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Influence of different concentrations of Auxins and a broad spectrum fungicide on pre harvest fruit drop, physical and biochemical parameters of Kinnow Mandarin (*Citrus reticulata* Blanco)

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Abstract
Kinnow (*Citrus reticulata* Blanco) is high yield mandarin hybrid of two citrus cultivars 'king' (*Citrus nobilis*) and 'willow leaf' (*Citrus deliciosa*). Commercially, kinnow is one of the most successful fruit as it contributes good percentage to citrus fruit income. According to Nationnal Horticulture Database (2016-17) Punjab is the leading kinnow producing state of India. In Punjab progressive farmers prefer to grow kinnow because of its high yielding characteristics and its attractive quality that possesses the potential to give lucrative return in form of profit. Although kinnow cultivation is a profitable endeavor, it is liable to certain limitations resulting in less profit or even loss under worst circumstances. Pre-harvest fruit drop is major problem experienced by almost all farmers of kinnow plantation area. According to an estimate nearly 30-76% yield loss occurs due to pre-harvest fruit drop. There are large numbers of physiological and pathological reason responsible for pre-harvest fruit drop. The present study reports formulation of growth regulator and fungicide for reducing pre-harvest fruit drop in kinnow. Synthetic auxin Naphthaleneacetic acid (NAA) and 2,4-Dichlorophenoxyacetic acid were used as a growth regulator. Carbendazim, broad spectrum benzimidazole fungicide was used to control fungi. Foliar application of formulation consisting of 20 ppm NAA and 0.2% carbendazim reduced the pre-harvest fruit drop by 18.92% as compared to untreated plants. Aforementioned formulation also increased the juice yield significantly. Thus, suggesting that combination naphthalene-1-acetic acid and carbendazim can be applied to reduce fruit drop in kinnow.

Keywords: Kinnow, Auxin, Carbendazim, NAA and 2,4-D.

1. Introduction

Kinnow is a high yield mandarin hybrid (*Citrus nobilis* × *Citrus deliciosa*), scientifically known as *Citrus reticulata* Blanco. Botanically Kinnow belongs to family Rutaceae. Kinnow is renowned citrus fruit favoured for its pleasant flavor,appearance, color, taste, good yield, high processing value, therapeutic applications, delicious juice, smoothing character, vitamin C source, wider adaptability to various agro-climatic condition and high nutritive value (Ahmed *et al*., 2007) [1]. Citrus fruits have a major role in the economy of world. Brazil is the leading citrus fruit producer followed by USA, China and Mexico. Average yield of Brazil is 21.64 tones ha^{-1}, USA 25.98 tones ha^{-1} and of Turkey 26.73 tones ha^{-1}. In India Kinnow contributes good percentage to citrus fruit income. The major Kinnow producing states are Punjab, Maharashtra, West Bengal, Maharashtra, Haryana, Madhya Pradesh, Himachal Pradesh,and Karnataka (Horticulture at a glance 2017). National Horticulture Board database 2016-17 shows that in India 4,29,290 hactare area was under Kinnow plantation with total yield of 47,53,830 MT. According to Horticulture Statistics (2016-17) Madhya Pradesh is a highest producer of Kinnow followed by Punjab. In Madhya Pradesh Kinnow plantation covers 1,15,835 hactare area and produces 14,37,976 MT of fruit. Punjab produces 11,82,109 MT of Kinnow annually and nearly 51,063 hactare of land are under Kinnow plantation.Hoshiarpur, Muktsar and Fazilka are main citrus growing belt of Punjab(Kaur and Singla, 2016; Davinder *et al*., 2017) [45, 13].

Although kinnow cultivation is a profitable endeavor, it is liable to certain limitations resulting in decrease in profit or even loss under worst circumstances. Some of the common problems faced by farmers are excessive bearing leading to tree decline, viral diseases like Citrus tristeza, bacterial diseases like Citrus canker, fungal diseases likeand fruit rot and pre-harvest fruit drop (Mazhar *et al*., 2015; Timmer *et al*., 2004) [26, 39]. According to an estimate nearly 30-76% yield loss occurs due to pre-harvest fruit drop (Chundavat *et al*., 1975; Daulta *et al*., 1986) [10, 11]. There are large numbers of physiological and pathological reason responsible for pre-harvest fruit drop (Rizzuti *et al*., 2015; Zheng *et al*., 2017) [3, 44]. Some commonly experienced regions are abscission formation, inadequate pollination, nitrogen stress, high temperature, water stress, inappropriate nutrient availability, ageing, pest attack and hormonal imbalance (Browing, 1986; Marinho *et al*., 2005) [9, 27]. However, leading kinnow producing countries like Brazil and USA are able to maintain high average yield by controlling the pre-harvest fruit drop (Kumar *et al*., 2017) [29]. Researchers have reported several methods for controlling physiological and pathological reasons responsible for pre-harvest fruit drop. Growth regulators are well documented to enhance fruit quality by altering physiological process (Adouli *et al*., 2018; Li *et al*., 2016) [7, 20]. Thus, in most of the reported preventive treatment growth regulators are central to formulation. Growth regulators modulate plant physiology and nutrients mobilization during fruit development (Li *et al*., 2016) [20].

2. Review of literature

Plant growth regulators (PGRs) consist of organic molecules, produced synthetically and used to alter the growth of plant. They have ability to accelerate or retard the growth of plant. The hormone which is produce in plant is called as phytohormone (Thimann, 1948) [38] The PGRs can be biostimulant or bioinhibitors and are active even at very low concentrations in plant cells and have ability to alter the growth and development. The plant growth regulators represent various categories as American Society for Horticultural Science also divides the plant growth regulators into six classes including gibberellins, auxins, cytokinins, ethylene genetors, growth inhibitors and growth retardants. For altering physiological factors in citrus plant 2,4-D, GA_3 and NAA are widely used pre harvest application. Auxin (2,4-D and NAA) has better role in reducing pre harvest fruit drop than gibberllins. Application of 2,4-D and NAA dramatically increases total no. of fruit buds per plant, total soluble solids, acidity, vitamin c, reducing sugar (Nawaz*et al*., 2008) [28]. Application of 2,4-D increases auxin levels, which control the formation of the abscission layer between the fruit pedicel and stem and it leads to decrease in fruit drop (Kavinprashanth *et al*. 2021) [24]. Higher amounts of auxins in the plant and fruit were reported to enhance the mobilization of food and nutrient (Kumar 2021) [23]. Fruit drop in citrus occurs due to imbalance in nutrient and pest attack (Shivankar *et al*., 2000) [37]. Therefore treatment with 2,4-D and salicyclic acid, K, Zn amend the yield and quality of kinnow in punjab. K and Zn stimulates the fruit weight, juice content and control fruit drop (Ashraf *et al*., 2012; Nasir *et al*., 2016) [5, 46]. Exogenous application of GA_3, 2,4-D in different concentration on blood red and after three month of fruit set there is no effect seen on seed health and fruit diameter. The study of Bharti (2020) [8] revealed that application of NAA significantly improved fruit length as compared to control. Fruit weight was decreased by most of the growth regulator treatments compared with control. Juice quantity, TSS, total sugars and reducing sugars were improved by most of the treatments compared with control but in case of acidity and vit. C, there was not any clear cut trend of treatment effect (Saleem *et al*., 2007) [36]. Jian *et al*., and Ullah *et al*., (2014) [40] indicated that 2,4-D significantlly improves soluble solids, TSS, ascorbic acids and fruit yield of plant. Ullah *et al*., (2014) [40] found that GA_3 reduce percentge of fruit drop. Various growth regulators have been found to control fruit drop but 2,4-D has been found to controlling better resulting in fruit under various agroclimatic regions when used as foliar spray and improves quality of kinnow mandarin (Verma *et al*.,(2018) [42] suggested that at pea and gravel stages of kinnow application of 20 ppm NAA increases 45% fruit retention and similarly 2% spray of urea increases weight of 100 fruit upto 16.70 kg. Coggins and lovatt *et al*., (2004) [31] studied that 2,4-D and salicyclic acid effective against fruit drop in citrus and other included 2,4-5 trichlorophenoxypropionic acid NAA & GA_3 helps in synthesis of protein, opening of stomata & activation of enzyme. Ibrahim *et al*., (2007) [17] indicated the deficiencies of micronutrients like Zn, Cu, Fe and Mn in citrus orchards of Pakistan and among them Zn is more acute. Literature indicated that the treatment of Zn is beneficial againstthe fruit yield and quality (Rodríguez *et al*., 2005) [33]. So the perfect combination of macro-, micronutrients and growth regulators mask the effect offruit drop and improve the citrus fruit yield and its quality (Doberman and Fairhurst, 2000;Saleem *et al*., 2005) [12, 34]. Similarly in a study by Kaur *et al*., (2000) growth regulators treatments 2,4-D, GA_3, NAA at 15 and 20 ppm concentrations respectively reduced fruit drop in Kinnow mandarin. The highest fruit drop control was exhibited by 2,4-D resulting in high yield and quality. It was reported by Gomez-Cadenas *et al*., (2000) [16] that exogenous application of gibberellins had no effect on abscission in citrus. Application of different growth regulators (GA_3, 2,4-D and NAA alone and in combination) on 'Pera' orange had no influence on the development of the fruit such as length; diameter and fresh fruit mass (Almeida *et al*., 2004) [2].

