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Synergistic Effects of Plant Growth Regulators and Micronutrients on Vegetative and Reproductive Traits of Kinnow Mandarin



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ABSTRACT

Aim: This study aimed to assess the effects of foliar application of micronutrients and PGR's on the growth, cropping, and fruit quality of Kinnow mandarin.

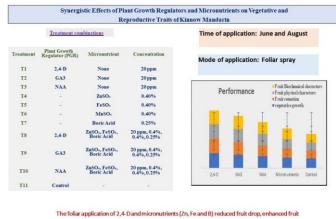
Methodology: The study was carried out on 4-year-old Kinnow plants, planted at a spacing of $2 \times 2m$ at the experimental farm of the Department of Fruit Science, COHF, Neri, during the year 2020-2021. In this experiment, individual PGR's (2,4-D, GA₃, NAA @ 20 ppm), micronutrients (NAA @ 20 ppm, ZnSO₄ @ 0.4 %, FeSO₄ @ 0.4 %, Boric acid @ 0.25 %) and their combinations comprising each PGR along with all the micronutrients were applied as treatments in two foliar sprays during June and August.

Results: Observational studies on vegetative and reproductive parameters under different foliar treatments of growth regulators indicated that the application of 2,4-D @ 20 ppm along with the essential micronutrients ($ZnSO_4$ @ 0.4 %, FeSO_4 @ 0.4 % and Boric acid @ 0.25 %) showed the best overall characteristics.

Interpretation: The combined application of plant growth regulators and micronutrients is essential to reduce fruit drop, enhance fruit quality and achieve optimal harvest of Kinnow mandarin under High-density plantation.

Keywords: Micronutrients, Fruit set, Fruit retention, foliar.

Graphical abstract



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1. Introduction

Citrus, a prominent member of the Rutaceae family, encompasses significant species, such as Sweet orange, Mandarin, Lime, Lemon, Sweet lime, and Grapefruit. Among these,Kinnow mandarin, a hybrid species (*Citrus nobilis*Lour. x *Citrus deliciosa* Tenore), now classified botanically as *Citrus deliciosa* Tenore, possesses significant commercial value. First developed by H. B. Frost in 1915 and introduced to India in 1959

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DOI: https://doi.org/10.21276/AATCCReview.2025.13.01.189 © 2025 by the authors. The license of AATCC Review. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). as virus-free bud wood from the University of California, theKinnow is optimally cultivated in regions like Punjab, Haryana, the lower Himalayan regions of Himachal Pradesh, Uttar Pradesh and some parts of Karnataka, Kerala and Tamil Nadu, flourishing at elevations ranging from 500 to 1500 meters.

Kinnow mandarin is esteemed for its elevated vitamin C concentration, distinctive aroma, reliable processing quality, nutrient richness, early fruiting and production of mediumsized, globose to oblate fruits. It is typically harvested between January and February, producing approximately 50-60 kilograms of fruit annually per tree [1]. Despite favorable agroclimatic conditions and rising demand, the cultivation of Kinnow is affected by erratic bearing, a decline in fruit production, significant fruit drop and reduced fruit size. These challenges are often ascribed to temperature fluctuations, inadequate irrigation, pest infestations and climatic shifts, which influence fruit set and quality.

Water stress, hormonal imbalance, inadequate nutrition levels and the influence of plant growth regulators further exacerbate issues inKinnow production. Furthermore, the concentrations of hormones, particularly auxin and abscisic acid (ABA), play a significant role in retaining fruits and flowers in Kinnow trees [23]. Addressing nutrient deficiencies, such as B, Cu, Mg, Mn, and Zn, through efficient foliar spraying methods rather than soil application, particularly in high-density cropping systems, is vital for optimal growth. Additionally, balancing essential nutrients and growth hormones, such as auxins, gibberellins and cytokinins, is crucial for enhancing citrus health and performance [10; 19]. In light of these challenges, the purpose of this study was to investigate the effect of plant growth regulators and micronutrients on reducing fruit drop, enhancing fruit quality, yield and achieving optimal harvest maturity inKinnow mandarin within the subtropical regions of Himachal Pradesh under HDP conditions.

