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Study of foliage nutrient status and plant growth in Kinnow mandarin as influenced by growth regulators (2, 4-D & NAA) and micronutrients (Zn & B)

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Abstract
The present investigation was conducted at Experimental farm, ICAR-Central Institute for Arid Horticulture, Beechwal, Bikaner (Rajasthan), during the year 2016. The experiment was laid out in Randomized Block Design (RBD) having 15 treatments with three replications each. Foliar application of plant growth regulators and micro nutrients *viz.* NAA (20 & 40 ppm), 2,4-D (10 & 20 ppm), Zinc sulphate (0.5%), Borax (0.5%) alone and in combinations were applied twice in the month of April and May on 14 years old Kinnow trees. The initial vegetative growth of plant was recorded before imposing treatments in the month of April, 2016 and final at harvest in the month of December, 2016. Statistically, growth parameters increased maximum in terms of height and plant spread with treatment NAA 20 ppm + $ZnSO_4$ 0.5% as compared to control. Maximum zinc content (40.09ppm) was recorded in the leaves of Kinnow with foliar spray of NAA 20 ppm + $ZnSO_4$ 0.5% while maximum boron content (41.52 ppm) was recorded with foliar spray 2,4-D 10 ppm + Borax 0.5% as compared to control.

Keywords: Kinnow mandarin, growth regulators, micronutrients

Introduction
In India *Citrus* fruits have a prominent place among popular fruits and being extensively grown under tropical and subtropical conditions. Among the various *Citrus* species, mandarin, sweet orange and lime are the common fruits having 50, 21 and 15 per cent of total area under cultivation, respectively.

Mandarin (*Citrus reticulata* Blanco) is considered to be one of the most important cultivated species among *Citrus* and is being commercially grown in specific regions of the country, like "Nagpur mandarin" in Central India, "Khasi mandarin" in North Eastern regions and "Coorg mandarin" in Southern States of Karnataka (Coorg, Chikmangaloor, Hassan), Kerala (Wyanad), Tamil Nadu (Shevray Hills, Lower Palani Hills) and Kinnow mandarin in North-Western India. Mandarin juice is refreshing and nutritious due to its ascorbic acid content, sweet - acid taste and appealing colour.

Kinnow (*Citrus reticulata* Blanco), a mandarin hybrid belongs to the family Rutaceae and sub-family Aurantoideae. It is a potential *Citrus* fruit plant of irrigated semi-arid and arid region of the country. Kinnow is hybrid between King orange (*Citrus nobilis* Lour) and Williow- Leaf mandarin (*Citrus deliciosa* Tenore). *Citrus* is the third most important fruit crop after mango and banana in area and production in the world. In Rajasthan, the total area under Kinnow cultivation is about 14,890 ha^{-1} with production 2,51,200 metric tonnes (Anon., 2016). For Kinnow, Sri-Ganganagar district is on prime position with area and production followed by Hanumangarh and Bikaner district of North-West Rajasthan.

Among the various *Citrus* species (more than 162 species), Kinnow is an economically important sub-tropical fruit, which is grown almost all over the arid and semi-arid regions of India. In view of its prolific bearing quality coupled with relative tolerance to disease and pests, Kinnow has gained popularity amongst the growers and now it is commercially cultivated in Punjab, Haryana, Himachal Pradesh, Jammu & Kashmir and Uttar Pradesh and to some extent in the states of Karnataka and Tamil Nadu.

It is one of the most important fruits because of its pleasant flavor, juicy and sour-sweet taste. It is a subacidic fruit, freshly eaten and also used for extracting commercial pectin, refreshing drink and in making cosmetics (Bose and Mitra, 1996) [4]. Kinnow fruits are not only delicious and refreshing but also possess great nutritive value and used as a desert fruit as it contains 20-30 mg/100g vitamin C, 11.5 -13.5% T.S.S., 0.50-56% acidity, 87.8% moisture, 50.0 mg/100g calcium, 20.2 mg/100g phosphorus and 100mg/100g iron. Unfortunately, proper attention has not been paid in solving various problems faced by the Kinnow growers for successful and profitable production. It is commonly observed that the fruit growers are mostly using some cultural practices based on the experience of the other fellow orchardist & without technical knowledge. One of the important factor, which can ensure lucrative income from Kinnow growing regularly over number of years is proper feeding and judicious nutrients management.