3. Materials and Methods

The present research work was conducted in the Kinnow orchard of Mr. Satpal Singh located at village Khiala, Jalandhar (31º25'37.8"N 75º48'34.8"E) and the experiment was conducted in the horticulture laboratory of Sant Baba Bhag Singh University, Khiala, Punjab in the year 2019. The orchard has 3 year old Kinnow plants that were planted with spacing of 6m×3m. The plants were budded on Jatti Khatti rootstock (Centre of Excellence for Fruit Research in Citrus, Khanaura, Punjab). Orchard has faced problem of fruit dropping in previous fruiting season. Orchard was maintained as per standard recommendation (Rattanpal *et al*., 2017)[32]. For controlling pre-harvest fruit drop in kinnow, the plants were treated with various concentrations and combinations of growth regulator and fungicide. Growth regulators used were synthetic auxin NAA and 2,4-D (Naphthaleneacetic acid and 2,4-dichlorophenoxyacetic acid), broad spectrum benzimidazole fungicide carbendazim was used to control fungus. During experimentation total of three treatments were given at span of 30 days and subsequent observation was made after 15 days of treatments. The experiments were organized as Randomized complete Block Design (RCBD). The details of treatment were given in table. The details of treatment were given in table 1.

Table 1: Treatment Details

Treatments	Dozes	Treatment Code
NAA	10 ppm	T_1
NAA	20 ppm	T_2
NAA	30 ppm	T_3
2,4-D	10 ppm	T_4
2,4-D	20 ppm	T_5
2,4-D	30 ppm	T_6
Carbendazim	0.2%	T_7
NAA+Carbendazim	20 ppm+0.2%	T_8
2,4-D+Carbendazim	20 ppm+0.2%	T_9
Control		T_{10}

Randomly 5 branches were selected from a tree (both treated and untreated). Total number of fruits present on each branch was recorded at intervals of 15 days after each treatment. Difference between the respective readings at interval of fifteens days was applied to calculate the fruit drop. Percentage of kinnow fruit drop was calculated as:

$$\text{Fruit Drop (\%)} = \frac{\text{Total no. of fruits dropped from a tree}}{\text{Initial no. of fruits prsent on tree}} \times 100$$

Fruit yield on the basis of fruit weight was recorded at the time of harvest and reported as Kg/tree. Also total number of fruits present on tree was counted on each treated and untreated trees. Later was reported as number of fruits per tree.

Before juice extraction seeds were removed by help of needle and forceps. Seeds collected were counted and weight. Juice yield percentage kinnow fruit was given as:

$$\text{Juice Yield(\%)} = \frac{\text{Juice weight in Gram}}{\text{Total Fruit Weight in Gram}} \times 100$$

Total soluble solid (TSS) measures total sugar content in the kinnow fruit juice. Juice extracted from kinnow fruit were filtered through Whatman qualitative filter paper No. 1. Filtrates were applied over the window of hand Refactrometer (ERMA Inc. Tokyo Japan) and readings were recorded. TSS of fruit juice was reported as ˚Brix at room temperature.

The pH of fruit juice was measured by digital pH meter (Comsys Technologies, India). Before experiment the pH meter was calibrated with standard pH buffers (pH 4.0, pH 7.0 and pH 9.0).

The fruit juice was diluted 5 times with distilled water. An aliquot (10 ml) was taken from the diluted fruit juice and titrated against standard 0.1 N NaOH solutions. Phenolphthalein was used as indicator. The titrable acidity was expressed as percentage of citric acid equivalent following below mentioned equation:-

$$\text{Total Titrable Acidity (\%)} = \frac{\text{Titre X DilutionX Eq.Weight of Cirtic acid}}{\text{Volume of Sample X Weight of sample X 1000}} \text{ X } 100$$

Amount of ascorbic acid present in fruit juice was determined by redox titration using Iodine. An aliquot of 20 ml kinnow fruit juice was taken in 250 ml Erlenmayer flask containing 150 ml of distilled water. Starch solution (0.5% w/v) was used as an indicator. The endpoint of titration was determined as the first permanent appearance of a dark blue-black color due to starch-iodine complex formation. The amount of ascorbic acid present in juice was calculated by comparing the titration with standard ascorbic acid solution.

Folin-Ciocalteu Phenol method was used for determining the phenol content in the kinnow fruit juice. An aliquot of (1ml) of fruit juice was added to 25 ml volumetric flask containing 9 ml of distilled water. To the diluted fruited juice Folin- Ciocalteu phenol reagent was added and mixed properly. After 5 minutes 10 ml of 7% Na_2CO_3 solution was added to the mixture. Then distilled water was added to make up the volume. After 90 minutes of incubation at room temperature, the absorbance was taken at 550 nm using Microprocessor UV-VIS single beam spectrophotometer (Advance Lab Equipments PVT LTD, India). Water with reagents was taken as blank. Total phenolics content was expressed as Gallic acid Equivalents (GAE) in mg.

Total sugar content of kinnow fruit juice was determined by phenol-sulfuric acid method. An aliquot of 1ml was taken from the diluted kinnow fruit juice in test tube. To fruit juice 1ml phenol solution (5% w/v) and 5ml sulphuric acid added. After cooling the test tube at room temperature, absorbance was taken at 490nm using Microprocessor UV-VIS single beam spectrophotometer (Advance Lab Equipments PVT LTD, India). The concentration of total sugar was calculated from dextrose standard curve.

Lane and Eynon method was used for estimating the reducing sugar in kinnow fruit juice with slight modification. Five times diluted and filtered fruit juice (50 ml) was titrated against hot Fehling's solution (10 ml) till the blue colour faded. Then few drop of methyl blue indicator (1%) was added to solution. Titration was further carried to obtain a brick red color. The titrate value of fruit juice was compared with the known concentration of invert solution. The results were reported as percentage of reducing sugar. Non reducing sugar was calculated by subtracting reducing sugar from total sugar.

Seeds collected form fruits were rinsed twice with water. Washed seeds were scarified and soaked in 2,3,5 triphenyl tetrazolium chloride solution (0.1% w/v) solution for one day. After one day of incubation at 30˚C, seeds were washed and treated with clearing solution (Lactic acid: phenol: glycerine: water in ratio of 1:1:2:1) for 1 hour. Seeds were washed and observed under magnifying glass for pink or red to grayish red stain. Seeds with pink or red to grayish red stains were scored as viable and non-stained seeds were scored as non-viable seeds. Heat killed seeds were taken as negative control.

The data was analyzed using the Microsoft Office Excel 2007 program. The values given are mean ± standard deviation.

4. Results and Discussion

4.1 Soil Analysis

Soil samples from each site were collected up to 60 cm depth and analyzed for pH (pHs) and electrical conductivity (EC). Other chemical analysis like that for organic matter (Nelson & Sommers, 1982) [30] total N (Kjeldhal method), Na, K, Ca2+, Mg2+, CO_3, HCO_3 and P was carried out as described by theand Jackson (1962) [18].

