MAJOR RESEARCH PROJECT

UNRAVELING THE PHYSIOLOGICAL BASIS OF GROWTH SUBSTANCE MEDIATED QUALITY AND QUANTITY IMPROVEMENT OF KINNOW MANDARIN

FINAL REPORT

SUBMITTED TO

UNIVERSITY GRANTS COMMISSION

Department of Botany
College of Basic Sciences and Humanities
Punjab Agricultural University, Ludhiana -141004
2019

Annexure- IX

University Grants Commisssion

(Ministry of Human Resource Development, Govt. of India)

Bahadurshah Zafar Marg, New Delhi – 110002

1.	**Title of the Project**	"Unraveling the physiological basis of growth substance mediated quality and quantity improvement of Kinnow mandarin"
2.	**Name and Address of Principal Investigator**	Dr. Nirmaljit Kaur, Senior Botanist Office: Department of Botany, Punjab Agricultural University, Ludhiana- 141004, Punjab Residential: H.NO. 9/9, PAU Campus, Punjab Agricultural University, Ludhiana- 141004, Punjab
		E-mail: nirmalkaur@pau.edu Mobile No: 09417766864
3.	**Name and Address of the Institution**	Department of Botany Punjab Agricultural University, Ludhiana
4.	**UGC approval letter no. and date**	F NO. 43-128/2014 (SR)
5.	**Date of Implementation**	01.07.2015
6.	**Tenure of the Project**	3 years from 01.07.2015 to 01.07.2018
7.	**Total grant allocated**	Rs. 10,55,000/-

8.	**Total grant received**	Rs. 8,06,013/-
9.	**Final expenditure**	Rs. 8,03,560/-
10.	**Title of the Project**	Unraveling the physiological basis of growth substance mediated quality and quantity improvement of Kinnow mandarin
11.	**Objectives of the project**	The present study was planned with following objectives: **i.** To identify patterns of flowering and fruit drop in Kinnow mandarin in two locations, i.e., Abohar and Ludhiana **ii.** To monitor the effect of weather parameters on flowering and fruit drop **iii.** To identify specific plant growth substances and nutrients for reducing fruit drop and to study their impact on fruit yield and quality **iv.** To unravel biochemical and physiological changes associated with flowering and fruit drop
12.	**Whether objectives were achieved (give details)**	Yes
13.	**Achievements from the project**	Attached (Annexure –A)
14.	**Summary of the Findings**	The present study with the objective to improve the quality and quantity in Kinnow with Salicylic acid, 2, 4-D and its combination with KNO_3 & $ZnSO_4$ was conducted at the Fruit Research Farm of Department of Fruit Science and in the Laboratories; Department of Botany, PAU, Ludhiana 2015-18. ➢ Physiological fruit drop limits the quality and quantity of Kinnow returns. To reduce this disorder, foliar application of synthetic auxins (2,4-D @ 10 and 20 ppm), Phenolic compound Salicylic acid (10 ppm), Zinc ($ZnSO_4$ @ 0.3 %) and combination of 2,4-D (20 ppm) with KNO_3 and ZnSO4 (0.3 %) were given to the Kinnow mandarin trees (As in Table 1). ➢ The foliar application of 2, 4 D (10 ppm) during mid-

		April, mid – May and August resulted in minimum fruit drop and maximum fruit yield (number/tree). This was closely followed by foliar application of SA (10 ppm) during mid-April, Mid-June and mid-September. ➢ It was concluded that the ***foliar application of 10 ppm SA during mid-April, Mid-June and mid-September is at par with 2,4-D (20 ppm) and may be suggested as an alternate to 2,4-D in areas where cotton is grown around Kinnow orchards***. ➢2, 4–D prevents the formation of abscission zone and Salicylic acid acts as a signalling molecule and provides thermotolerance. ➢Comparative studies were made in the fruit pedicel of healthy and dropped Kinnow mandarin fruits. The periderm width, phloem fiber width and xylem width were higher in healthy pedicels as compared to dropped ones**,** whereas, pith diameter was higher in dropped pedicels. ➢The activity of cell wall degrading enzymes cellulase and polygalactouranase was low in healthy pedicels. During cell wall degradation, the walls appear to swell and become highly flexible and wall of some of the cells under zone of separation invaginated during advanced stage of cell wall degradation which ultemately collapsed. ➢The current results suggest that cell elongation accompanied by decrease in cell and cell wall width with expansion of middle lamella width in abscission zone cells that separate later. The data demonstrate the important role of polygalacturonase and cellulase in pedicel abscission. The activity of cellulase was found to increase substantially in the pedicel abscission zone during fruit drop. ➢The endogenous IAA and Salicylic acid was higher in the healthy pedicels as compared to the dropped ones. The Auxins, Gibberlins and SA are considered as inhibitors of