'Kinnow' (*Citrus nobilis* Lour × *Citrus deliciosa* Tenora) mandarin is the most prominent *citrus* cultivar for large scale cultivation. Its area and production has been increasing overtime, however, its life span is decreasing due to many biotic and abiotic factors and its average life seldom exceeds 25 years. The average production is far below than other countries (Ibrahim *et al*. 2011). In some countries the productive life of *Citrus* tree is 50 years and in some cases it lives 100 years or more depending upon good management practices. In Pakistan, *Citrus* tree takes 8-9 years to bear fruit commercially, whereas, in Australia it takes only 6 years. Moreover, fruit from young trees also contains less TSS contents (Khalid *et al*., 2012), hence, rejected by the processers. Due to these reasons, fruit from young trees are often sold in local market at very low price. Growers of 'Kinnow' mandarin in this respect are highly disadvantaged because exporters are reluctant to buy fruit from young orchards and after 15-20 years their orchard starts declining and need replantation. So there is a need to increase the productive window of 'Kinnow' mandarin trees and it can be increased by improving quality of young orchards or extending life span of old orchards. A lot of research work has been done in extending life span of old orchards (Batool *et al*., 2007) [3], but little information is available about the improvement of fruit quality of young orchards.

Ample research has been done on the use of plant growth regulators (PGRs) to improve fruit size, delay in fruit maturity and overcome rind staining in *Citrus*. However, limited studies have been conducted to evaluate the complete profile of fruit quality in response to growth regulators application to *Citrus* during fruit development. The use of growth regulators has become an important component of agro-technical procedures for most of the cultivated plants and especially, for fruit plants (Monselise, 1979) [12].

PGR_s are being used in *Citrus* orchards to manipulate vegetative and reproductive growth, to modify fruit set and fruit growth and to improve fruit quality (Saleem *et al*., 2005) [18]. In case of *Citrus* fruit plant, micronutrients play an important role on trees growth and their importance has attracted the attention of Agricultural Scientists. Sometimes, the micronutrient deficiencies are likely to become more acute. In recent years, the importance of micronutrients to correct the deficiencies symptoms in plants has been greatly realized in the country. The plant nutrients are one of the key factors influencing the yield and quality of fruits. Rodriguez *et al*. (2005) [16] reported that the higher levels of zinc fertilizer increased the production of medium and big fruits of Valencia orange. Foliar application of zinc was positively correlated with the production of big and medium size fruits and negatively correlated with the production of small fruits. Besides the major nutrients (NPK) and secondary elements (Ca, Mg and S), essential micronutrients also play a vital role to increase the plant growth and fruit quality.

Materials and Method

The experiment was laid out at at ICAR- Central Institute for Arid Horticulture Bikaner (Rajasthan) and Department of Horticulture, College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner during 2016-17. Forty five plants of Kinnow mandarin having uniform size, vigour and of bearing stages at 14 years age were selected for the investigation. A single tree was kept as one replicate in a unit for each treatment. There were 15 treatments in the study having three replications. All experimental plants were maintained with common cultural practices during the course of study. The orchard was irrigated through drip irrigation.

The micronutrients *viz*, Zinc (Zn) applied as foliar spray through their sulphate salt. The concentration of Zn and boron used were 0.50% and 0.50%, respectively. In order to check the burning effect of nutrient salt, hydrate lime was added in all treatments solution. The amount of lime used was half the amount by weight of the nutrient salt in the solution. Teepol (0.1%) was used to get proper sticking of the solution on the foliage for spray. PGR solutions were prepared on the day of application of treatment. All the 45 trees were sprayed according to treatments with help of the sprayer. The first spray was done in first week of April, 2016, followed by second spray after a month interval.