Table 2: Soil analysis

Characteristics	**Quantity**
Type of soil	Sandy loam
Ph	8.2
Electric Conductivity (hos/cm)	0.12
Organic carbon %	0.160
Phosphorous (ppm)	13.6
Potassium (ppm)	48
Zinc (ppm)	1.80
Ferrous (ppm)	4.76
Magnese (ppm)	2.84
Copper (ppm)	0.42

Table 3: Total fruit drop percentage

	30 day	**60 day**	**90 day**	**Total**
NAA 10 ppm	5.71	9.09	6.66	21.46
NAA 20 ppm	4.75	8.32	3.62	16.69
NAA 30 ppm	8.69	4.76	5.01	18.46
2,4-D 10 ppm	5.01	5.26	8.33	18.60
2,4-D 20 ppm	5.16	9.09	5.99	20.24
2,4-D 30 ppm	5.26	5.55	1.96	12.77
carb 0.2%	3.84	1.01	6	10.85
NAA 20 ppm+carb 0.2%	5.32	-	3.77	9.09
2,4-D 20 ppm+carb 0.2%	5.84	6.25	3.33	15.42
Control	7.93	9.18	10.9	28.01

4.2 Fruit drop percentage

In present study maximum decrease in fruit drop 9.09% was observed in treatment having combination of 20 ppm NAA and carbendazim 0.2%. In untreated trees fruit drop observed was 28.01% (Table 2). Individually 16.69% fruit drop was found in 20ppm NAA and 10.85% fruit drop was found in carbendazim 0.2 Our results were agreed with (Anthony and Coggins, 2001) [6] who observed that the NAA and 3,5,6-TPA were reducing abscission of mature citrus fruit. Similarly Kumar *et al.*, 2018 [22] reportedapplication NAA with zinc reduces pre harvest fruit drop in mandarin.

4.3 Fruit Yield

On 0 day maximum yield 883.86 gm/10 fruits was found in case of carbendzim 0.2% and minimum 684.72gm/10 fruits was recorded in control (Figure 1). The yield was rising sharply from 883.86gm to 2052.50gm. As highest yield 2052.50 gm/10 fruits was observed in tree treated with 10 ppm NAA and minimum 1465.47gm/10 fruits found in control. Earlier, Verma *et al*., (2018) [42] reported maximum yield in Nagpur mandarin after treatment with NAA (10 ppm) and 2,4-D (10 ppm).

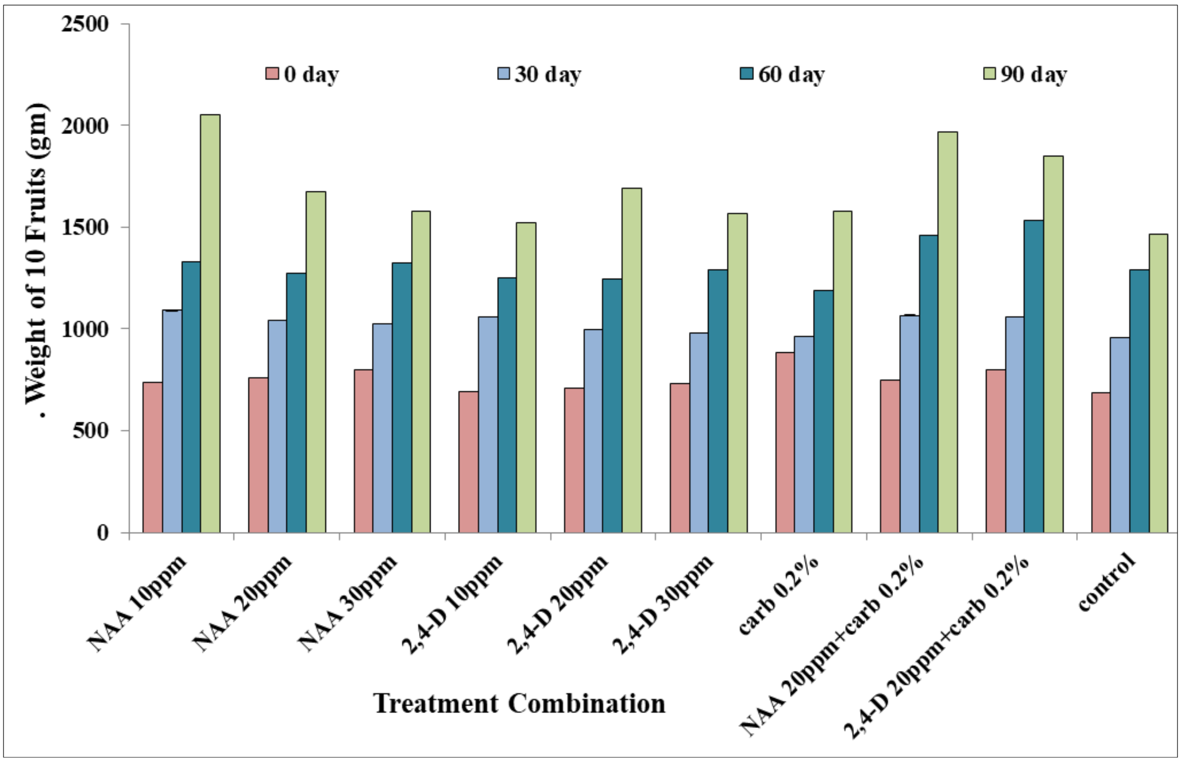

Fig 1: Average weight (gm) of 10 fruits obtained from experimental plants at interval of 30 days. Values given are mean ± SD of three plants.

4.4 Fruit Weight

Fruit weight is an important factor for capturing attention of peoples in market. On 0 day observation maximum fruit weight 88.40gm had gained by treatment of carbendazim 0.2% and minimum fruit weight 66.37gm had gained by treatment of 10 ppm 2,4-D (Table 3). On 90 day observation 10 ppm NAA was attained maximum fruit weight 205.29gm and least weight of fruit 146.53gm was found where no treatment had given. The result was not to be in agreement with that of Saraswathi *et al*., (2003) [35] who investigated that 2,4-D and GA_3 significantly influenced the fruit weight. Ghosh *et al.* (2012)[15] reported increase in fruit size in Sweet orange with the spraying of auxins at 15 ppm.

Table 4: Fruit weight in grams

	0 day	30 day	60 day	90 day
NAA 10 ppm	73.55±0.05	109.13±0.60	133.10±0.08	205.29±0.23
NAA 20 ppm	76.29±0.19	104.21±0.18	127.41±0.21	167.55±0.21
NAA 30 ppm	80.23±0.08	102.56±0.13	132.43±0.16	157.81±0.06
2,4-D 10 ppm	66.37±0.22	106.15±0.16	125.26±0.16	152.30±0.13
2,4-D 20 ppm	70.53±0.20	99.85±0.13	124.63±0.10	169.32±0.25
2,4-D 30 ppm	73.27±0.13	97.93±0.04	128.80±0.01	156.53±0.04
carb 0.2%	88.4±0.23	84.48±0.36	119.36±0.26	157.79±0.10
NAA 20 ppm+carb 0.2%	75.24±0.35	106.71±0.22	145.72±0.29	196.82±0.05
2,4-D 20 ppm+carb 0.2%	79.72±0.21	105.92±0.12	153.56±0.11	184.77±0.18
Control	68.42±0.27	95.81±0.14	128.93±0.06	146.53±0.35

Values given are mean ± SD of three plants.

4.4 Fruit Length

There had not been any significant changes found in fruit length (Table 4). Maximum treatment was statistically similar to each other from 0 to 90 day. 30 ppm NAA gave maximum fruit length 4.99cm and 10 ppm NAA gave minimum fruit length 4.40cm on 0 day. 20 ppm NAA and carbendazim 0.2% gained maximum fruit length 6.50cm and minimum fruit length 5.69cm gained by 30 ppm NAA on 90 day. NAA at 20 ppm produced maximum fruit length and breadth **(**Uniyal *et al.*, 2012) [41].

Table 5: Fruit length in cm

	0 day	30 day	60 day	90 day
NAA 10 ppm	4.40±0.22	5.41±0.23	6.07±0.13	6.26±0.05
NAA 20 ppm	4.82±0.16	5.54±0.35	6.27±0.16	6.28±0.35
NAA 30 ppm	4.99±0.01	5.49±0.05	6.08±0.14	5.69±0.17
2,4-D 10 ppm	4.55±0.11	5.51±0.08	5.52±0.09	5.83±0.15
2,4-D 20 ppm	4.89±0.04	5.37±0.09	5.68±0.16	6.18±0.42
2,4-D 30 ppm	4.95±0.03	5.33±0.20	6.05±0.04	6.06±0.04
carb 0.2%	4.98±0.05	5.05±0.05	5.57±0.22	5.97±0.06
NAA 20 ppm+carb 0.2%	4.87±0.01	5.23±0.30	6.55±0.21	6.50±0.05
2,4-D 20 ppm+carb 0.2%	4.84±0.06	5.37±0.15	6.48±0.17	6.37±0.01
Control	4.76±0.02	4.99±0.58	6.06±0.05	5.85±0.03

Values given are mean ± SD of three plants.