		abscission. With application of Auxin and Auxin analogue (2,4-D), there is a decrease in abscission by reducing the senstivity of abscission zone to ethylene. The level of sugars in the pedicel is very critical to prevent abscission and carbohydrate shortage accelarate fruit abscission. ➢ Hence, understanding of the mechanism underlying abscission can help in regulation of fruit production. However, further molecular research on abscission could help to elucidate new molecular markers for improved genetic breeding programmes.
15.	**Contribution to the Society**	Kinnow is a hybrid of King and Willow Leaf mandarin. Its fruits are large, globular, orange, having 12 to 25 seeds. This is an "easy peel" fruit and has assumed special economic importance and demand due to its high juice content, special flavor and being a rich source of Vitamin C. The Kinnow tree is generally a heavy bearer and its cultivation faces some unanswered queries, viz., irregular flowering behavior, overbearing and fruit drop. Kinnow comes to bearing during the third year and gives the first commercial crop during fifth year of its plantation. The citrus industry encounters a considerable threat in the form of fruit drop during various stages of fruit growth that results in decline of ultimate yield. Fruit let abscission/ drop is a common phenomenon that occurs in many crop plants in response to developmental and environmental cues leading to significant crop losses. Irregular flowering and fruit setting followed by reduced fruit retention are the major factors responsible for affecting the qualitative and quantitative fruit production. The effective replenishing of nutrients and plant growth regulators is necessary to produce high quality citrus fruits and control excessive citrus fruit drop. Hence the present investigation will help to control of excessive fruit drop and bring about improvement in Kinnow fruit yield and quality. The information generated will be of practical application to the horticultural industry for consistent high quality Kinnow production.

		Reduction of physiological fruit drop in Kinnow mandarin with 2,4-D and Salicylic acid can boost the production and economic returns of Kinnow growers.
16.	**Whether any PhD enrolled/produced out of the Project**	No
17.	**Number of Publications out of the Project**	Papers Published : One Papers Submitted : One Abstracts Published:Four (Annexure B)

(PRINCIPAL INVESTIGATOR) **(REGISTRAR/PRINCIPAL)**

(Seal)

(CO-INVESTIGATOR)

Annexure A

1. **ACHIEVEMENTS FROM THE PROJECT**

<u>Experiment 1:</u> Effect of plant growth substances and nutrients on fruit drop, yield and quality in Kinnow mandarin

Citrus is a major fruit of India which covers an area of 1003 Mha with a production of 12546 million MT. In Punjab (India), citrus orchards capture 55,620 hectares with a major share in national production and Kinnow is grown in an area of 51,637 hectares with an annual production of 12,08,140 MT. Fruit drop or abscission, natural separation of organs from the parent plant, is a serious problem in mandarins, which start from blooming and continue till harvesting, particularly the summer drop and pre harvest drop. Fruit drop and final fruit retention are mostly varietal characters, although water and nutritional stress increases fruit drop in susceptible trees. The poor tree health also plays a significant role in enhancing fruit drop due to depletion of nutrient supply to the demanding sinks. Plant growth regulators (PGRs) play a major role in fruit growth and abscission and have been used in citrus fruit production for influencing flowering, fruit set and fruit drop. These regulators have also been used to influence fruit quality factors like peel quality and colour, fruit size, juice quality, and to improve total soluble solids in different citrus species. Among PGRs, the auxins have direct effect on abscission by causing a delay of abscission resulting in improvement in fruit quality and yield in citrus. Zn affects formation of the growth substance Indole Acetic Acid (IAA) through its influence on the synthesis of tryptophan which is the precursor of IAA. Potassium plays a regulatory role in physiological and biochemical processes of citrus plants and is involved in the formation and functioning of protein, fats, carbohydrates, chlorophyll and maintaining the balance of salts and water in plant cells. Salicylic acid (SA) is a phenolic compound found in plants with a role in plant growth, development, photosynthesis, transpiration as well as uptake and transport of nutrients. The present study was undertaken to test the efficacy of synthetic auxin 2,4 –D, SA, $ZnSO_4$ and combination of 2,4-D with KNO_3 and $ZnSO_4$ on fruit retention, physical and biochemical parameters of fruits and ultimate fruit yield in Kinnow mandarin.

Methodology:

The present studies were conducted at the Fruit Research Farm of Department of Fruit Science and in the Laboratories of Department of Botany, Punjab Agricultural University, Ludhiana during 2015-2018. The cultural practices for orchard were kept uniform for all the trees as per package of practices, P.A.U. The Kinnow plants which were 10 years old at the start of experiment and planted at a distance of 20 x 20 m, budded on Jatti Khatti rootstock were selected as experimental plants. The treatments were applied as foliar application to the selected experimental Kinnow plants at the New orchard as per Table 1.1. The experimental design was Randomized Block Design with single tree as an experimental unit replicated three times.