2. Material and Method

Site of experiment and planting material

This study was conducted at the experimental farm of the Department of Fruit Science, College of Horticulture and Forestry, Neri, Hamirpur, from 2020 to 2021. Geographically, this region is located at $31^{0}41'47.6''$ N latitude and $76^{0}28'6.3''$ E longitude, with an elevation of 650 m above mean sea level, falling under the lower Himalayan and southern Shivalik ranges. Agro-climatically, this region experiences a hot summer with an average temperature of 36° c followed by a wet monsoon from late June to early September with an average annual rainfall of 1000-1200mm. The study was conducted on 4-year-old Kinnow plants, which were planted in High-Density Planting with a spacing of 2 x 2m.

Experimental Methodology

The study was conducted in a Randomized Block Design with 11 treatments at three replicate levels. The treatments consisted of three different Plant Growth Regulators and four essential micronutrients, which were applied individually as well as in combinations consisting each PGR along with the four different micronutrients as in a way that the treatments were T_1 (2, 4-D (20 ppm), T_2 (GA₃(20ppm), T_3 (NAA (20ppm), T_4 (ZnSO₄ (0.4%), T_5 (FeSO₄ (0.4%), T_6 (MnSO₄ (0.4%) + FeSO₄ (0.4%) + Boric acid (0.25%), T_9 (GA₃(20 ppm) + ZnSO₄ (0.4%) + FeSO₄ (0.4%) + Boric acid (0.25%), T_9 (GA₃(20 ppm) + ZnSO₄ (0.4%) + FeSO₄ (0.4%) + Boric acid (0.25%) T₁₀ (NAA (20 ppm) + ZnSO₄ (0.4%) + FeSO₄ (0.4%) + Boric acid (0.25%) and T_{11} (Control). These treatments were applied as foliar applications in two sprays, first during the last week of February and second during the third week of August. *Observations Recorded*

Vegetative growth parameters, included plant height, which was measured from ground level at the base of the tree to the topmost tip, tree spread in two directions, that is, E-W and N-S and shoot extension, which was obtained by subtracting the initial length of shoot from the final length of the shoot. The reproductive growth parameters were observed on selected shoots in all four directions, the fruit set percentage was measured using

Fruit drop, which was observed during the months of July, August, and October and the final fruit drop per centage during December, which was calculated using Fruit

drop (%)
$$\frac{\text{Final fruit retention - Number of fruit set}}{\text{Number of fruit set}} X 100;$$

the physiochemical characteristics of ten different fruits at random locations in different directions per replication were analyzed. Fruit length (cm) and breadth (cm) were measured using a Vernier caliper and fruit weight (g) was measured using an analytical weighing balance with 0.001 g precision. Fruit volume was measured using the water displacement method and fruit yield (kg/tree) was determined by weighing the total harvested fruits per tree using an electronic weighing balance. The number of seeds per fruit was counted manually and the rind thickness of the fruit was measured using a Vernier caliper. TSS ([°]Brix),Titratable Acidity (%), TSS: acid, ascorbic acid (mg/100 g), reducing sugar (%) and total sugars (%) were estimated using the procedures suggested by Ranganna [14]. *Statistical Analysis*

Observational data collected during the experiment were collected, tabulated and analyzed in MS Excel. The data were further subjected to analysis of variance for a Randomized Block Design at 5 percent level of significance using the procedure proposed by Gomez and Gomez [3].

3. Results and Discussion

The foliar sprays of PGR's, along with the micronutrients, significantly affected the tree's vegetative and reproductive growth and fruit physiochemical characteristics. The analyzed observational data on the influence of these PGR's and micronutrients on different parameters are discussed below:

Vegetative growth

The vegetative growth parameters such as plant height, shoot diameter, trunk girth, tree spread andleaf area were all significantly influenced by the individual application of PGR's (2,4-D,GA₃, NAA), micronutrients (Zn, Fe, Mn) over control (Table 1.). The foliar application of 2,4-D at 20 ppmshowed the maximum increase in all the vegetative parameters other than tree spread. In the combined application of these PGR's and micronutrients, the application of 2,4-D (20 ppm) + $ZnSO_4(0.4\%) + FeSO_4(0.4\%) + Boric acid (0.25\%)$ resulted in a maximum increase in plant height (66.75 cm), trunk girth (3.15 cm) and leaf area (36.42 cm²). From the correlational studies in Fig.1. it was evident that $T_8[2,4-D (20 \text{ ppm}) + \text{ZnSO}_4 (0.4\%) +$ $FeSO_4$ (0.4%) + Boric acid (0.25%)] and $T_1[2, 4-D (20 \text{ ppm})]$ had a strong positive correlation with vegetative parameters. This increase in vegetative growth in treated plants over the control is due to the effect of micronutrients and PGR's, which play crucial roles in various physiological and metabolic processes in plants, exerting catalytic or stimulatory effects. When the micronutrients were combined with PGR's, they exhibited a synergistic effect that collectively enhanced plant growth in T_{av} T_9 and T_{10} treatments. Moreover, 2,4-D which acts as an elongation hormone combined with the essential nutrients (Zn, Fe and B) in T_{μ} , resulted in an overall increase in the vegetative growth of the plant[12]. The increase in leaf size in the treated trees might be due to the active involvement of Zn in the synthesis of tryptophan, a precursor of indole acetic acid, which consequently increases tissue growth and development [6,24].

Flowering

It is evident from the data shown in Fig. 2 that micronutrients and plant growth regulators played a major role in improving fruit set and reducing fruit drop, which eventually increased the fruit retention of Kinnow plants. The combinational application of PGR's and micronutrients resulted in highest flowering buds (60.75) and fruit set percent (68.35), with treatment $T_{\rm s}$ [2,4-D (20 ppm) + ZnSO₄ (0.4%) + FeSO₄ (0.4%) + Boric acid (0.25%)],followed by T₉, whereas, the least number of flowering buds (40.50) and fruit set (51.99) % respectively was observed in Control ($T_{\rm 11}$). Foliar application of chemicals did not show any significant effect on fruit retention after the initial fruit set during the month of June but there was a significant change in the retention of fruits in the later months during August, October and December with the maximum fruit retention

observed in T₈ where 2,4-D at @20 ppm was applied along with the micronutrients, which was statistically at par with the application of GA_3 at 20 ppm and micronutrients (T_9) Fig. 2and 3. The response of these flowering characteristics to micronutrient application may be due to the greater translocation of hormones and food substances that stimulate fruit formation from the ovary. In addition, these micronutrients promote photosynthesis, regulate fruit drop and improve fruit size and quality[16]. Boron plays a positive response to transport of carbohydrates, auxins synthesis and increased pollen viability[4]. Foliar application of zinc, a growthpromoting element, together with 2,4-D, helped to retard the formation of the abscission zone by elevating the levels of auxin maturity [21; 22], which helped in maintaining the prominent characteristics of a greater number of fruit sets and the maximum retention of fruits until in T_{s} . [5].

