg L^{-1} were applied at fruit setting (FS) stage to 'Kinnow' mandarin trees and their effect on fruit quality was evaluated immediately after harvest (D1) and after seven days of shelf (D7) at ambient conditions (20±2ºC; 60-65% RH). The PGRs alone had significant influence on juice mass (%), rag mass (%), ascorbic acid (AA mg 100 mL^{-1}) and reducing sugars (%) whereas, rind mass (%), TSS, titratable acidity (TA), TSS:TA and total sugars (%) were not affected by PGRs applications. Irrespective of shelf duration the fruit harvested from young trees treated with 10 mg L^{-1} GA_3, 30 mg L^{-1} BA and kinetin at FS showed significantly higher juice contents (50.53%, 49.8% & 51.64%) and lower rag contents (26.5, 26.6 & 25.83%), respectively in comparison with control. Maximum reducing sugars (1.62%) was observed with 20 mg L^{-1} kinetin at flowering and maximum AA contents (58.45 mg 100 mL^{-1}) of juice were observed with control. Interaction of PGRs and shelf duration had significant effect on reducing sugars (%), non reducing sugars (%) and AA (mg 100 mL^{-1}) contents. All fruit quality parameters except non reducing sugars (%) were significantly affected by shelf duration. Our results validated that fruit quality parameters except AA contents of young 'Kinnow' mandarin trees could be improved by the exogenous application of kinetin.

Key Words: Cytokinins; Fruit quality; Gibberellic acid; 'Kinnow' mandarin; Young trees

INTRODUCTION

'Kinnow' mandarin (*Citrus nobilis* Lour × *Citrus deliciosa* Tenora) is the major fruit crop of Pakistan in terms of area, production and export (Anonymous, 2009). It provides 95% share of annual citrus exports from Pakistan (Anwar and Ibrahim, 1994). Pakistan is the fifth largest mandarin exporter in the world on the basis of export volume and on tenth position in terms of value earned from exports due to low unit price (US $ 222 $tonne^{-1}$) in international market (FAO, 2008), owing mainly to poor fruit quality and presentation.

So far identified and well addressed quality related factors include; pruning, irrigation, fertilizer application, disease and plant protection measures (Mahmood & Sheikh, 2006; Anwar *et al.,* 2011). Besides these factors low tree age (15-20 years) is becoming a threat to citrus industry of Pakistan. The life span of 'Kinnow' mandarin tree is declining due to many biotic and abiotic factors (Ahmad *et al*., 2006) and seldom exceeds 25 years (Ibrahim, 1994). In more than 40% cases tree decline starts at the age of 10 year, which is the prime age of production (Ahmad *et al*., 2006). However, in other countries economic life of citrus tree is 50-100 years depending on good management practices (Chaudhary, 1994). In Pakistan, citrus tree takes 8-9 years for quality and commercial fruit production, where as in other countries like Australia this period is only six years (Johanson, 2006). Currently exporters are reluctant to take fruit from young (less than eight years old) orchards due to quality concerns in terms of low juice and high rind and rag contents (Malik personal communications). Moreover, fruit from young trees also contains less total soluble solids (Hearn, 1993), hence rejected by the processers. Due to these reasons fruit from young trees are often sold in local market at very low price thus reducing the income of grower.

Therefore, it is essential to extend the productive window of 'Kinnow' mandarin orchard beyond 10-15 years.

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There is an urgent need to address the following two issues: (1) decline related factor (2) fruit quality improvement in young orchards. Lot of research work has been done to overcome the first possibility by tree health management (Batool *et al*., 2007; Chung & Brlansky, 2005; Razi *et al.,* 2011), whereas limited information is available for improving fruit quality of young orchards.

Application of PGRs including GA_3 and cytokinins for quality fruit production is well documented in mature trees of 'Satsuma' mandarin (Garcia-Luis *et al*., 1985), 'Sunbrust' mandarin (Pozo *et al*., 2000), grapefruit (EI-Zeftawi, 1980), 'Hamlin', 'Valencia', and 'Navel' oranges (Fidelibus *et al*., 2002). However, the work on exogenous application of PGRs on fruit quality of young citrus trees is rare. Some studies revealed that young plants are low in endogenous GA_3 (Wadhi & Ram, 1967) and cytokinins (Hendry *et al*., 1982) as compared to mature plants. This showed the difference in endogenous levels of PGRs between young and mature plants, which might be a possible reason for poor quality of fruit in young orchards. Therefore, the present study was designed to explore the potential of exogenous application of PGRs such as cytokinins [benzyladenine (BA) and kinetin] and gibberellic acid (GA_3) in improving the physico-chemical quality attributes of fruit from young 'Kinnow' mandarin trees.