4.5 Fruit Diameter

None of the treatments had any significant effect on fruit diameter (Table 5). Diameter of the fruit had increased with the passage of time. Maximum fruit diameter 7.75cm gained by 10 ppm NAA on 90 day. Minimum fruit diameter 5.10cm was reported in treatment of 10ppm 2,4-D on 0 day. Erner *et al.*, (1993) [14] reported 2,4-D influenced the fruit diameter in Velanica Orange. NAA at 20 ppm produced maximum fruit length and breadth (Uniyal *et al.*, 2012) [41].

Table 6: Fruit diameter in cm

	0 day	30 day	60 day	90 day
NAA 10 ppm	5.53±0.04	6.25±0.09	7.11±0.04	7.75±0.11
NAA 20 ppm	5.58±0.09	5.47±0.02	6.91±0.08	7.39±0.16
NAA 30 ppm	5.51±0.13	5.95±0.04	7.20±0.07	7.11±0.06
2,4-D 10 ppm	5.10±0.08	6.29±0.25	6.57±0.18	6.92±0.04
2,4-D 20 ppm	5.28±0.12	6.13±0.07	6.93±0.03	7.48±0.16
2,4-D 30 ppm	5.49±0.16	6.19±0.09	6.85±0.07	6.92±0.08
carb 0.2%	5.75±0.14	5.67±0.13	6.95±0.03	7.17±0.04
NAA 20 ppm+carb 0.2%	5.37±0.10	6.09±0.11	7.60±0.15	7.56±0.08
2,4-D 20 ppm+carb 0.2%	5.41±0.32	6.16±0.04	7.52±0.05	7.39±0.15
Control	5.26±0.03	5.94±0.02	6.80±0.22	6.72±0.19

Values given are mean ± SD of three plants.

4.6 Juice Percentage

On 0 day observation 10 ppm NAA gave minimum juice percentage i.e 44.75% and maximum juice percentage 63.16 in case of carbendazim 0.2%. On 90 day observation minimum juice percentage 64.83 was reported in case of carbendazim 0.2% and maximum 70.70% juice was attained by 20 ppm NAA and carbendazim 0.2% (Figure 2). Lima and Davis, (1984) [25] reported none of the significant changes in juice percentage after treatment with 2,4-D and GA_3 in citrus.

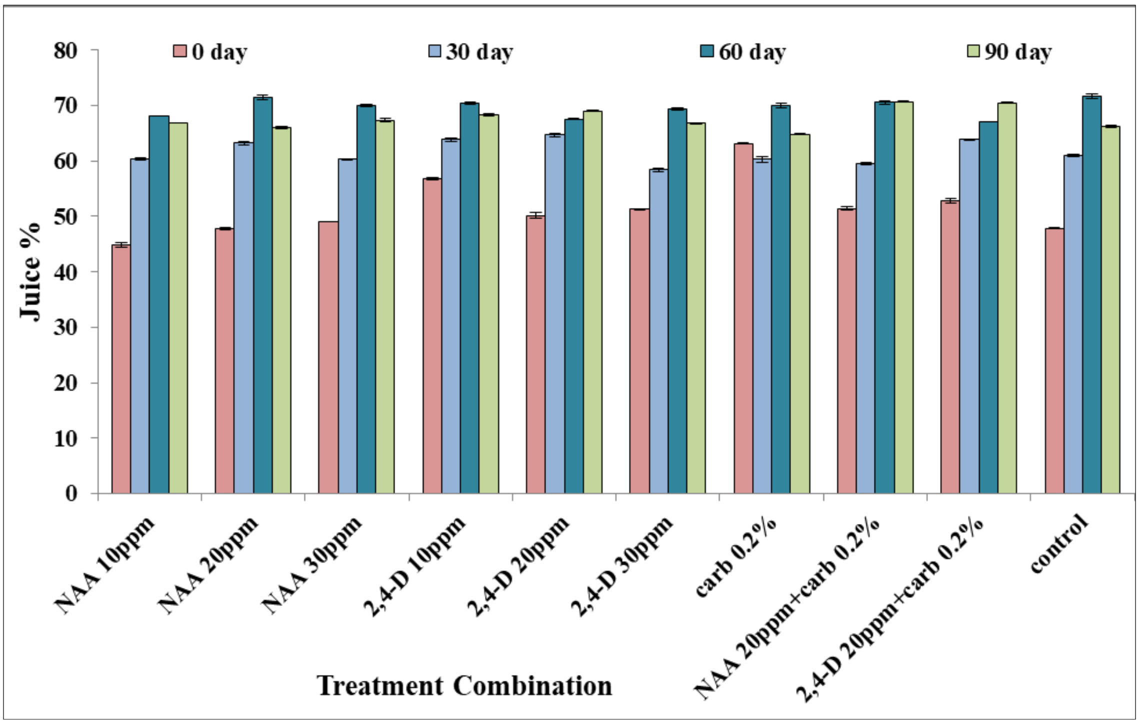

Fig 2: Average juice (%) of kinnow fruit after treatment with synthetic auxins and fungicide. Values given are mean ±SD of three plants.

4.7 Titratable Acidity

The data with respect to titratable acidity of different treatments were estimated and their mean values have been displayed graphically in figure 3. A critical analysis of data showed that the treatments exerted significant variation in improving acidity level of the fruits. The minimum acidity (0.16%) was obtained with 10 ppm 2,4-D sprayed on 90 day followed by 0.2% carbendazim (0.18%). The acidity level of the fruits was recorded maximum (2.6%) in fruits obtained from control treatment on 0 day. Foliar spray of NAA was found to be helpful in reduction of acidity (Otmani *et al*., 2004; Xiao *et al*., 2005) [31, 43].

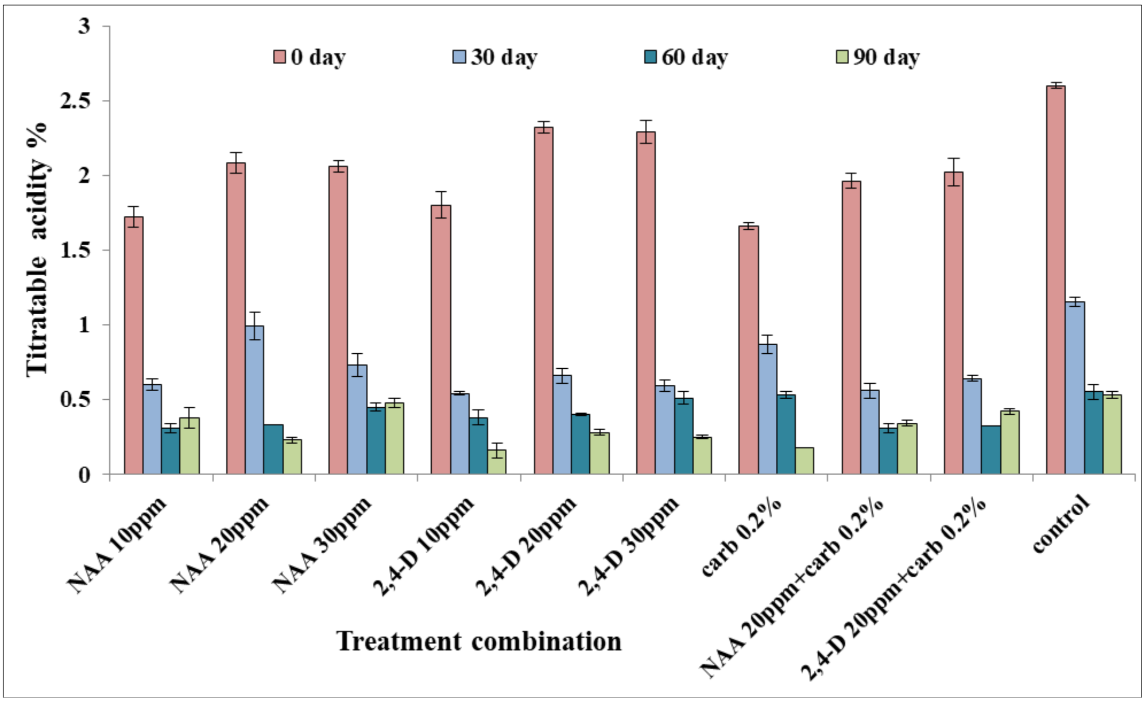

Fig 3: Average titratable acidity (%) of kinnow fruit after treatment with synthetic auxins and funigicide. Values given are mean ± SD of three plants.