Table 1: Details of treatments and their symbol

S.N	Treatments	Symbol
1	Control(Water spray)	T_0
2	NAA 20 ppm	T_1
3	NAA 40 ppm	T_2
4	2,4-D 10 ppm	T_3
5	2,4-D 20 ppm	T_4
6	$ZnSO_4$ 0.5%	T_5
7	Borax 0.5%	T_6
8	NAA 20ppm + $ZnSO_4$ 0.5%	T_7
9	NAA 40ppm + $ZnSO_4$ 0.5%	T_8
10	2,4-D 10ppm + $ZnSO_4$ 0.5%	T_9
11	2,4-D 20ppm + $ZnSO_4$ 0.5%	T_{10}
12	NAA 20ppm + Borax 0.5%	T_{11}
13	NAA 40ppm + Borax 0.5%	T_{12}
14	2,4-D 10ppm + Borax 0.5%	T_{13}
15	2,4-D 20ppm + Borax 0.5%	T_{14}

Results

Effect of plant growth regulators and micronutrients on growth parameters of kinnow tree

Growth parameters *i.e.* tree height and spread of Kinnow were measured before spray during April and six months after sprays treatment (November). The results obtained for the above parameters are described here under.

Height of tree

The maximum tree height (5.68 m.) Was recorded under the treatment T_7 (NAA 20 ppm+ $ZnSO_4$ 0.5%) followed by T_5 (5.67 m) and T_1. However, treatments applied T_2, T_3, T_4, T_5, T_{11}, T_{13}, and T_{14} were observed at par with each other. The maximum per cent increase in tree's height was recorded in treatment T_7 (13.60%) followed by T_3 2, 4-D, 10 ppm (12.97%) and minimum in T_8 (2.16%) followed by T_{10}

(3.79%), while per cent increase in height of tree was (9.88%) under control (water spray).

Plant Spread (N-S; E-W)

There was maximum spread *e.g.*, N-S (5.79 m) and E-W (5.80 m) under the treatment T_7 (NAA 20 ppm+ $ZnSO_4$ 0.5%) and it was the minimum under control *i.e.* 5.48m (N-S) and 5.52 m (E-W), respectively). The maximum per cent increase in plant spread (E-W) was obtained in T_7 (12.98%) followed by T_1 (10.57%). Foliar application of NAA 20ppm + 0.5% Zinc sulphate was found best treatment in terms of good plant growth in Kinnow.

Effect of Plant Growth Regulators And Micronutrients On Zinc And Boron Contents In Kinnow Leaves

Nutrients contents *i.e.* zinc and boron were analyzed in samples collected before spray and after I & II spray treatments in leaves samples. Observations were recorded after two foliar sprays *i.e.* first spray followed by second spray after a month period. Both samples were analysed for availability of micronutrients *viz*., Zn and B content.

Zinc content in leaves

Zinc content in leaf samples collected from Kinnow tree during May 2016 after sprays. During the month of May, 2016, leaf sampling was done after foliar sprays and were analysed for Zn content. The maximum zinc content (40.09ppm) was observed in treatment T_7 (NAA 20ppm + $ZnSO_4$ 0.5%) followed by T_5 (39.11ppm). However, it was the minimum in control (35.07ppm). Among the treatments, zinc content was significantly higher with T_7 as compared to NAA 40ppm + $ZnSO_4$ 0.5 per cent (T_8).

Boron content in leaves

Boron content in leaf samples collected from Kinnow trees during May 2016 after twice foliar sprays. The maximum boron content (41.52 ppm) was observed in treatment T_{13} (2,4-D 10 ppm + Borax 0.5%) followed by T_{14}, T_{12} and T_{10}. However, it was the minimum in control (32.14 ppm). Among the treatments, boron content was significantly higher with T_6 as compared to control. It was also noticed that the treatments having borax alone or in combination significantly increased boron content in leaves in comparison to other treatments.