Table 1.1 Concentration and time of application of mineral nutrients and growth regulators

S.No.	Treatments	Concentration	Time of Application
T_1	Control	Water spray	End March, End April, Mid August, Mid September
T_2	2,4-Dichlorophenoxy acetic acid + KNO_3	20 ppm + 5%	60 days after full bloom
T_3	2,4-Dichlorophenoxy acetic acid + $ZnSO_4$	20 ppm+0.3%	Mid-April, Mid-June, Mid- September
T_4	$ZnSO_4$	0.3%	Mid-April, Mid-June, Mid-September
T_5	Salicylic acid	10 ppm	Mid-April, Mid-June, Mid-September
T_6	2,4-Dichlorophenoxy acetic acid	10 ppm	Mid- April, Mid - May, Mid August
T_7	2,4-Dichlorophenoxy acetic acid	20 ppm	End March, End April, Mid August, Mid September

Ten fruits selected randomly per replication from each treatment were brought to the laboratory, washed and dried at room temperature and analyzed for the biochemical parameters. Percent fruit drop and per cent fruit retention were calculated. Fruit yield on the basis of fruit weight per tree was recorded at the time of harvest from each tree. The average fruit weight and fruit size were computed. For estimation of juice (%), peel (%), TSS, Vitamin C, Acidity, reducing sugars standard procedures were followed.

Salient Achievements:

During the three years of investigation, there was a significant reduction in the per cent fruit drop (Table 1.2) with almost all the treatments as compared to control. Minimum mean fruit drop (49.39%) was recorded with the foliar application of 2,4-D (20ppm) which was 20.74% less as compared to control. The foliar application of Salicylic acid (10ppm) also resulted in reduction of mean fruit drop (52.73%) and registered 15.38% reduction in fruit drop as compared to control. The abscission/dropping of fruit might be due to low auxin activity or limited supply of auxin to developing fruit as reported in Nagpur mandarin and in Navel orange. So, decrease in fruit drop in this study by the application of growth regulators can be attributed by making up the deficiency of endogenous auxin preventing the formation of abscission layer possibly through the inhibition of enzymatic activity such as pectinase, cellulase and polygalacturonase. Salicylic acid has been reported to regulate physiological process in plant and has been found to be effective improving the yield of fruits in different fruit crops.

Table 1.2 Effect of different treatments on per cent fruit drop in Kinnow mandarin

Treatments	Concentration	Per cent fruit drop				Per cent Decrease in fruit drop
		2015	2016	2017	Average	
Control	Water spray	63.39	62.49	61.09	62.32	--
2,4-D+KNO_3	20 ppm+5%	44.79	67.13	71.05	60.99	2.13
2,4-D+$ZnSO_4$	20ppm+0.3%	51.11	54.96	55.88	53.98	13.70
$ZnSO_4$	0.3%	50.31	52.07	52.73	51.70	17.04
SA	10ppm	50.71	54.14	53.35	52.73	15.38
2,4-D	10ppm	50.46	52.43	52.55	51.81	16.84
2,4-D	20ppm	46.42	51.06	50.69	49.39	20.74
CD(P=0.05)		6.80	9.13	9.78	---	-----

There was a significant increase in fruit retention percent (Table 1.3) with all the treatments. The average fruit retention was maximum (49.22%) with 2,4-D (20ppm). The increased fruit retention leads to increase in fruit yield. Earlier reports on increase in fruit yield by enhancing the fruit retention and reduction of fruit drop in citrus support the present investigation. The increase in number of fruits retained per tree with 2, 4-D is probably because auxins lead to enhancement of sucrose translocation to fruits.

Table1.3. Effect of different treatments on per cent fruit retention in Kinnow mandarin

Treatments	Concentration	Per cent Fruit retention			
		2015	2016	2017	Average
Control	Water spray	36.60	37.51	38.91	37.67
2,4-D+KNO_3	20 ppm+5%	55.21	32.87	28.95	39.01
2,4-D+$ZnSO_4$	20ppm+0.3%	48.80	45.04	44.12	45.98
$ZnSO_4$	0.3%	49.72	47.93	47.27	48.30
SA	10ppm	49.22	45.86	46.65	47.24
2,4-D	10ppm	53.53	47.86	42.55	47.88
2,4-D	20ppm	49.43	47.57	49.31	49.22
CD(P=0.05)		6.04	5.93	5.81	----

The data presented in table 1.4 shows that there was a significant increase in fruit yield on the basis of number of fruits/tree with all the treatments during 2015. The mean fruit yield was maximum (498.11fruits/tree) with the foliar application of 20ppm 2, 4-D. Consequently, the mean fruit yield (kg/tree) was maximum (94.59 kg/tree) with the foliar application of 20 ppm 2,4-D. This was closely followed by 10 ppm 2,4-D (90 Kg/tree) and SA (85 kg/tree) (Fig. 1.1). The increase in yield with (20ppm) 2, 4-D was due to decrease in fruit drop by this treatment.