MATERIALS AND METHODS

This study was carried out on young (3-5 years old) 'Kinnow' mandarin trees at Silanwali tehsil of Sargodha district (32º03'N; 72º40'E), Pakistan. The experiment was laid out under randomized complete block design with three replicates and single tree was treated as treatment unit. Thirty trees with similar vigour budded on rough lemon (*Citrus jambheri* Lush) rootstock were selected to investigate the effect of BA, kinetin and GA_3 on 'Kinnow' mandarin fruit quality. Aqueous solution of all the treatments was prepared and sprayed on whole trees to the point of run off at fruit setting (FS) and flowering (FL). The treatments included T_1=Control, T_2=10 mg L^{-1} BA at FS stage, T_3=20 mg L^{-1} BA at flowering FL stage, T_4=20 mg L^{-1}BA at FS stage, T_5=30 mg L^{-1} BA at FS stage, T_6=10 mg L^{-1} kinetin at FS stage, T_7=20 mg L^{-1} kinetin at FL stage, T_8=20 mg L^{-1} kinetin at FS stage, T_9=30 mg L^{-1} kinetin at FS stage, T_{10}=10 mg L^{-1} GA_3 at FS stage. A non-ionic wetting agent Tween 20 (0.1%) was added as a surfactant. Control plants were treated with simple water containing Tween 20.

Fruit sampling and data collection: At optimum fruit maturity twenty fruit from each experimental unit were harvested carefully and transported to Postharvest Research and Training Centre (PRTC), Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan. On arrival, the fruit of all treatments were washed in tap water air dried and divided into two lots i.e., for immediate analysis (D1) and assessments after seven days of shelf (D7) at ambient conditions (20±2ºC; 60-65% RH) for various fruit quality attributes.

Data regarding fruit physical quality attributes such as rind mass (%), rag mass (%), juice mass (%) and rind thickness (mm) were determined as described by Saleem *et al*. (2008). Rind, rag and juice were weighed separately and their quantities were expressed in percentage. Rind thickness (mm) was measured with the help of Vernier caliper. Juice total soluble solids (TSS), titratable acidity (TA) and TSS:TA ratio were determined by the method described by Malik and Singh (2003). The TSS (ºBrix) of the juice was determined by using hand refractometer (Atago, ATC-1, Tokyo, Japan) from a pooled juice sample squeezed from 10 fruit. The TA (%) of the juice was determined by titration method using 0.1 N NaOH and was expressed in percentage. The TSS:TA was determined by dividing TSS by analogous TA value. The AA (mg 100 mL^{-1}) was determined by titration method using 2, 6 dichlorophenolindophenol dye. Reducing, non-reducing and total sugars (%) were determined by the method outlined earlier by Maqbool and Malik (2008).

Statistical analysis: The response of different treatments on fruit quality was determined by statistical analysis of the data using software MSTAT-C (Freed, 1994), while Duncan's Multiple Range (DMR) test was used to compare the differences among the treatment means (Steel *et al.,* 1997).

RESULTS

Fruit physical quality parameters: Plant growth regulators alone decreased rag mass (%) and maximum reduction (25.83%) was observed with 30 mg L^{-1} of kinetin at FS stage as compared with control (Table I). However, the interaction of PGRs and shelf duration showed no significant impact on rag mass (%). rag mass (%) was reduced significantly during shelf period at ambient conditions (20±2ºC; 60-65% RH). Rind mass (%) was not significantly affected by the application of PGRs (Table I). The interactive effect of PGRs and shelf duration had no significant effect on rind mass (%). However, significant decline in rind mass (%) was observed during shelf life period at ambient conditions. Juice mass (%) was slightly improved by PGRs application (Table II). Kinetin application (30 mg L^{-1}) at FS stage produced fruit with maximum juice mass (51.64%) as compared with control. There is no significant effect of PGRs and their interaction with shelf duration on juice mass (%). Juice mass (%) increased during shelf duration at ambient conditions. PGRs application alone and their interaction (PGRs x shelf duration) had no significant effect on rind thickness (mm). However, rind thickness was significantly reduced during shelf duration (Table II).