4.8 pH

Tha data of pH of fruit juice graphically represent in figure 4 indicate that minimum pH 2.33 observed in 10 ppm 2,4-D and maximum pH 3.16 in control. Significant increase in level of pH was recorded during 60 day. On 60 day minimum pH 6.06 was observed in 30 ppm NAA and maximum pH 6.56 was observed in control. pH of fruit juice has been decreased again on observation recorded on 90 day. Thus, formulation of growth regulators and fungicides effect the level of pH.

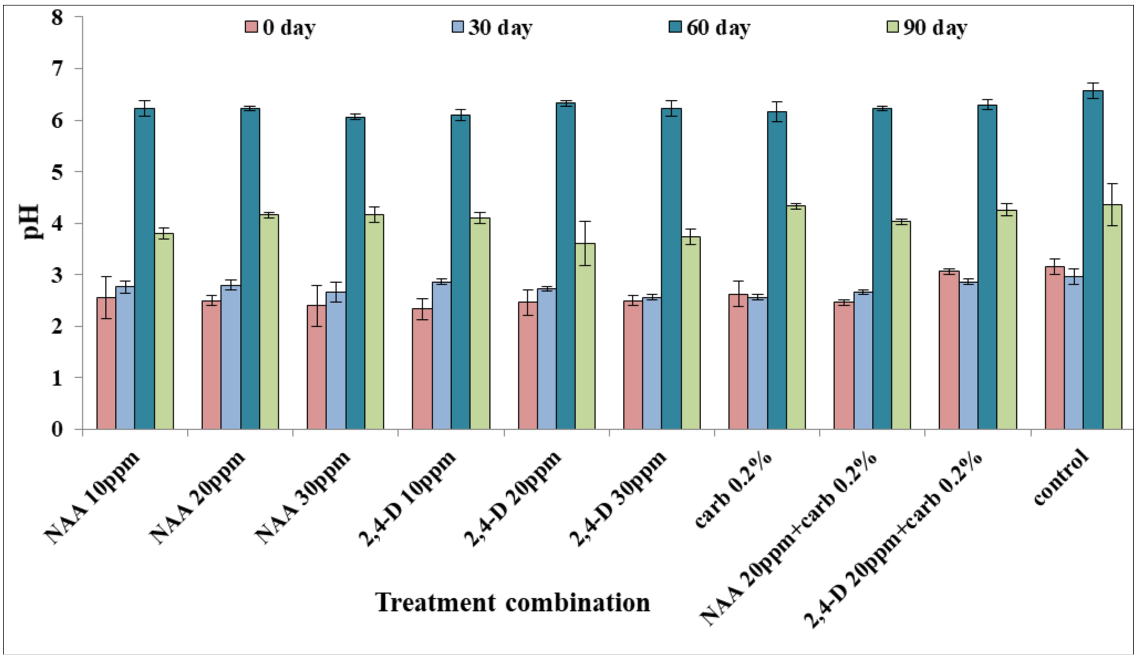

Fig 4: Average pH of kinnow fruit after treatment with synthetic auxins and funigicide. Values given are mean ±SD of three plants.

4.9 Total Soluble Solids (TSS)

The data concerning to total soluble solids content of fruit pulp for different treatments are represented graphically in figure 5 which clearly shows that maximum T.S.S.(10.10°brix) was recorded with 10 ppm 2,4-D sprayed on 90 day. This was followed by 30 ppm NAA sprayed on 90 day (9.63°brix). The statistical analysis with respect to treatments and control showed that minimum T.S.S 5.96, 6.56, 6.36 and8.16°brix were observed on 0, 30, 60 and 90 day respectively. Application of auxin (NAA, 2,4-D) can increased the total soluble solids in citrus species **(**Atawia & El Desouky, 1997) [4].

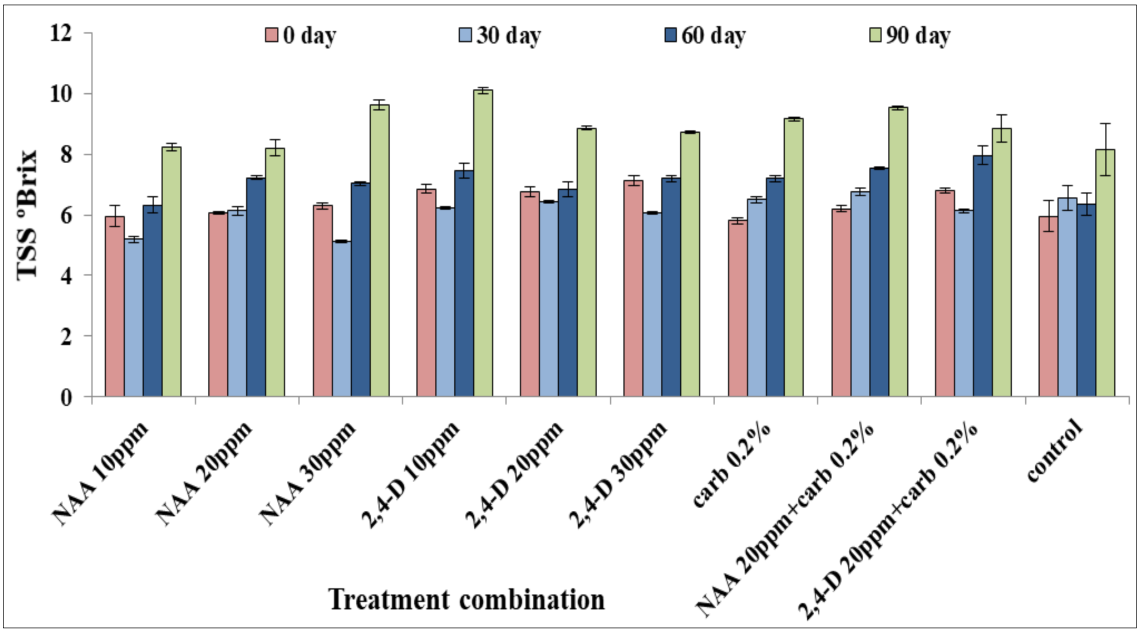

Fig 5: Average total soluble solids (ºbrix) of kinnow fruit after treatment with synthetic auxins and funigicide. Values given are mean ±SD of three plants.

4.10 Ascorbic Acid

The data with respect to ascorbic acid content are presented in figure 6indicates that the maximum ascorbic acid content (86.48 mg/100ml) was observed with NAA at 20 ppm sprayed on 30 day which was superior over control (43.11 mg/ 100ml).while on 90 day maximum ascorbic acid content (68.37 mg/100ml) was found in fruit from tree sprayed with 30 ppm 2,4-D.The minimum ascorbic acid content (14.42 mg/100g), (43.11 mg/100 ml), (35.92mg/100ml) and (36.64 mg/100ml) was found in control on 0, 30, 60 and 90 day respectively. Xiao *et al*., 2005 reported that application of PGR increased the ascorbic acid content in citrus.