Table 1.4 Effect of different treatments on fruit yield (No. /tree) in Kinnow mandarin

Treatments	Concentration	Fruit Yield (No./tree)			
		2015	2016	2017	Average
Control	Water spray	346.33	341.66	333.66	340.55
2,4-D+KNO_3	20 ppm+5%	629.00	233.00	231.00	364.33
2,4-D+$ZnSO_4$	20ppm+0.3%	425.66	334.00	332.00	363.88
$ZnSO_4$	0.3%	578.33	431.33	423.33	477.66
SA	10ppm	562.33	422.33	416.66	467.10
2,4-D	10ppm	570.66	445.66	437.00	484.44
2,4-D	20ppm	599.00	450.33	445.00	498.11
CD(P=0.05)		67.93	32.46	29.36	---

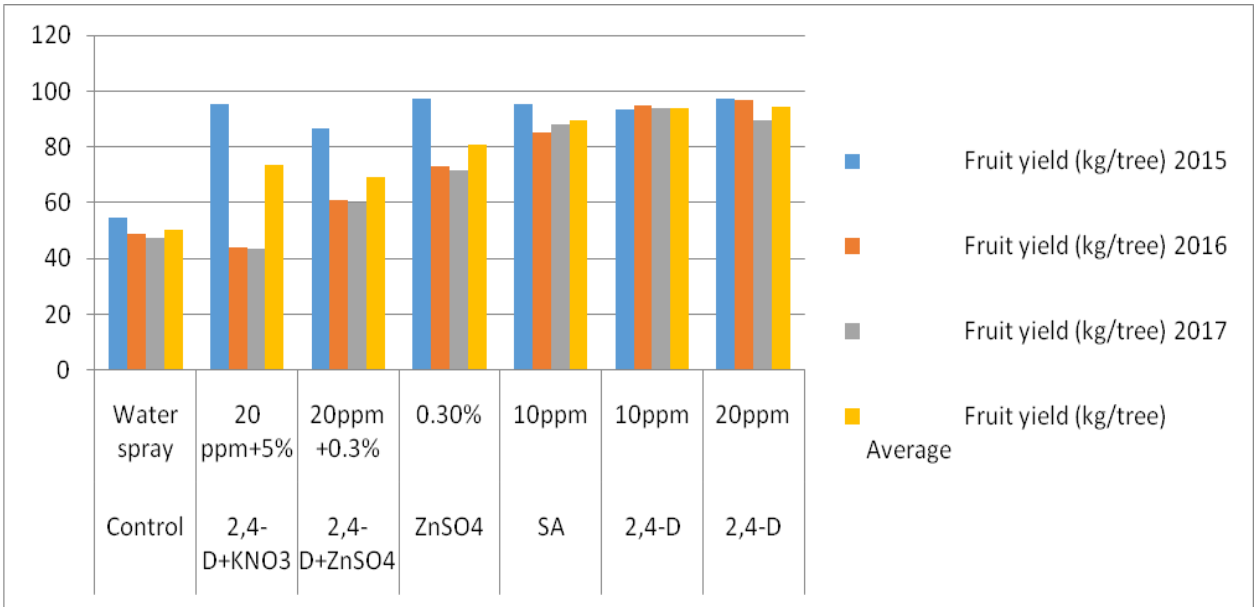


Fig.1.1 Effect of different treatments on of fruit yield (kg/tree) in Kinnow mandarin

There was significant increase in fruit weight (Table 1.5) with all the treatments except (0.3%) $ZnSO_4$. The maximum fruit weight (215.35g) was attained in the trees treated with 2,4-D (10ppm)

which was followed by 10ppm Salicylic acid treated trees (212.30 g). The increased fruit weight due to different treatments is attributed to the increase in fruit size with the treatments. Increase in fruit weight and fruit size was observed to be maximum with 2, 4-D (20ppm) treatment in an earlier reports also. There was significant increase in fruit length and breadth with all the treatments. The highest fruit length (6.70 cm) was observed in 2, 4-D (20ppm) treated trees followed by 10 ppm 2,4-D (6.50 cm). The application of auxins at the onset of cell enlargement stage increase final fruit size. In vegetative organs, the activation of cell division and cell enlargement processes leads to increase in fruit size. The zinc is required for synthesis of tryptophan, a precursor of synthesis of IAA, which is involved during fruit growth and development. There was significant decrease in peel per cent with all the treatments (Table 1.5). The least peel per cent was of fruits picked from trees treated with 2,4-D (10ppm) which was 23.24% followed by 2,4-D(20ppm) and SA(10ppm). The maximum peel per cent was recorded from control trees (29.45%). All the treatments significantly increased the juice per cent as compared to control and maximum juice (53.78%) was recovered in 2,4-D (20ppm) treated trees followed by 10ppm 2,4-D (51.78%) and 10 ppm Salicylic acid (51.22%). Minimum juice per cent was retained in fruits of control trees (43.07%).