Fruit biochemical quality parameters: The PGRs alone and their interaction with shelf duration had statistically significant effect on AA (mg 100 mL^{-1}) contents of juice (Fig. 1). Maximum AA (58.45 mg 100 mL^{-1}) was recorded

Table I: Effect of plant growth regulators on rind and rag contents of fruit harvested from young 'Kinnow' mandarin trees

Treatments	Rind mass (%)			Rag mass (%)		
	*D1	**D7	Mean (Treatment)	*D1	**D7	Mean (Treatment)
Control	24.95	21.38	23.17	32.42	26.12	29.27ab
10 mg L^{-1} BA @ FS stage	25.91	22.66	24.28	32.98	28.21	30.59a
20 mg L^{-1} BA @ FL stage	26.43	23.54	24.99	29.89	24.50	27.20bc
20 mg L^{-1} BA @ FS stage	26.72	23.34	25.03	30.20	24.56	27.38bc
30 mg L^{-1} BA @ FS stage	24.14	22.95	23.54	28.76	24.50	26.63bc
10 mg L^{-1} kinetin @ FS stage	24.98	21.57	23.28	29.12	24.44	26.78bc
20 mg L^{-1} kinetin @ FL stage	27.44	21.77	24.66	28.99	26.03	27.51bc
20 mg L^{-1} kinetin @ FS stage	28.82	24.31	26.57	31.92	25.44	28.68abc
30 mg L^{-1} kinetin @ FS stage	22.85	22.21	22.53	26.48	25.19	25.83c
10 mg L^{-1} GA_3 @ FS stage	23.93	21.85	22.89	26.73	26.43	26.58bc
Mean (Shelf duration)	25.62a	22.56b		29.75a	25.52a	
LSD value ($P \leq 0.05$)		Rind mass (%)			Rag mass (%)	
PGRs (LSD)		NS			2.66	
Shelf duration (*P* value)		0.00			0.00	
PGRs x Shelf duration (LSD)		NS			NS	

Table II: Juice rag (%) and rind thickness (mm) of 'Kinnow' mandarin affected by plant growth regulators

Treatments	Juice mass (%)			Rind thickness (mm)		
	*D1	**D7	Mean (Treatment)	*D1	**D7	Mean (Treatment)
Control	42.63	52.50	47.56ab	4.10	3.51	3.80
10 mg L^{-1} BA @ FS stage	41.12	49.12	45.12b	4.43	3.61	4.02
20 mg L^{-1} BA @ FL stage	43.63	51.95	47.82ab	4.26	3.70	3.98
20 mg L^{-1} BA @ FS stage	43.08	52.10	47.59ab	4.10	3.66	3.88
30 mg L^{-1} BA @ FS stage	47.10	52.55	49.83a	4.09	3.57	3.83
10 mg L^{-1} kinetin @ FS stage	45.90	53.99	49.95a	4.12	3.38	3.76
20 mg L^{-1} kinetin @ FL stage	43.58	52.20	47.89ab	4.33	3.36	3.84
20 mg L^{-1} kinetin @ FS stage	39.26	50.25	44.76b	4.50	3.89	4.20
30 mg L^{-1} kinetin @ FS stage	50.67	52.60	51.64a	4.11	3.61	3.86
10 mg L^{-1} GA_3 @ FS stage	49.34	51.71	50.53a	4.52	3.64	4.08
Mean (Shelf duration)	44.63b	51.90a		4.26a	3.59b	
LSD value ($P \leq 0.05$)		Juice mass (%)			Rind thickness (mm)	
PGRs (LSD)		3.96			NS	
Shelf duration (*P* value)		0.00			0.00	
PGRs x Shelf duration (LSD)		NS			NS	

Any two means not sharing a common letter are significantly different ($P \leq 0.05$)