Fruit Physical characteristics

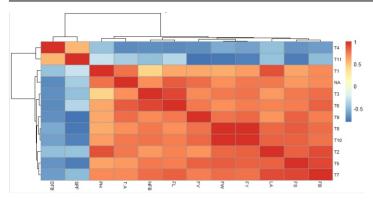
In citrus fruit size is directly proportional to the physical quality of the fruit; in contrast, small-sized fruits are of low quality. In this study, Kinnow plants treated with foliar application of PGR's and micronutrients showed a significant difference in fruit physical characteristics compared with the control (Table 2.). Application of 2,4-D and GA_3 along with micronutrients in T_8 and T_a helped in retaining the maximum number of fruits per plant (141.5, 141.5). 2,4-D @20ppmalong with ZnSO₄@ 0.4%, FeSO₄@0.4% and Boric acid@ 0.25% (T_8) showed the maximum increase in fruit size (7.13 cm), Fruit weight (192.4 g) and fruit volume (256.7 cm³) which was closely followed by $T_{0}[GA_{3}]$ (20 ppm) + ZnSO₄ (0.4%) + FeSO₄ (0.4%) + Boric acid (0.25%)] with a fruit size of 7.1 cm², fruit weight of 183.3 g and fruit volume of 253 cm³. When comparing the number of seeds and rind thickness of fruit in the control and with the rest of the treatments, it is evident that chemical sprays had a significant role in optimizing the seed count and the thickness of the rind with the minimum number of seeds per fruit (18.25 seeds/fruit) and rind thickness (2.9 mm) recorded in T_aandthe maximum number of seeds per fruit (26.00 seeds/fruit) and rind thickness (3.69 mm) recorded in control T_{11} . Among the different treatments T₈, recorded the maximum yield (27.23 kg/plant) and was at par with T_{q} in which the yield obtained was 25.94 kg/plant. This increase in yield in the plants treated with 2,4-D (20 ppm) along with other micronutrients is due to the better physical quality of the fruits in comparison with other treatments. Based on the correlation analysis (Figure 1.), treatment T8, which included a combination of 2,4-D and micronutrients (Zn, Fe, and B), appeared to show the best results and the most positive correlations between yield and quality parameters such as fruit size, fruit volume, fruit volume and fruit yield in the studied plants. The positive correlation between T_8 and yield suggested that this treatment was conducive to higher fruit production. Additionally, the strong positive correlation with leaf area implies that plants treated with T₈ exhibited increased leaf growth, which can contribute to higher photosynthetic activity. This increase in photosynthetic activity could be linked to improved quality parameters, such as fruit size, volume, and yield. The improved quality parameters could be a result of enhanced assimilate partitioning in T8 plants, where more photosynthetic products are directed toward fruit development and quality enhancement rather than

other plant processes. Overall, these findings suggest that treatment T8 had a direct positive impact on photosynthetic activity and assimilate partitioning, leading to improved yield and fruit quality in citrus plants. Moreover, treatments T_{q} $(GA_3Zn, Fe, and B)$ also showed significant results, similar to T_8 . The increase in fruit quality is due to the application of GA₃ and 2,4-D which promoted fruit growth as these growth regulators increase the plasticity of the cell wall, followed by the hydrolysis of starch into sugars[17]. Thus, it reduced the cell water potential, resulting in the entry of water into the cell and elongation. The improvement in fruit weight might be due to the availability of zinc and other nutrients to the plants, which enhances the formation and translocation of carbohydrates [13]. Fruits harvested from plants sprayed with plant growth regulators have thinner peels due to cell expansion [15]. All together the application GA₃ and 2,4-D with micronutrients helped in enhancing the fruit's physical characteristics [9; 7; 20] in Kinnow.

Fruit Biochemical Characteristics

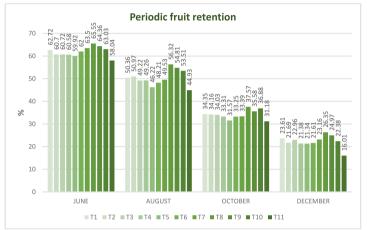
The foliar application of PGR's in combination with micronutrients during June after fruit set and in August at the developmental period, showed an increased trend toward the biochemical characteristics(Table 3.). The highest total soluble solids (12.17 "Brix) and reducing sugars (6.62%) were recorded in plants which were subjected to the application2,4-D along with micronutrients (Zn, Fe and B) Whereas, the maximum values of TSS:Acid(17.86), total sugar (10.65%) and a minimal value of acidity (0.67%) was found where, GA₃ was applied with micronutrients (Zn, Fe and B)to the plants, followed by the combined application of 2, 4-D,Zn, Fe and B. The maximum value of 39.29 mg/100 g of ascorbic acid was found in the treatment of NAA and micronutrients (Zn, Fe and B) closely followed by the integrated application of GA₃, Zn, Fe and B (38.1 mg/100 g)and 2, 4-D Zn, Fe and B (38.64 mg/100 g).The increased photosynthetic activity and chlorophyll synthesis in the leaves, which lead to increased carbohydrate accumulation in the fruit, are probably the cause of the observed rise in total soluble solids (TSS) inKinnow fruit after micronutrient application, especially Zn [2] TSS levels are considerably raised when micronutrients and 2,4-D are applied together [9; 18]. Since growth regulators are known to aid in the conversion of glucose-6-phosphate, a precursor of vitamin C, the rise in ascorbic acid levels seen in this treatment group may be due to the catalytic effects of growth regulators on ascorbic acid production. By promoting ascorbic acid accumulation and lowering enzymatic degradation, micronutrients contribute to organic acid metabolism, which raises the fruit's ascorbic acid concentration overall [8]. In addition, the active synthesis of tryptophan in the presence of zinc, which is a precursor to indole-3-acetic acid (IAA), that stimulates various physiological processes in plant tissues, may be the cause of the observed rise in sugar content. The combined effects of 2,4-D and micronutrients are responsible for improved results in terms of fruit quality attributes. In particular, 2,4-D appears to delay the abscission zone's formation by raising auxin levels, which facilitate effective nutrient transfer and reinforce the pedicel, extending the fruit's time on the plant. Fruit quality is enhanced at harvest as a result of this prolonged fruit retention time, which promotes complete physiochemical development [11].