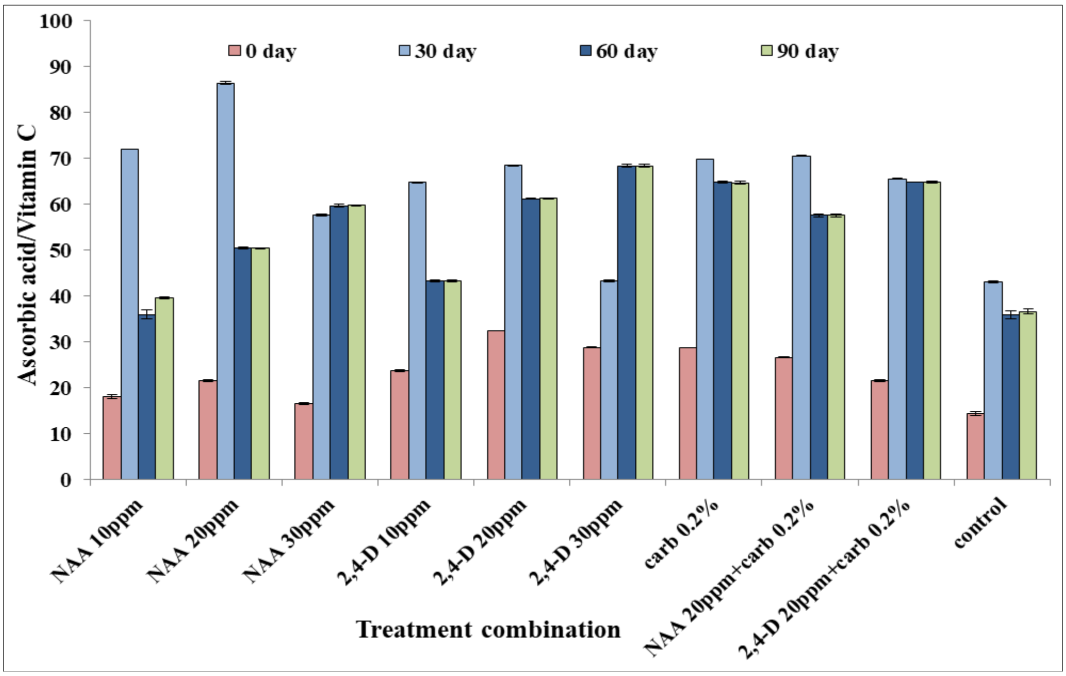

Fig 6: Average ascorbic acid content (mg/100ml) of kinnow fruit after treatment with synthetic auxins and funigicide. Values given are mean ±SD of three plants.

4.11 Total Sugar

Sugars are important constituents of kinnow fruit juice. Fruits, usually level of sugar in fruit juice used as an indicator of harvestable maturity. Amount of sugar present in juice determine taste and palatability of fruit.

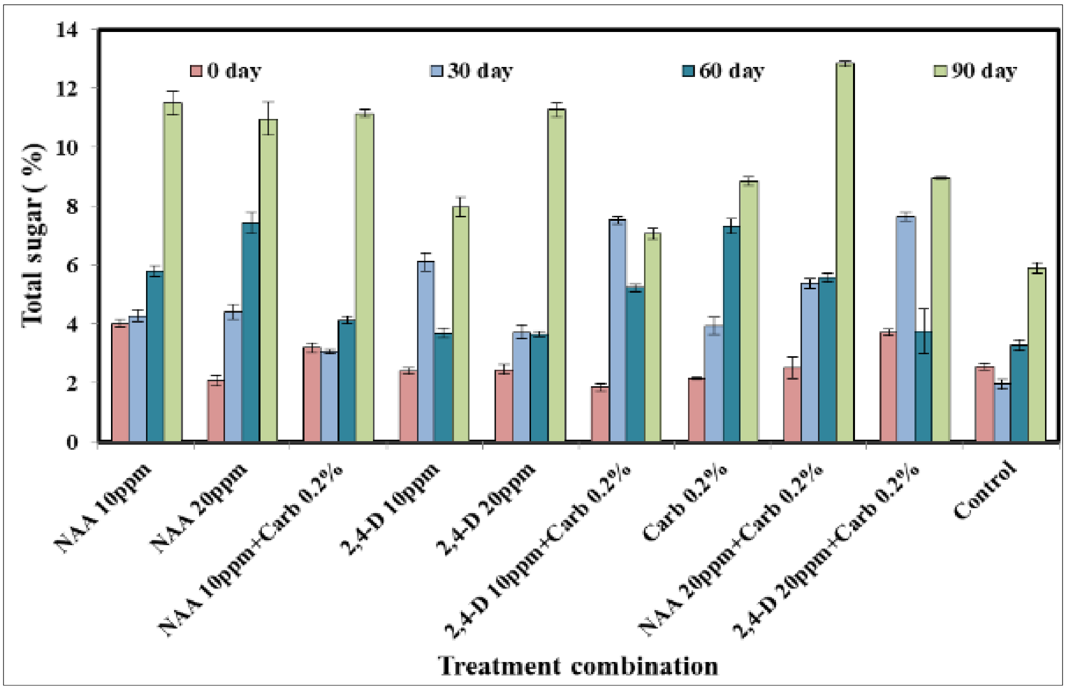

Fig 7: Average total sugar (%) of kinnow fruit after treatment with synthetic auxins and funigicide. Values given are mean ±SD of three plants.

Fully ripe kinnow fruit have good concentration of sugar in its juice. Sucrose, gluctose and fructose are the major sugars present in fully ripened kinnow fruits. Sucrose is the primary non- reducing sugar present in fruit juice and its concentration grasually increase with maturation. Sugar act as bulking agent, anticoagulant, enhance color, preservative and adds viscosity in fruit juice. The data concerning to total sugar content of fruit pulp for different plant growth regulators treatments are pictorially given in figure 7. The statistical analysis of data clearly indicated that different treatment had significant variation in total sugar. Maximum total sugar (12.85%) was observed in fruits collected from plant treated with 20 ppm NAA and Carbendazim 0.2% while minimum (8.95%) was observed in untreated plants on 90th day. Thus it was found that treatments have significant effects on total sugar content of fruit juice. Ingleand Wang reported application of 2,4-D and GA_3 and some other growth regulators increased the sugar content in citrus species.

4.12 Reducing Sugar

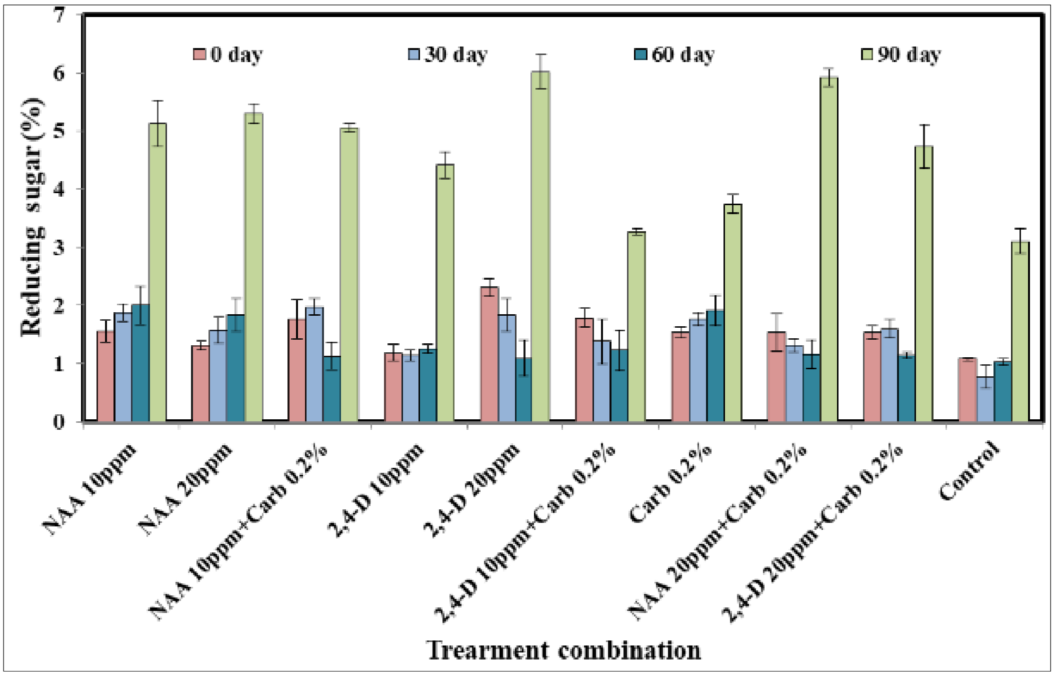

Fig 8: Average reducing sugar (%) of kinnow fruit after treatment with synthetic auxins and funigicide. Values given are mean ±SD of three plants.

The data pertaining to reducing sugar percentage has been graphically represented infigure 8 and showed that maximum reducing sugar was observed on 90 days while minimum reducing sugar was observed on 0 day. Maximum reducing sugar was observed in the juice of fruit obtained from plant treated with 20 ppm 2,4-D and minimum reducing sugar was observed in untreated plant on 90th day (Figure 8). Growth regulators increased the reducing sugar contents in various mandarin and sweet orange cultivars.

4.13Non- Reducing Sugar

The data concerning to non-reducing sugar content of fruit juice for different plant growth regulators treatments are pictorially given in figure 9. The statistical analysis of data clearly indicated that maximum non reducing sugar was observed on 90th day while minimum non reducing sugar was observed on 0 day.