*D1 (Immediately after harvest), **D7 (Seven days after harvest), BA (benzyladenine), GA_3 (gibberellic acid), FL (flowering), FS (fruit setting), PGRs (plant growth regulators)

with control, while minimum AA contents (34.88 mg 100 mL^{-1}) were observed with 20 mg L^{-1} kinetin at FS stage. Control gave maximum AA contents both on D1 and D7 with 61.67 and 55.24 mg 100 mL^{-1}, respectively as compared to PGR applications. The AA contents were significantly decreased after seven days shelf time. The PGRs showed statistically similar effect on TSS, TA (%) and TSS:TA in comparison to with control (Table III). Interaction of PGRs with seven days shelf time had no significant effect on TSS, TA (%) and TSS:TA. The TA (%) was significantly reduced whereas TSS and TSS:TA increased during shelf period at ambient conditions. Total sugars (%) significantly increased at ambient conditions during shelf period (Table IV). However, PGRs alone and PGRs interaction with seven days ambient time showed statistically similar effects on total sugars (%). Reducing sugars (%) was significantly increased by PGRs applications (Table IV). Tree sprayed with 20 mg L^{-1} kinetin at FL stage produced fruit with significantly higher reducing sugars (1.62%) in comparison to control. Interaction results showed that all kinetin applications significantly enhanced the level of reducing sugars (%) in contrast to control on D7. Reducing sugars (%) increased significantly during shelf duration at ambient conditions. Non reducing sugars (%) were not affected by PGRs application (Table IV). The PGRs interaction with shelf duration showed significant effect on non reducing sugars (%). Kinetin application at 10 mg L^{-1} on FS stage gave maximum non reducing sugars (6.14%) on D1, whereas on D7 this treatment gave minimum non reducing sugars (4.39%) in comparison to control. Shelf duration had no significant influence on non reducing sugars (%).

DISCUSSION

The use of PGRs in improving citrus fruit quality is well documented in literature (Pozo *et al*., 2000; Fidelibus *et al*., 2002; Saleem *et al*., 2008). In this study, GA_3 and higher concentrations of BA and kinetin significantly increased juice mass (%) and decreased the rag mass (%) as

Table III: Plant growth regulators applications affect on acidity (%) and TSS contents of young 'Kinnow' mandarin trees

Treatments	TSS			Acidity (%)			TSS:TA		
	*D1	**D7	Mean (Treatment)	*D1	**D7	Mean (Treatment)	*D1	**D7	Mean (Treatment)
Control	8.60	10.03	9.32	0.78	0.70	0.75	10.96	14.49	12.78
10 mg L^{-1} BA @ FS stage	9.57	10.20	9.88	0.96	0.79	0.88	9.94	13.01	11.48
20 mg L^{-1} BA @ FL stage	9.87	9.87	9.87	0.86	0.75	0.81	11.38	13.38	12.38
20 mg L^{-1} BA @ FS stage	9.50	9.83	9.67	0.84	0.73	0.78	11.38	13.54	12.46
30 mg L^{-1} BA @ FS stage	10.47	10.80	10.63	0.86	0.80	0.83	12.09	13.58	12.83
10 mg L^{-1} kinetin @ FS stage	10.80	9.57	10.18	0.86	0.76	0.81	12.55	12.68	12.62
20 mg L^{-1} kinetin @ FL stage	10.03	10.03	10.03	0.92	0.64	0.78	10.98	15.51	13.25
20 mg L^{-1} kinetin @ FS stage	9.80	10.37	10.08	0.85	0.88	0.86	11.68	11.98	11.83
30 mg L^{-1} kinetin @ FS stage	8.83	9.83	9.33	0.85	0.73	0.79	10.56	13.64	12.10
10 mg L^{-1} GA_3 @ FS stage	9.37	10.23	9.80	0.79	0.80	0.80	12.92	12.94	12.93
Mean (Shelf duration)	9.68b	10.1a		0.86a	0.76b		11.44b	13.84a	
value ($P \leq 0.05$)		TSS			Acidity (%)			TSS:TA	
PGRs (LSD)		NS			NS			NS	
Shelf duration (*P* value)		0.04			0.00			0.00	
PGRs x Shelf duration (LSD)		NS			NS			NS	

Table IV: Total, reducing, and non reducing sugars (%) of young 'Kinnow' mandarin trees as affected by plant growth regulators application