Discussion

Growth parameters

The experimental results indicated that foliar applications of plant growth regulators and micronutrients (NAA, 2,4-D and Zn, B) were found useful in increasing growth parameters in terms of plant height and plant spread (N-S and E-W). The spray treatments significantly influenced the height of tree. The maximum per cent increase in tree's height was recorded in treatment T_7 (13.60%) followed by T_3 (12.97%) and minimum in T_8 (2.16%) followed by T_{10} (3.79%), while per cent increase in height of tree was (9.88%), under control. Increase in height and spread of plant may be due to better availability and uptake of nutrients and also growth promoting substance especially auxins.

Foliar sprays of growth regulators and micronutrients significantly affected the plant spread (E-W; N-S) in Kinnow. The combined application of NAA, 2,4-D, Zn and B was superior to individual application of plant growth regulators and micronutrients in respect to vegetative growth. This could perhaps be due to their synergistic or additive effect on various growth parameters. The role of plant growth regulators particularly auxins such as NAA, IAA, 2,4-D are well known for enhancing growth in different fruit plants. The maximum per cent increase in plant spread was measured in trees sprayed with NAA 20ppm and 2,4-D 10ppm. Micronutrients *viz.* zinc and boron improves the content in leaves as well as act as catalyst to mobilize to improve growth. Similarly, foliar application of zinc and boron activate the normal nitrate reduction phenomenon for the synthesis of protein, which is reported to protect chlorophyll destruction (Rabinowitch, 1945; Rossel and Ulrich, 1964) [15, 17]. Zinc and boron as micronutrients and PGR (NAA and 2,4-D) also promotes synthesis of indole acetic acid through tryptophan, which serves as a precursor for auxin synthesis and directly affect the growth parameters. The present results are similar to the findings of Bakshi *et al.* (1973) [2] in trifoliate orange, Singh and Ram (1982) [19]; Kumar *et al.*, (2015) in guava and Malik *et al.* (1999) [11]; Kaur *et al. (*2015) [9] in Kinnow.

Micronutrients contents of leaves

Zinc

Foliar application of micronutrients and plant growth regulators alone or in combinations enhanced Zn content in leaves. This is evident from the fact that the sample analyzed under various treatments after two sprays of treatments indicate that the Zn content of leaves increased to a great extent under different treatments. Zn content of leaves under T_7 (NAA 20 ppm + ZnSO4 0.5 per cent) was recorded to be maximum (40.10ppm) followed by T_5 (39.11%), while it was 35.07% in control. It is in conformity to the findings of Kanwar and Dhingra (1962) [7] in *Citrus,* Dube and Saxena (1971) [6] in sweet orange, Nanaya *et al.* (1985) [13] in coorg mandarin and Chandra *et al.* (1987) [5] in mango. Zinc is a important micronutrients to accelerate the physiological activities of the plants. It is easily absorbed by the plants to correct the nutrient deficiency, which might be the possible reason in increasing of Zn content in leaves.

Boron

Spray of micronutrients and plant growth regulators alone or in combinations improved B content in leaves. This is evident from the fact that the sample analyzed under various treatments after two sprays indicate that the B content of leaves increased to a great extent under different treatments. The maximum boron content (41.52 ppm) was observed in treatment T_{13} (2,4-D 10 ppm +Borax 0.5%) followed by T_{14} (40.42%). However, it was minimum in control (32.14 ppm). Among the treatments, boron content was significantly higher with T_6 as compared to higher dose T_{10} and T_{12}. It was also observed that only treatments having boron alone or in combination significantly increased boron content in Kinnow leaves in comparison to other treatments. It may be due to available boron content as well as availability through foliar feeding in plants. Similar findings have been reported by Khan *et al.* (2015) [10] in Kinnow mandarin.