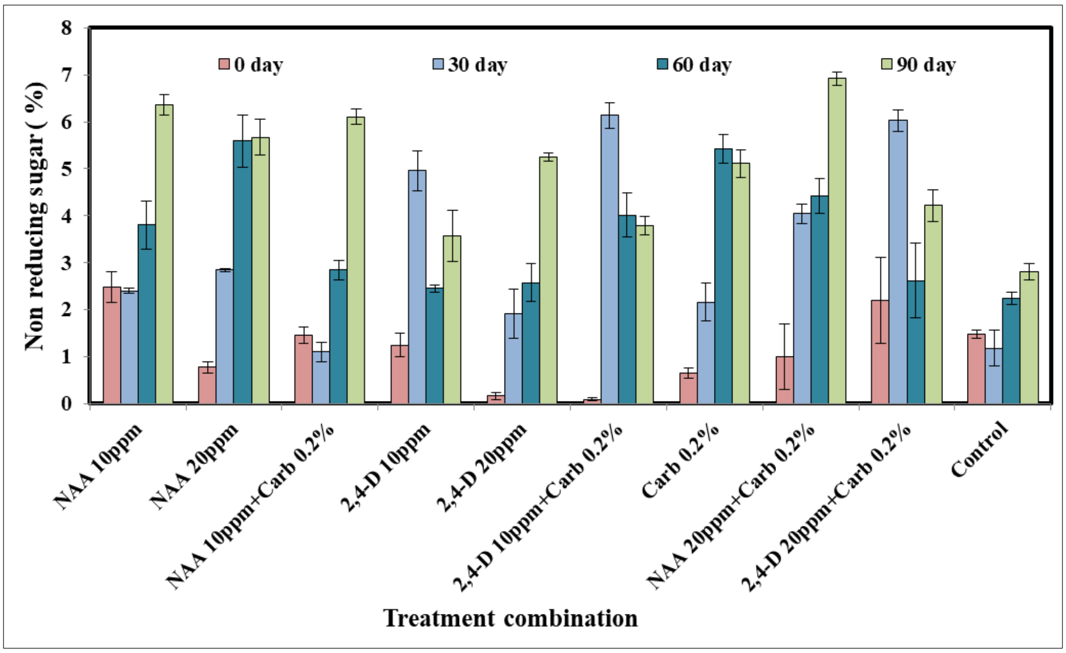

Fig 9: Average non reducing sugar (%) of kinnow fruit after treatment with synthetic auxins and funigicide. Values given are mean ±SD of three plants.

Maximum non reducing sugar was observed in the juice of fruit obtained from plant treated with formulation of 20 ppm NAA and Carbendazim 0.2% and minimum reducing sugar was observed in untreated plant on 90th day. Reported increase in non-reducing sugar with the treatment of GA_3 and 2,4-D in kinnow mandarin.

4.14 Polyphenol Content

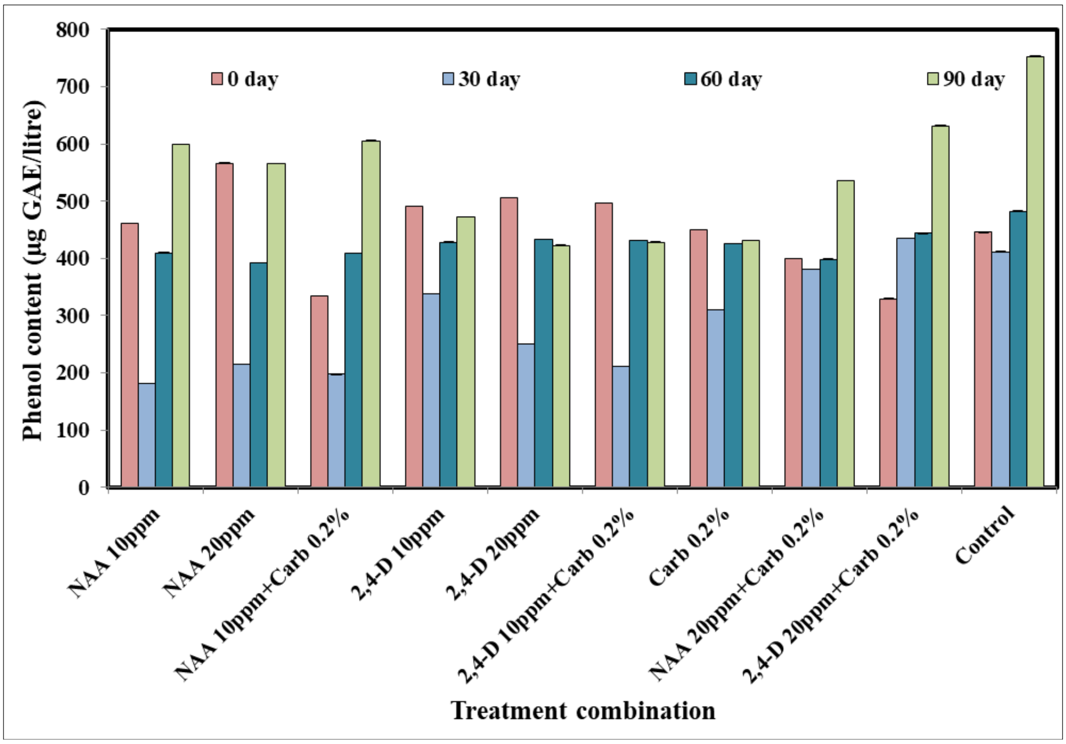

Fig 10: Average phenol content (µg GAE/litre) of kinnow fruit after treatment with synthetic auxins and funigicide. Values given are mean ±SD of three plants.

Postharvest cold storage increases the phenolic content of kinnow fruit. Growing and storage conditions significantly influence phenolic content of fruit pulp. In present study at time of harvest, maximum phenol content was seen in untreated plants while minimum was observed in plant treated with 20 ppm 2,4-D (Figure 10).

Overall result suggests that combination of synthetic auxin and fungicide can be used as foliar spray to increase fruit yield and decrease pre-harvest fruit drop. Formulation consisting of NAA and Carbendazim is effective in controlling pre-harvest fruit drop and increasing fruit yield. Combination of 2,4-D and Carbendazim is helpful in enhancing the kinnow fruit quality by altering biochemical properties.Thus, farmers can increase yield as well as fruit quality by exogenous application of synthetic auxin and Carbendazim.

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6. References

1. Ahmed W, Ziaf K, Nawaz MA, Saleem BA, Ayyub CM. Studies on combining ability of citrus hybrids with commercial indigenous cultivars. Pak J Bot. 2007;39(1):47-55.
2. Almeida IML de, Rodrigues JD, Ono EO. Application of plant growth regulators at pre-harvest for fruit development of 'Pera' oranges. Braz Arch Biol Technol. 2004;47(4):511-520.
3. Rizzuti A, Aguilera-Sáez LM, Gallo V, Cafagna I, Mastrorilli P, Latronico M, *et al*. On the use of Ethephon as abscising agent in cv. Crimson Seedless table grape production: Combination of Fruit Detachment Force, Fruit Drop and metabolomics. Food Chem. 2015;171:341-350.
4. Atawia AR, El-Desouky SA. Trials for improving fruit set, yield and fruit quality of Washington Navel Orange by application of some growth regulators and yeast extract as a natural source of phytohormones. Ann Agr Sci. 1997;35(3):1613-1632.
5. Ashraf MY, Yaqub M, Akhtar J, Khan MA, Ali-Khan A, Ebert G. Control of excessive fruit drop and improvement in yield and juice Khalid S, Malik AU, Khan AS, Razzaq K, Naseer M. 2012.
6. Anthony MF, Coggins CWJ. Effect of NAA and 3,5,6-TPA on pre-harvest drop in naval orange and grapefruit. Hort Science Alexandria, Va: The American society for Horticultural Science. 2001 Dec;36(7):1296-1299.
7. Adouli B, Zamani Z, Fattahi-Mohgadam MR, Golein B, Rezaei K. Effects of alternate bearing and 2,4-D application on fruit growth pattern, abscission enzymes activity, ACC content of calyx and carbohydrates partitioning of fruits in Satsuma mandarin (*Citrus unshiu* Marc.) cv. Miyagawa. Sci Hortic. 2018;238:58-65.
8. Bharti A. Studies on pre-harvest fruit drop and improvement in physical parameters of Kinnow mandarin through exogenous application of plant growth regulators. Int J Con Sci. 2020;8(1):1036-1040.
9. Browning G. The physiology of fruit set. In: Wright CU, ed. Manipulation of Fruiting. Butter Wareh: C.U. Wright; c1986. p. 195-198.
10. Chundawat BS, Gupta OP, Arora RK. Studies on fruit drop in kinnow, a mandarin hybrid cultivars. Haryana J Hort Sci. 1975;4:11-15.
11. Daulta BS, Kumar R, Singh D. Effect of ZnSO4, cytozyne and 2,4-D on fruit retention and quality of