Treatments	Total sugars (%)			Reducing sugars (%)			Non reducing sugars (%)		
	*D1	**D7	Mean (Treatment)	*D1	**D7	Mean (Treatment)	*D1	**D7	Mean (Treatment)
Control	6.02	7.02	6.52	1.13cd	1.35bcd	1.24bcd	4.64cd	5.39abc	5.01
10 mg L^{-1} BA @ FS stage	6.70	7.14	6.92	1.15cd	1.21bcd	1.18d	5.27abcd	5.63abc	5.45
20 mg L^{-1} BA @ FL stage	6.91	6.91	6.91	1.13cd	1.28bcd	1.20cd	5.49abc	5.35abcd	5.42
20 mg L^{-1} BA @ FS stage	6.65	6.88	6.77	1.36bcd	1.06d	1.21cd	5.02bcd	5.53abc	5.28
30 mg L^{-1} BA @ FS stage	7.33	7.56	7.45	0.92d	1.60abc	1.26bcd	6.09a	5.66ab	5.88
10 mg L^{-1} kinetin @ FS stage	7.56	6.67	7.13	1.09cd	2.07a	1.59ab	6.14a	4.39d	5.26
20 mg L^{-1} kinetin @ FL stage	7.02	7.02	7.02	1.19bcd	2.05a	1.62a	5.54abc	4.72bcd	5.13
20 mg L^{-1} kinetin @ FS stage	6.86	7.26	7.06	1.04d	2.07a	1.56abc	5.53abc	4.92bcd	5.23
30 mg L^{-1} kinetin @ FS stage	6.18	6.88	6.53	1.08cd	1.97a	1.52abcd	4.85bcd	4.67bcd	4.76
10 mg L^{-1} GA_3 @ FS stage	6.56	7.16	6.86	1.30bcd	1.68ab	1.49abcd	4.99bcd	5.20abcd	5.10
Mean (Shelf duration)	6.69b	7.14a		1.14b	1.63a		5.35	5.14	
value ($P \leq 0.05$)		Total sugars (%)			Reducing sugars (%)			Non reducing sugars (%)	
PGRs (LSD)		NS			0.31			NS	
Shelf duration (*P* value)		0.03			0.00			NS	
PGRs x Shelf duration (LSD)		NS			0.44			0.82	

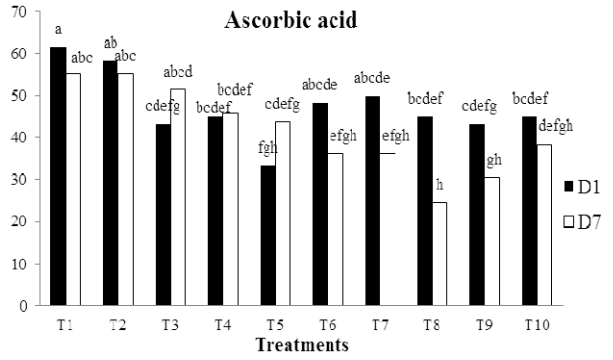
Any two means not sharing a common letter are significantly different ($P \leq 0.05$)

*D1 (Immediately after harvest), **D7 (Seven days after harvest), BA (benzyladenine), GA_3 (gibberellic acid), FL (flowering), FS (fruit setting), PGRs (plant growth regulators)

compared with control. This might be due to the increased vascularization in the pedicel (Guardiola *et al*., 1993) and/or due to the increased sink strength (Mauk *et al*., 1986) and/or reduced senescence and respiration from the fruit (Wade & Bradey, 1971; Dhillion *et al*., 1985) by the application of these growth regulators. Concurrent effects of GA_3 on juice yield were reported in 'Satsuma' mandarins (Garcia-Luis *et al*., 1985) 'Sunbrust' mandarin (Pozo *et al*., 2000) and 'Hamlin' orange (Davies *et al*., 2001; Fidelibus *et al*., 2002). However in grapefruit (EI-Zeftawi, 1980) GA_3 reduced juice mass (%), while in 'Clementine' mandarin it has slight effect on juice contents (Guardiola *et al*., 1981). After seven days storage rind mass (%), rag (%) and rind thickness (mm) decreased, whereas juice mass (%) increased. This might be due to more loss of moisture from rind as compared to the flesh of the fruit (Ladaniya, 2008) hence resulted in increase of juice mass (%). Similar results were reported in lemon during storage at 13ºC by Cohen *et al*. (1990).