Table 1.5 Effect of different treatments on physical parameters of the Kinnow mandarin fruits

Treatment	Concentration	Fruit Weight (g)	Fruit Length (cm)	Fruit Breadth (cm)	Peel (%)	Juice (%)
Control	Water spray	143.70	5.70	6.60	29.45	43.07
2,4-D+KNO_3	20 ppm+5%	189.90	6.20	7.20	25.37	50.58
2,4-D+$ZnSO_4$	20ppm+0.3%	182.00	6.25	7.30	26.32	50.52
$ZnSO_4$	0.3%	169.75	6.25	7.35	26.68	49.88
SA	10ppm	212.30	6.30	7.45	25.17	51.22
2,4-D	10ppm	215.35	6.50	7.25	23.24	51.78
2,4-D	20ppm	201.90	6.70	7.70	24.61	53.78
CD(P=0.05)		29.43	0.45	0.51	1.23	0.68

The data presented on quality parameters in table 1.6 reveals that the different treatments increased the TSS per cent as compared to control (except 0.3% $ZnSO_4$). However, the increase was non-significant. The maximum TSS (11.0%) was recorded from the fruits treated with 2,4-D (20ppm) followed by 10ppm Salicylic acid (10.90%) and minimum TSS (10.50%) was recorded in $ZnSO_4$ (0.3%) and control trees. Increase in TSS content with the application of nutrients and plant growth regulators might be due to increase in photosynthetic activity and chlorophyll content of leaves which might have resulted in production of total soluble solids in fruit juice. The data on the impact of nutrients and plant growth regulators on acidity content of Kinnow fruit juice is presented in table 1.6

The acidity of juice reduced significantly with different treatments and the minimum acidity per cent was observed with (20ppm) 2,4-D (0.703%). Corresponding to high TSS and low acidity with the foliar application of nutrients and plant growth regulators, there was increase in TSS: acid ratio with all the treatments. The maximum TSS: Acid ratio (15.64) was observed with the foliar application of 2,4-D (20 ppm) followed by (10 ppm) 2,4-D (14.69%).

The changes recorded in reducing sugars and Vitamin C content with the application of plant growth regulators and nutrients are presented in Table 1.6.There was a significant increase in reducing sugars and Vitamin C due to foliar application of plant growth regulators and nutrients. The maximum reducing sugars (4.08%) were recorded in the juice of fruits given foliar application of 2, 4-D (20 ppm) which was closely followed by 10ppm Salicylic acid (3.85%) and 10 ppm 2,4-D (3.85%). The maximum vitamin C (41.37 mg/100ml juice) was observed in 2, 4-D (20ppm) treated trees, whereas least was observed with control trees (29.41 mg/100 ml juice).

Table 1.6 Effect of different treatments on quality parameters of the Kinnow mandarin fruits

Treatment	Concentration	TSS (%)	Acidity (%)	TSS: Acid Ratio	Reducing Sugars (%)	Vitamin C (mg/ 100 ml juice)
Control	Water spray	10.50	0.990	10.60	1.96	29.41
2,4-D+KNO_3	20 ppm+5%	10.80	0.761	14.19	3.03	35.67
2,4-D+$ZnSO_4$	20ppm+0.3%	10.75	0.831	12.93	3.36	39.43
$ZnSO_4$	0.3%	10.50	0.850	12.35	2.63	30.36
SA	10ppm	10.90	0.755	14.43	3.85	32.45
2,4-D	10ppm	10.80	0.735	14.69	3.85	32.45
2,4-D	20ppm	11.00	0.703	15.64	4.08	41.37
CD(P=0.05)		NS	0.29	--	0.703	0.619

<u>Experiment 2</u>. Biochemical, physiological and anatomical changes associated with flowering and fruit drop in Kinnow mandarin

Fruit drop/abscission comprises of process of natural separation of organs from the parent plant. Like in other citrus species, fruit drop is a serious problem in mandarins, which start from blooming and continue till harvesting, particularly the summer drop and pre harvest drop. Assessment of such losses in mandarins revealed that out of forty to eighty thousand flowers, only two to seven hundred reach maturities. High or low temperatures, rains, malnutrition, seeds in fruits, and pests and diseases are various causal agents associated with fruit drop in different species. Fruit drop and final fruit retention are mostly varietal characters, although water and nutritional stress increases fruit drop in susceptible trees. Information on management of excessive fruit drop in citrus is scattered and many of the studies have been based on only the extent and pattern of fruit drop. The present experiment was planned to monitor the periodical fruit

drop, study of biochemical entities and structure of pedicel of healthy and dropped fruits of Kinnow mandarin so as to unravel physiological and anatomical basis of fruit drop.

Methodology

The present investigation was conducted in the Fruit Research Farm of Department of Fruit Science and in the Laboratories of Department of Botany, Punjab Agricultural University, Ludhiana. The cultural practices for orchard were kept uniform for all the trees as per package of practices. The Kinnow plants which were 10 years old and planted at a distance of 20 x 20 m, budded on Jatti Khatti rootstock were selected as experimental plants. The periodic fruit drop and fruit retention was computed for Kinnow mandarin.

Ten fruits were selected randomly from each replication of each treatment during May-June. The healthy and dropped fruits bearing pedicels were brought to the laboratory, washed and dried at room temperature and pedicels were analyzed for total Soluble Sugars (Dubois *et al* 1956), Starch (McCready *et al* 1950), total Soluble Proteins (Lowry *et al* 1951), free Amino Acids (Lee and Takahashi 1966). The pedicel of the Kinnow fruit samples from each treatment collected during May-June were sectioned transversely using a diamond knife on a rotary microtome (Porter Blum JB4) to create two micrometer sections. Sections were stained with Toluidine Blue (0.5% solution in 0.1% sodium carbonate, pH 11.1) and bright field images were taken at both 10x and 60x using a Nikon TE 300 Inverted Microscope with an attached CCD camera (Quantix Cool snap; Reper Scientific). The endogenous level of IAA, Gibberellic acid and Salicylic acid was determined through HPLC coupled with FLD, NIPER, Mohali.