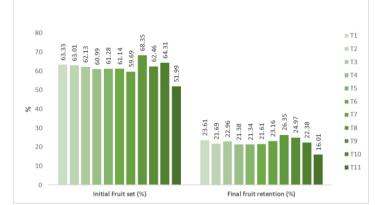
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DFB: days for full bloom, SPF: seed per fruit, PH: plant height,T:A: TSS: acid, NFB: number of flowering buds, FL: Fruit length, FV: fruit volume, FW: fruit volume, FY: Fuit yield, LA: leaf area, FS: Fruit set, FB: Fruit breadth

Fig.1. Dendrogram showing correlation studies of different parameters in kinnow mandarin.





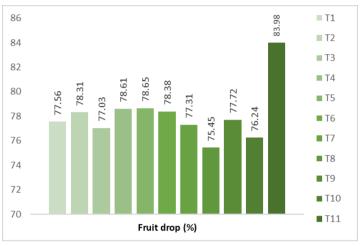


Fig. 3. Effect of micronutrientys and PGR's on percent fruit set, retention and drop in Kinnow mandarin.

Treatments		Increase in plant height	Increase in shoot diameter	Increase in trunk girth	Increase in (ci	Leaf area	
		(cm)	(cm)	(cm)	E-W	N-S	(cm ²)
T ₁	2,4-D (20 ppm)	60.75	1.11	2.89	45.5	38.25	35.07
T ₂	GA3(20 ppm)	51.75	1.05	2.73	43	36.25	32.91
T ₃	NAA (20 ppm)	42.5	0.94	2.61	35.75	47.25	30.46
T4	ZnSO ₄ (0.4%)	48.5 0.96		2.62	46.75	37.75	30.69
T 5	FeSO ₄ (0.4%)	49.25	1.01	2.81	37.25	34.25	33.66
T ₆	MnSO ₄ (0.4%)	45	0.8	2.42	39	31.5	31.09
T ₇	Boric acid (0.25 %)	58 0.81		2.47	37	40.5	32.93
T ₈	2,4-D (20 ppm) + ZnSO ₄ (0.4%) + FeSO ₄ (0.4%) + Boric acid (0.25%)		1.17	3.15	49	44.75	36.42

Fig. 2. Effect of micronutrientys and PGR's on periodic fruit retention in Kinnow mandarin. Table 1.Effect of micronutrientys and PGR's on vegetative growth in Kinnow mandarin.

T 9	GA3(20 ppm) + ZnSO4 (0.4%) + FeSO4 (0.4%)+ Boric acid (0.25%)	64.5	1.21	2.77	52.5	51	35.48
T1 0	NAA (20 ppm) + ZnSO4 (0.4%) + FeSO4(0.4 %) + Boric acid (0.25 %)	51.25	1.15	2.8	40.5	42.5	34.1
T ₁	Control	42	0.78	2.35	35.75	30	27.56
	C.D	6.63	NS	0.17	NS	NS	4.35
	S.E.(m)	2.28	0.16	0.06	8.22	11.38	1.5