PGRs alone and their interaction with storage had no effect on TA, TSS, TSS:TA ratios and total sugars. Similar results on TSS with the application of GA_3 were described in grapefruit (Ferguson *et al*., 1982) and in other fruits like apples with the application of GA_3 and BA (Koukourikou-Petridou *et al*., 2007) and by BA alone in guava (Jayachandran *et al*., 2007). GA_3 had no or inconsistent effects on TA of 'Sunbrust' mandarin (Pozo *et al*., 2000) and 'Hamlin' orange (Davis *et al*., 1997). Although the concentration of TSS and total sugars were higher in PGRs treatments when analyzed after harvest but after seven days at ambient conditions some of the PGRs treatments showed less increment in TSS and total sugars as compared to control. This might be due to the treatment effect on physiological ageing and change in metabolism (Bhardwaj *et al.,* 2010), which eventually resulted in more retention of TSS and total sugars during storage. TA

Fig. 1: Influence of PGRs and shelf duration on ascorbic acid (mg 100 mL^{-1}) contents of 'Kinnow' mandarin. T1 (Control), T2 (10 mg L^{-1} BA@ FS stage), T3 (20 mg L^{-1} BA@ FL stage), T4 (20 mg L^{-1} BA@ FS stage), T5 (30 mg L^{-1} BA@ FS stage), T6 (10 mg L^{-1} kinetin @ FS stage), T7 (20 mg L^{-1} kinetin @ FL stage), T8 (20 mg L^{-1} kinetin @ FS stage), T9 (30 mg L^{-1} kinetin @ FS stage), T10 (10 mg L^{-1} GA_3 @ FS stage, D1 (Immediately after harvest), D7 (Seven days after harvest)



decreased during storage irrespective of the treatments. Reduction in TA during ambient storage was due to the consumption of acids in respiration process and conversion into sugar and salts (Rutter *et al*., 1975). Increase in TSS might be due to conversion of carbohydrate into simple sugars (Rub *et al*., 2010) and TSS:TA might be due to increase in TSS and decrease in TA (%) during shelf period.

The PGRs significantly increased reducing sugars (%) in comparison to control. Increase in reducing sugars might be due to the effect of cytokinin (Roitsch & Gonzalez, 2004) and gibberellic acid (Tymowska-Lalanne & Kreis, 1998) on the activity of invertase enzyme, which break down sucrose into fructose and glucose, hence resulting in increased reducing sugars. Higher non reducing sugars (%) in 10 mg L^{-1} kinetin at FS stage on D1 might be due to more production of total sugars (%) in this treatment and reduction of non reducing sugars (%) on D7 is due formation of more reducing sugars (%) in this treatment.

Reduction in AA contents in PGRs treated trees might be due to their positive influence on sink strength (reproductive growth) as evidenced by more TSS and juice mass (%) in fruit of PGRs treated trees in comparison with control. Conklin and Barth (2006) reported that AA deficient *Arabidopsis* mutant *vtc1* showed delayed reproductive growth as compared to wild type. The influence of PGRs on growth phase might be the reason of low juice AA contents in PGRs treated 'Kinnow' trees. Contrary to our results (Saleem *et al*., 2008) found higher ascorbic acid contents due to gibberellic acid application in 'Blood red' sweet orange as compared to control. This might be due to the difference in cultivar and age of the plant. However, some scientists reported inconsistent effect of PGRs on ascorbic acid contents of citrus fruit (Lima & Davies, 1984).

In conclusion, application of plant growth regulators exhibited significant effects on fruit quality of young 'Kinnow' mandarin trees. Cytokinin especially kinetin positively affected fruit quality parameters like juice mass (%), rag mass (%) and reducing sugars (%), except AA (mg 100 mL^{-1}) contents thus showing its potential for improving fruit quality of young 'Kinnow' mandarin trees. An improvement in fruit quality of young orchards (3-5 years) can help to extend the productive window of 'Kinnow' mandarin orchards.

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