Salient Achievements:

Periodic fruit drop percentage in Kinnow was recorded during investigation (Fig. 2.1). During first year of investigation, maximum drop percentage was recorded in May (65.31%). In 2018, maximum fruit drop percentage was again observed in May (63.52%). Mean fruit drop percentage was maximum in May (64.41%), followed by June (23.35%), July (4.82%) and August (2.24) and it was very low in the later months. The results depicted maximum fruit drop in May which is in agreement with earlier observations.

The different physiological and biochemical traits of pedicels of healthy and dropped fruits of Kinnow were compared. The total soluble sugars, starch, total soluble proteins, free amino acids, were high in healthy pedicels as compared to dropped ones. The sugar metabolizing and protein hydrolysis enzymes were high in healthy pedicels however; cell wall degrading enzymes was high in dropped pedicels (Table 2.1). The HPLC analysis of endogenous level of GA, IAA and SA of the pedicels (Table 2.2. Fig 2.2) revealed higher level of all PGR's in the healthy as compared to the dropped counterparts.

The abscission zone of the fruit pedicel of Kinnow mandarin was studied with special reference to the structural and physiological changes in the walls of cells during process of abscission. Sections

through the abscission zone of the fruit pedicel of Kinnow mandarin were studied through light microscopy. The periderm width, phloem fiber width and xylem width was higher in healthy pedicel, whereas, pith diameter was higher in dropped pedicels (Table 2.3;Plate 2.1). During cell wall degradation, the walls appear to swell and become highly flexible and wall of some of the cells under zone of separation invaginated during advanced stage of cell wall degradation which ultemately collapsed. The current results suggest that cell elongation accompanied by decrease in cell and cell wall width with expansion of middle lamina width in abscission zone cells that separate later.

The data demonstrate the important role of Polygalacturonase and cellulase in petiole abscision. Many workers have reported correlation between changes in expression of cell wall degrading enzymes and abscission. The activity of cellulase was found to increase substantially in the petiole abscission zone during fruit drop.The stages of abscission are proceeded by production in transport to abscising fruits. Hence fruit abscission is mainly caused by limitation in carbohydrate supply.

The Auxins, Gibberlins and SA are considered as inhibitors of abscission. With application of Auxin and auxin analogue, there is a decrease in abscission by reducing the senstivity of abscission zone to ethylene. The level of sugars in the pedicel is very critical to prevent abscission and carbohydrate shortage accelarate fruit abscission. Deeper understanding of the mechanism underlying abscission can help in regulation of fruit production. Hence further molecular research on abscission could help to elucidate new molecular markers for improved genetic breeding programmes.

Table 2.1 Physiological and biochemical characters of healthy and dropped Kinnow mandarinfruit pedicel

Characters	**Healthy Pedicel**	**Dropped Pedicel**
Total soluble sugars (mg/g DW)	0.937	0.827
Total starch (mg/g DW)	1.87	0.920
Total soluble proteins (mg/g DW)	12.34	13.97
Free amino acids (mg/g DW)	10.63	11.33
Protease activity(µg proteins degraded/g FW/min)	5.03	1.870
Invertase activity (µmoles sucrose hydrolyzed/g FW/min)	5.03	4.690
Cellulase activity (µg D-glucose released/g FW/min)	4.06	3.690
Polygalacturonase activity (µg D-galacturonic acid released/g FW/min)	3.72	3.550

Table 2.2 Endogenous growth hormones in healthy and dropped Kinnow mandarin fruit pedicel

HPLC analysis: Plant growth regulators in Kinnow	Healthy Pedicel	Dropped Pedicel
SA (µg/g FW pedicel)	0.514	0.429
IAA (µg/g FW pedicel)	1.020	0.521
GA (µg/g FW pedicel)	0.837	0.522

Table 2.3 Anatomical characters of healthy and dropped pedicel of Kinnow mandarin fruits

Anatomical characters	Healthy Pedicel	Dropped Pedicel
Periderm width (µm)	103.24	98.62
Phloem fibre width (µm)	400.77	263.02
Xylem width (µm)	1129.09	670.65
Pith diameter (µm)	1187.25	1346.56