Summary and conclusion

The study included the application of plant growth regulators (NAA 20, 40 ppm), (2,4-D 10, 20 ppm) and micronutrients (Zinc sulphate and Borax @0.5 per cent). Vegetative growth parameters in terms of height and plant spread (North-South and East-West) increased with foliar spray of NAA, 2,4-D, Zn and B in Kinnow mandarin. Statistically maximum tree height (5.68m), plant spread N-S (5.79 m) and E-W (5.80 m) were observed in plant receiving treatment T_7 (NAA 20 ppm+

$ZnSO_4$ 0.5%) during the period of study. Zinc and boron content in leaves increased with the foliar application of growth regulators and micronutrients (NAA & 2,4-D and Zn & B). Maximum zinc content (40.09ppm) was noted under T_7 (NAA 20 ppm + $ZnSO_4$ 0.5%) and boron content (41.52 ppm) in T_{13} (2-4-D 10 ppm +Borax 0.5%) followed by T_{14} (2,4-D 20 ppm +Borax 0.5%), respectively. However, the minimum micronutrient content was recorded in control.

Based on the result of the one year experimentation, it may be concluded that lower concentration of plant growth regulators and micronutrients *viz.* NAA 20 ppm + $ZnSO_4$ 0.5% significantly increased plant growth, nutrients status of Kinnow plant under arid conditions of western Rajasthan as compared to higher concentration and control.

Table 1: Effect of foliar spray of plant growth regulators and micronutrients (NAA, 2,4-D, Zn & B) on plant height of Kinnow tree

Symbols	Treatments	Before Spray(m)	After spray (m)	Per cent Increase
T_0	Control(water spray)	5.06	5.56	9.88
T_1	NAA 20 ppm	5.10	5.67	11.24
T_2	NAA 40 ppm	5.05	5.64	11.68
T_3	2,4-D 10 ppm	5.01	5.66	12.97
T_4	2,4-D 20 ppm	5.13	5.54	7.92
T_5	$ZnSO_4$ 0.5%	5.26	5.67	7.79
T_6	Borax 0.5%	5.00	5.40	7.99
T_7	NAA 20 ppm + $ZnSO_4$ 0.5%	5.01	5.68	13.60
T_8	NAA 40 ppm + $ZnSO_4$ 0.5%	5.41	5.53	2.16
T_9	2,4-D 10 ppm + $ZnSO_4$ 0.5%	5.03	5.51	9.47
T_{10}	2,4-D 20 ppm + $ZnSO_4$ 0.5%	5.18	5.38	3.79
T_{11}	NAA 20ppm + Borax 0.5%	5.26	5.66	7.54
T_{12}	NAA 40 ppm + Borax 0.5%	5.01	5.37	7.19
T_{13}	2,4-D 10 ppm + Borax 0.5%	5.08	5.67	11.68
T_{14}	2,4-D 20 ppm + Borax 0.5%	5.06	5.66	11.85
	S.Em. ±	0.18	0.07	-
	CD at 5%	NS	0.20	-

Table 2: Effect of foliar spray of plant growth regulators and micronutrients (NAA, 2,4-D, Zn & B) on plant spread of Kinnow tree