 $Table \ 2. \ Effect \ of \ micronutrientys \ and \ PGR's \ on \ fruit \ physical \ quality \ characteristics \ in \ Kinnow \ mandarin.$

	Treatments	Fruit length (cm)	Fruit breadth (cm)	Fruit weight (g)	Fruit volume (cm³)	No. of fruits per plant	Fruit yield/plant (kg)	No. of seeds per fruit	Rind Thickness (mm)
T ₁	2,4-D (20 ppm)	5.92	7.76	176.5	223.2	137.2	24.22	22.25	3.29
T ₂	GA3(20 ppm)	5.85	7.67	167.2	235.7	133.2	22.24	20.75	3.54
T 3	NAA (20 ppm)	5.95	7.67	168.4	234.8	132	22.22	20.75	3.63
T 4	ZnSO ₄ (0.4%),	5.94	7.47	175.2	244.7	138.2	24.22	19.25	3.32
T 5	FeSO ₄ (0.4%),	5.84	7.66	187.8	224.5	136	25.54	20.25	3.25
T ₆	MnSO ₄ (0.4%)	5.82	7.39	179.9	235.7	138	24.83	19.5	3.09
T ₇	Boric acid (0.25 %)	5.73	7.48	173.2	237	136.2	23.6	20.5	3.41
T ₈	2,4-D (20 ppm) + ZnSO4 (0.4%) + FeSO4 (0.4%) + Boric acid (0.25 %)	6.31	7.95	192.4	256.7	141.5	27.23	18.25	2.9
T9	GA3(20 ppm) + ZnSO4 (0.4%) + FeSO4 (0.4%)+ Boric acid (0.25 %)	6.36	7.85	183.3	253	141.5	25.94	21.75	3.37
T ₁₀	NAA (20 ppm) + ZnSO4 (0.4%) + FeSO4(0.4%) + Boric acid (0.25 %)	6.36	7.83	185.5	241.2	139	25.8	21.25	3.22
T ₁₁	Control	5.6	7.07	142.5	193	127.5	18.19	26	3.69
	C.D	NS	0.4	9.71	7.18	NS	3.08	NS	0.38
	S.E.(m)	0.16	0.14	3.34	2.47	5.37	1.06	1.43	0.13

 $Table \ 3. \ Effect \ of micronutrientys \ and \ PGR's \ on \ fruit \ Biochemical \ characteristics \ in \ Kinnow \ mandarin.$

TREATMENTS		TSS (⁰ B)	Acidity (%)	TSS: acid	Ascorbic acid (mg/100)	Reducing sugar (%)	Total sugar (%)
T ₁	2,4-D (20 ppm)	11.45	0.88	13.22	32.77	5.58	7.1
T ₂	GA3(20 ppm)	11.52	0.95	12.16	31.26	6.32	9.05
T ₃	NAA (20 ppm)	11.2	0.91	12.35	26.8	5.52	7.9
T 4	ZnSO4 (0.4%),	11.17	0.86	13.47	29.55	5.61	8.7
T 5	FeSO4 (0.4%),	11.25	0.92	12.22	30.16	5.31	8.15
T ₆	MnSO4 (0.4%)	11.12	0.88	12.69	26.08	5.38	8.41
T ₇	Boric acid (0.25 %)	11.8	0.76	15.79	34.77	5.47	8.98
T8	2,4-D (20 ppm) + ZnSO4 (0.4%) + FeSO4 (0.4%) + Boric acid (0.25 %)	12.17	0.71	17.5	38.64	6.62	9.97
T 9	GA3(20 ppm) + ZnSO4 (0.4%) + FeSO4 (0.4%)+ Boric acid (0.25 %)	11.8	0.67	17.86	38.1	6.57	10.65
T ₁₀	NAA (20 ppm) + ZnSO4 (0.4%) + FeSO4(0.4%) + Boric acid (0.25 %)	12.02	0.72	17.04	39.29	6.53	9.9
T ₁₁	Control	9.92	0.97	10.22	25.7	5.16	6.79
	C.D	0.83	0.18	3.47	3.93	1.05	1.93
	S.E.(m)	0.28	0.06	1.19	1.35	0.36	0.66

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