Figure 2.1: Periodic fruit drop in Kinnow mandarin

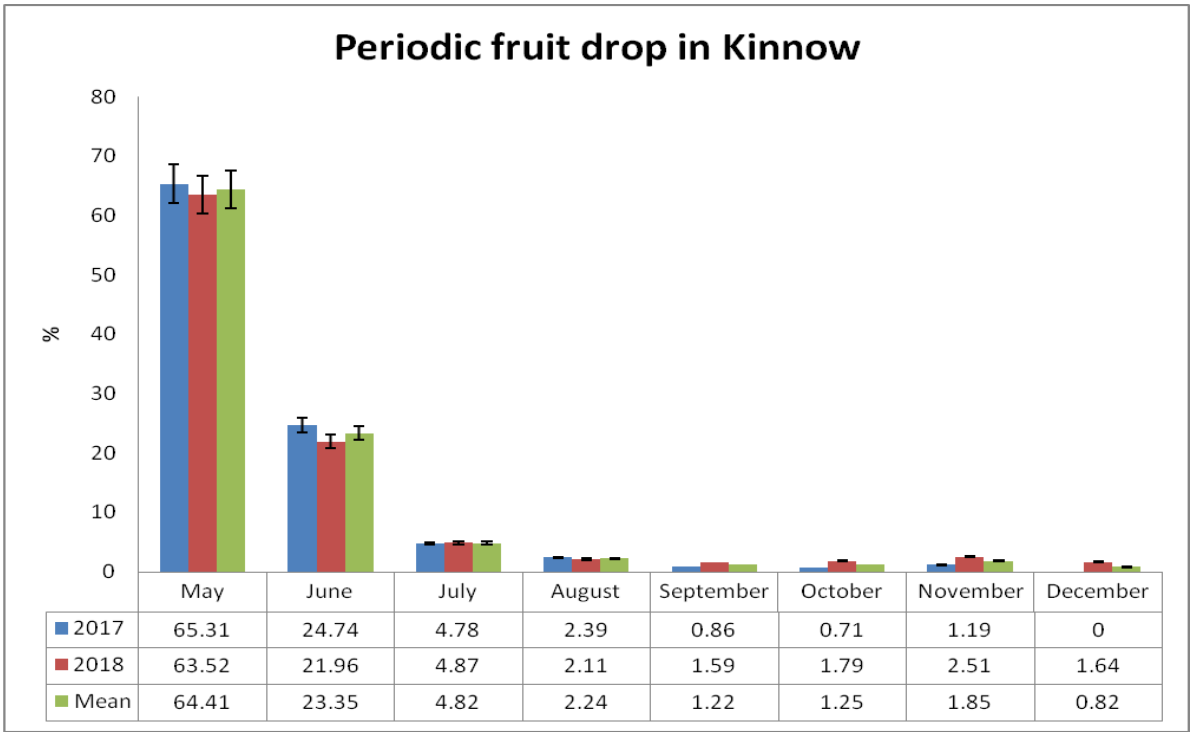
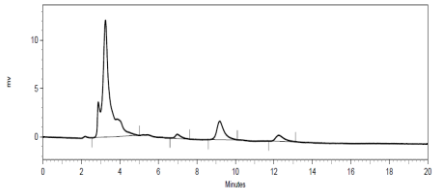
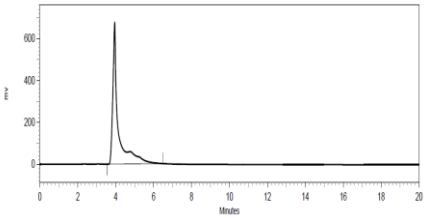
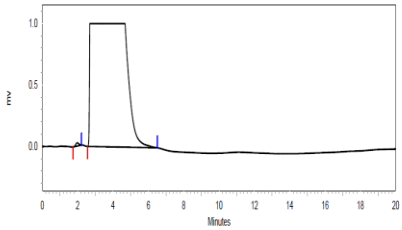
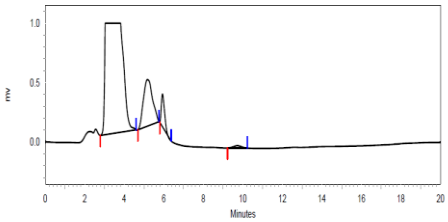
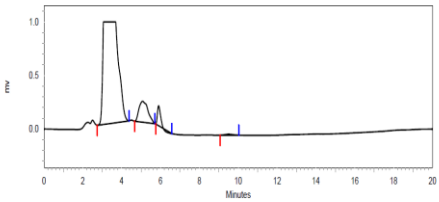
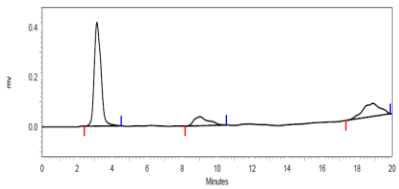


Figure 2.2: Graphical representation of Plant Growth Regulators in healthy and dropped pedicels of Kinnow mandarin fruits