Symbols	Treatments	N –S E- W					
		Before spray (m)	After spray (I & II)	% increase	Before spray (m)	After spray (I & II)	% increase
T_0	Control (water spray)	5.18	5.48	5.78	5.42	5.52	1.71
T_1	NAA 20 ppm	5.31	5.77	8.78	5.23	5.78	10.57
T_2	NAA 40 ppm	5.30	5.63	6.35	5.27	5.65	7.21
T_3	2,4-D 10 ppm	5.11	5.76	12.70	5.23	5.73	9.49
T_4	2,4-D 20 ppm	5.28	5.70	8.01	5.46	5.70	4.45
T_5	$ZnSO_4$ 0.5%	5.23	5.78	10.50	5.37	5.71	6.32
T_6	Borax 0.5%	5.36	5.69	6.08	5.34	5.68	6.42
T_7	NAA 20 ppm + $ZnSO_4$ 0.5%	5.50	5.79	5.33	5.13	5.80	12.98
T_8	NAA 40 ppm + $ZnSO_4$ 0.5%	5.27	5.65	7.13	5.41	5.63	3.93
T_9	2,4-D 10 ppm + $ZnSO_4$ 0.5%	5.21	5.64	8.31	5.40	5.72	5.92
T_{10}	2,4-D 20 ppm + $ZnSO_4$ 0.5%	5.31	5.61	5.58	5.45	5.60	2.62
T_{11}	NAA 20 ppm + Borax 0.5%	5.28	5.76	9.21	5.49	5.62	2.36
T_{12}	NAA 40 ppm + Borax 0.5%	5.32	5.60	5.26	5.33	5.61	5.18
T_{13}	2,4-D 10 ppm + Borax 0.5%	5.37	5.68	5.76	5.29	5.55	4.90
T_{14}	2,4-D 20 ppm + Borax 0.5%	5.18	5.47	5.53	5.19	5.48	5.64
	S.Em ±	0.19	0.06	-	0.21	0.05	-
	CD at 5%	NS	0.18	-	NS	0.15	-

Table 3: Effect of foliar spray of PGR and micro nutrients (NAA, 2,4-D, Zn & B) on Zinc content of Kinnow leaves

Symbols	Treatments	Zn content in leaves (ppm)	
		Before sprays	After sprays
T_0	Control (water spray)	27.84	35.07
T_1	NAA 20 ppm	29.42	38.33
T_2	NAA 40 ppm	26.88	33.07
T_3	2,4-D 10 ppm	28.75	37.35
T_4	2,4-D 20 ppm	27.46	35.13
T_5	$ZnSo_4$ 0.5%	29.95	39.11
T_6	Borax 0.5%	28.34	35.12
T_7	NAA 20 ppm + $ZnSO_4$ 0.5%	30.18	40.09
T_8	NAA 40 ppm + $ZnSO_4$ 0.5%	27.75	37.03
T_9	2,4-D 10 ppm + $ZnSO_4$ 0.5%	29.59	37.97
T_{10}	2,4-D 20 ppm + $ZnSO_4$ 0.5%	29.70	36.61
T_{11}	NAA 20 ppm + Borax 0.5%	30.06	38.37
T_{12}	NAA 40 ppm + Borax 0.5%	29.40	36.41

T_{13}	2,4-D 10 ppm + Borax 0.5%	28.47	36.20
T_{14}	2,4-D 20 ppm + Borax 0.5%	27.54	35.64
	S.Em. ±	1.10	0.89
	CD at 5%	NS	2.58

Table 4: Effect of foliar spray of PGR and micro nutrients (NAA, 2,4-D, Zn & B) on boron content of Kinnow leaves

Symbols	Treatments	Boron content in leaves(ppm)	
		Before spray	After spray
T_0	Control (water spray)	28.20	32.14
T_1	NAA 20 ppm	28.33	33.20
T_2	NAA 40 ppm	28.19	33.76
T_3	2,4-D 10 ppm	29.28	32.45
T_4	2,4-D 20 ppm	28.10	33.56
T_5	$ZnSO_4$ 0.5%	29.12	33.43
T_6	Borax 0.5%	30.52	39.32
T_7	NAA 20 ppm + $ZnSO_4$ 0.5%	30.20	33.65
T_8	NAA 40 ppm + $ZnSO_4$ 0.5%	30.22	33.69
T_9	2,4-D 10 ppm + $ZnSO_4$ 0.5%	30.60	32.16
T_{10}	2,4-D 20 ppm + $ZnSO_4$ 0.5%	28.18	40.10
T_{11}	NAA 20ppm + Borax 0.5%	30.72	33.50
T_{12}	NAA 40 ppm + Borax 0.5%	28.10	40.29
T_{13}	2-4mm -D 10 ppm + Borax 0.5%	30.11	41.52
T_{14}	2,4-D 20 ppm + Borax 0.5%	29.03	40.42
	S.Em. ±	1.31	1.18
	CD at 5%	NS	3.43

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