kinnow and mandarin hybrid. Haryana J Hort Sci. 1986;15(1/2):14-17.
12. Doberman A, Fairhurst T. Rice: Nutrients disorder and nutrients management. Potash and Phosphorus Institute of Canada and International Research Institute, Los Baffios, Phillipines. Plant Growth Regulators Application Time Influences Fruit Quality and Storage Potential of Young Kinnow' Mandarin Trees. Int J Agric Biol. 2000;18(3):623-629.
13. Davinder, Mirza A, Singh R, kumar A, partap S. Impact of zinc and boron on growth parameters of kinnow. J Pure Appl Microbiol;c2017. p.1-4.
14. Erner Y, Kaplan Y, Baracha A, Hamon M. Increasing fruit size using auxins and potassium. Acta Hortic. 1993;329:112-119.
15. Ghosh SN, Bera B, Roy S. Influence of plant growth regulators on fruit production of sweet orange. J Crop Weed. 2012;8(2):83-85.
16. Gomez-Cadenas A, Mehouachi J, Tadeo FR, Primo-Millo E, Talon M. Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus. Planta. 2000;210:636-643. Horticulture at a glance, 2017.
17. Ibrahim M, Ahmad N, Anwar SA, Majeed T. Effect of micronutrients on citrus fruit yield growing on calcareous soils. In: Fangsen XU, Goldbach HE, Brown PH, Bell RW, Fujiwara T, Hunt CD, Goldberg S, Shi L, eds. Advances in Plant and Animal Boron Nutrition. Springer Netherlands; c2007. p. 179-182.
18. Jackson ML. Soil Chemical Analysis. Constable and Company, Ltd; c1962.
19. Jain MC, Choudhary HD, Sharma MK, Singh B. Yield and quality attributes of Nagpur mandarin as effected by use of different plant growth regulators. 2014.
20. Li J, Liang C, Liu X, Huai B, Chen J, Yao Q, *et al*. Effect of Zn and NAA co-treatment on the occurrence of creasing fruit and the peel development of ‘Shatangju’ mandarin. Sci Hortic. 2016;201:230-237.
21. Kaur N, Monga PK, Thind SK, Thatai SK, Vij VK, Kaur N. The effect of growth regulators on tropical fruit drop in Kinnow mandarin. Haryana J Hort. Sci. 2000;29(1-2):39-41.
22. Kumar A, Mirza A, Singh B, Davinder, Partap S, Singh P. Effect of pruning, micronutrients and plant growth regulators on kinnow mandarin fruits. J Pure Appl Microbiol. 2018;12(4):2231-2238.
23. Kumar TS. Role of plant growth regulators on vegetative growth, yield and quality of sweet orange (*Citrus sinensis* L.) cv Sathgudi. The Pharma Innov J. 2021;10(3):1007-1009.
24. Kavinprashanth R, Paramaguru P, Aneesa Rani MS, Sujatha KB. Impact of foliar application of growth regulators and micronutrients on yield and quality of acid lime (*Citrus aurantifolia* Swingle). J Pharmacog Phytochem. 2021;10(1):2091-2093.
25. Lima JEO, Davies FS. Growth regulators, fruit drop, yield, and quality of Navel orange in Florida. J Amer Soc Hort Sci. 1984;109(1):81-84.
26. Abbas M, Khan MM, Mughal SM, Ji P. Comparison of infection of Citrus tristeza closterovirus in Kinnow mandarin (*Citrus reticulata*) and Mosambi sweet orange (*Citrus sinensis*) in Pakistan. Crop Prot. 2015;78:146-150.
27. Marinho CS, Oliveira L, Serrano JC, Carvalho J. Effects of gibberellic acid and fungicides on post-bloom fruit drop in Tahiti acid lime. Laranja. 2005;26(1):47-57.
28. Nawaz AM. Role of growth regulators on preharvest fruit drop, yield and quality in kinnow mandarin. Pak J Bot. 2008;40(5):1971-1981.
29. Kumar N, Duhan A, Bhatia J, Malik V. Economic Appraisal of Kinnow Production and its Marketing in Sirsa District of Haryana. Int J Curr Microbiol App Sci. 2017;6(11):4045-4053.
30. Nelson PW, Sommers LE. Total carbon, organic carbon and organic matter. In: Page AL, Miller RH, Keeney DR, eds. Methods of Soil Analysis. Am Soc Agron. 1982:539-579.
31. Otmani MEl, Ait-Oubahou A, Lovatt CJ, Hassainate F El. Effect of Gibberellic acid, Urea and KNO on yield, composition and nutritional quality of Clementine mandarin fruit juice. ISHS Acta Horticulturae. 2004;632:495-498.
32. Rattanpal HS, Singh G, Singh S, Arora A. Citrus cultivation in Punjab. Ludhiana, India: PAU; 2017.
33. Rodríguez VA, Mazza SM, Martínez GC, Ferrero AR. Zn and K influence on fruit sizes of Valencia orange. Rev Bras Frutic. 2005;27:132-135.
34. Saleem BA, Ziaf K, Farooq M, Ahmed W. Fruit set and drop patterns as affected by type and dose of fertilizer application in mandarin cultivars (*Citrus reticulata* Blanco). Int J Agri Biol. 2005;7(6):962-965.
35. Saraswathi T, Rangasamy P, Azhakiamanavalan RS. Effect of pre-harvest spray of growth regulators on fruit retention and quality of mandarins (*Citrus reticulata* Blanco). South Indian Hort. 2003;51(1/6):110-112.
36. Saleem BA, Malik AU, Farooq M. Effect of exogenous growth regulators application on June fruit drop and fruit quality in *Citrus sinensis* cv Blood Red. Pak J Agric Sci. 2007;44(2):289-294.
37. Shivankar VJ, Rao CN, Singh S. Studies on citrus psylla, Diaphorina citri Kuwayama: A review. Agric Rev. 2000;21:199-204.
38. Thimann KV. Plant growth hormones. The hormone: Physiology, chemistry and applications. New York: Academic Press Inc.; c1948.
39. Timmer LW, Mondal SN, Peres NAR, Bhatia A. Fungal Diseases of Fruit and Foliage of Citrus Trees. In: Naqvi SAMH, ed. Diseases of Fruits and Vegetables. Springer Netherlands; 2004:383-408.
40. Ullah R, Sajid M, Ahmed H, Luqman M, Razaq M, Nabi G, *et al*. Association of Gibberellic acid (GA3) with fruit set and fruit drop of Sweet Orange and farming system in the middle. Reseaches of the Journal of Biology Agricultural and Healthcare. 2014;4:23-29.
41. Uniyal S, Misra KK. Effect of plant growth regulators on fruit drop and quality of Bael under Tarai conditions of Uttarakhand. Indian J Hort. 2012;72(1):126-129.
42. Verma O, *et al*. Chemical control of preharvest fruit drop in Nagpur mandarin of Chhindwara district of M.P, India. Int J Curr Microbiol Appl Sci. 2018;7(6):2258-2263.
43. Xiao JX, Peng S, He-HuaPing, Li-JiangHai. Effects of calcium nitrate and IAA on calcium concentration and quality of Satsuma mandarin fruit. J Fruit Sci. 2005;22(3):211-215.

44. Zheng YQ, Yang Q, Jia XM, Liu YM, He SL, Deng L, *et al*. Ca(NO3)2 canopy spraying during physiological fruit drop period has a better influence on the tree character and fruit quality of Newhall navel orange (*Citrus sinensis* Osbeck). J Integr Agric. 2017;16(7):1513-1519.
45. Kaur M, Singla S, Mahajan R. Item analysis of in use multiple choice questions in pharmacology. International Journal of Applied and Basic Medical Research. 2016 Jul 1;6(3):170-3.
46. Nasir A, Nasir MS, Shauket I, Anwar S, Ayub I. Impact of samanduri drain on water resources of Faisalabad. Advances in Environmental Biology. 2016 Jan;10(1):155-160.