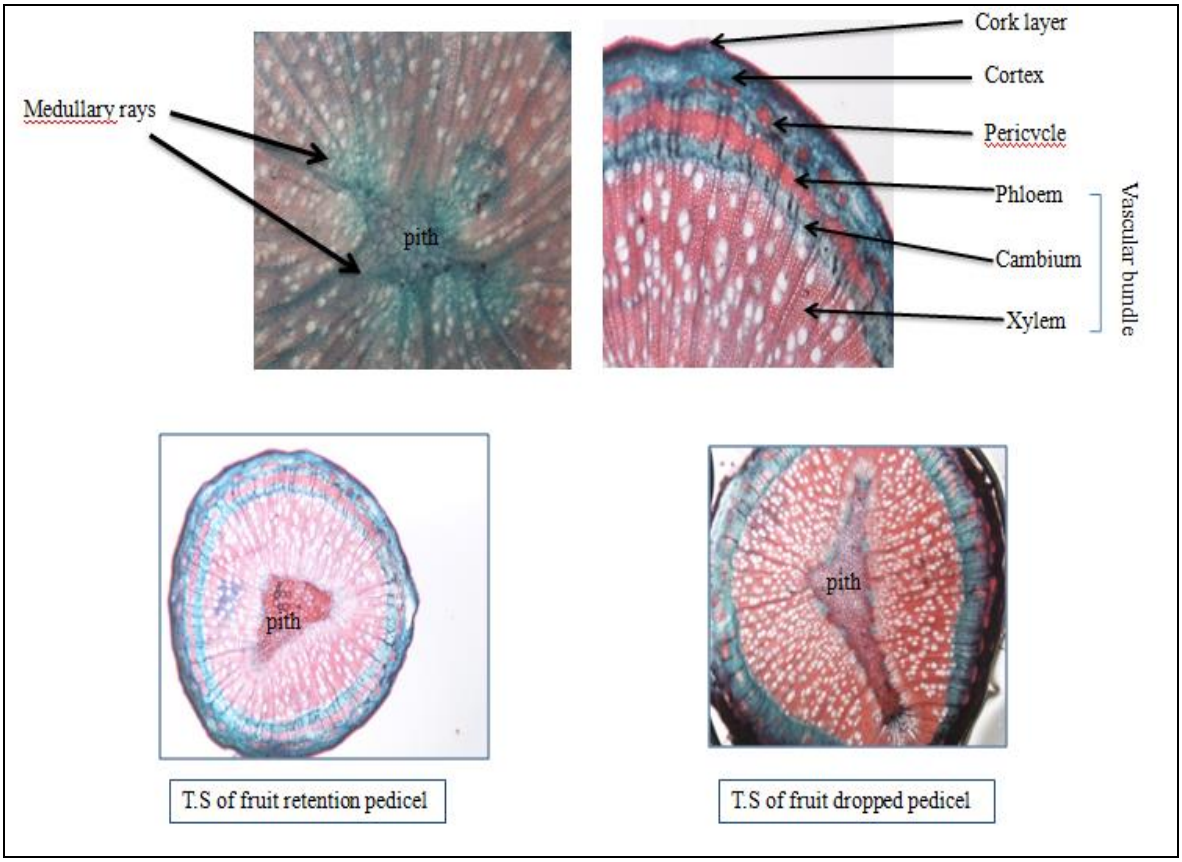
Healthy pedicel	Dropped pedicel
 SA	 SA
 IAA	 IAA
 GA	 GA

SA : Salicylic Acid

IAA : Indole Acetic Acid

GA : Gibberellic Acid

Plate 2.1: Anatomical characteristics of pedicel of Kinnow mandarin fruits



ANNEXURE B

PUBLICATIONS (Submitted/Accepted/Published)

PUBLISHED

1. Sidhu B, Nirmaljit Kaur, Rattanpal HS, Chawla N and Grewal IS (2017) Comparison of fruit set, fruit drop and quality attributes of Kinnow mandarin trees of different bearing age. *Green Farming* **8**(4): 895-900.

SUBMITTED

1. Nirmaljit Kaur, Ramandeep Kaur, HS Rattanpal and Anil Kumar (2018). Effect of plant growth substances on fruit drop, yield and quality of Kinnow mandarin. Submitted to *Indian Journal of Horticulture*..

ABSTRACTS PUBLISHED IN CONFERENCES/SYMPOSIA

1. RamandeepKaur, Nirmaljit Kaur, H S Rattanpal (2017). Performance of Kinnow mandarin in response to foliar application of plant growth regulators and nutrients. *8th Indian Youth Science Congress* held at University of Mumbai, Maharashtra. 16-18 Feb, 2017. Page no. 28
2. Nirmaljit Kaur, Ramandeep Kaur and HS Rattanpal (2018). Influence of Exogenous plant growth regulators and micronutrients on quality and quantity of Kinnow mandarin. 21st Punjab Science Congress held at Punjab Agricultural University, Ludhiana. 6-8 Feb, 2018. Abstract no. AP-135, P no. 89.
3. Ramandeep Kaur and Nirmaljit Kaur (2019)**.** Effect of plant growth regulators and macro and micronutrients influences NPK content content of Kinnow mandarin (*Citrus reticulata blanco*). Golden Jubilee National Symposium on Current Interventions to Plants and Microbes for Environmental and Agricultural Sustainability held at Guru Nanak Dev University, Amritsar. 6-8 March, 2019. Abstract no. PT069, Page no. 68.
4. Shabnam, Nirmaljit Kaur and H S Rattanpal (2019) Anatomical and physico-chemical characterization of mandarin (*Citrus reticulata* Blanco.) genotypes. *Indian Science Congress*, Punjab Agricultural University, Ludhiana (Punjab), 27 March 2019. (**Appreciation Award**)