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Effect of growth retardants on intermittent irrigation during water deficit stress period in *Mrig* (Monsoon flowering) crop of Nagpur mandarin (*Citrus reticulata* Blanco)

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Abstract

A field investigation was carried out at the experimental orchard of Nagpur mandarin (*Citrus reticulata* Blanco) at CCRI, Nagpur in block no. 44 during 2015. Breaking the seasonality of Nagpur mandarin for commercial production would not only substitute the import of fresh fruits, but also provide the incentive prices to the Nagpur mandarin growers. Withholding water, root exposure and root pruning are the common practices adopted to regulate flowering. In the recent years, chlormequat chloride and paclobutrazol, growth retardants have been used with considerable success to induce early flowering for off-season production in several fruit crops. Citrus trees are exposed to water deficit stress by withholding water for about 30-45 days before expected flowering. Many times this stress is untimely broken due to climate change related untimely rains resulting in to sparse or no flowering due loss of flowering stimulus due to untimely rains. Therefore, to assess the effectiveness of two plant growth regulators chlormequat chloride and paclobutrazol these studies were conducted. Flood irrigation was given during the stress period in second week of May and following treatments were applied to reimpose the stress broken by intermittent irrigation. Six years old trees of Nagpur mandarin were used as experimental plants. The trees were uniform in age and size and trained to single stem. The trees were grown under drip irrigation following common cultural practices during the study period. The experiment was laid out in randomized block design with seven treatments replicated four times. Four trees were used for each replication. The treatments were imposed four months before flowering (June). The treatments tried were chlormequat chloride 2000, 3000 and 4000 ppm as foliar spray, paclobutrazol 6 g, 9 g and 12 g as soil application along with control. Observations on soil moisture content (%) at weekly interval, leaf water potential (Ψ_L) at weekly interval, orchard microclimate: temperature, humidity and rainfall, were recorded. Paclobutrazol 12g showed the peak water stress in terms of leaf water potential (Ψ_L) which ranged from (-3.00 MPa to -3.17 MPa) till mid June and there was no flowering in June-July due to the high temperature (41.28°C), low rainfall (6 mm) and low humidity (40.71 %) during this period. The conditioning of microclimate in the orchard with sprinklers and irrigation can help in exploiting the flowering potential attained through growth retardant treatments.

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Introduction

Citrus is an important genus of the family *Rutaceae* in the plant kingdom. It occupies a place of considerable importance in the fruit economy of the country and comprises the third largest fruit industry. The most important commercial citrus cultivars in India are the mandarins, followed by Sweet orange and acid limes. Nagpur mandarin (*Citrus reticulata* Blanco) is considered to be one of the most important cultivated species among citrus and is being commercially grown in Central India; this crop occupies the first position among the citrus in India with respect to area and production. Nagpur mandarin is the principal citrus fruit grown commercially in Central India covering an area of 0.135 million ha with an estimated production of 7.4 million tonnes and productivity 5.5 MT/HA. (NHB 2014). Flowering is an important step in fruit production. The price of fruit depends on market demand, so therefore, induction of flowering at certain times is necessary. The flowers of Nagpur mandarin normally thrive in a year *i.e.*, *Ambia* bahar (Jan-Feb), *Mrig* bahar (June-July) and *Hasta* bahar (Sep-Oct). Among all these *Mrig* crop which comes to harvest during summer (March) gets highest remunerative price. Breaking the seasonality of Nagpur mandarin for commercial production would not only substitute the import of fresh fruits, but also provide the incentive prices to the Nagpur mandarin growers. Withholding water, root exposure and root pruning are the common practices adopted to regulate flowering. In the recent years, chlormequat chloride and paclobutrazol, growth retardants have been used with considerable success to induce early

flowering for off-season production in several fruit crops. Therefore, the present study was undertaken to determine the effectiveness of growth retardants such as chlormequat chloride and paclobutrazol of application in inducing flowering for off-season production of Nagpur mandarin under Central India conditions.

Flower expression in *Mrig* crop is more dependent on the prevailing weather conditions as described above in the absence of which there is risk of total crop failure leading to uncertainties in taking sustainable returns from the orchard. Many times, it happens that there are pre-monsoon rains or untimely rains of significant magnitude to the extent of 20-30 mm or more due to cyclonic disturbances in the Arabian Sea or Bay of Bengal which result in to untimely breakage of water deficit stress followed by normal summer temperatures with low humidity. Such plants fail to initiate flowering in June-July. Therefore, to offset the ill effects of these untimely rains it is necessary to reimpose the physiological (abiotic) stress with growth retardant (anti-gibberellin) induced stress. With this concept in mind this study was conducted by involving intermittent irrigation as substitute for untimely rains and use of growth retardants paclobutrazol and chlormequat chloride.

Paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol (Trade name - Luster)] is one of growth retardant which inhibits kaurene oxidase and thus blocks the oxidative reactions from ent-kaurene to entkaurenoic acid in the pathway leading to gibberellic acid. The mode of action of PBZ

has been associated with a decrease in transpiration, plant height, biomass and leaf area and increase in stomatal resistance. In case of induction of organ formation, there still have little attention. Soil application of paclobutrazol enhanced subsequent flowering but decreased new shoot sprouting and growth. The PBZ response in flowering induction under non inductive conditions, such as the occurrence of drought stress in period before flowering, can be variable due to factors pertaining to concentrations used, the absorption and the plant developmental stage at time of product application (Goldschmidt et al. 1998). Chlormequat chloride [(2-chloroethyltrimethylammonium chloride) (Trade name-Lihocin)] is also one of the retardants of plant Growth. It processes against gibberellic acid inside the plant when absorbed by organs (root, stem and leaves). The mode of action of CCC has been associated with it is absorbed by the leaves and acts on developing stem, shortening and thickening of internodes by inhibiting the cell elongations, restrain the elongation of cells, but have no influence on cell division; Restrain the growth of stem and leaves without affecting the development of sexual organs. It can make the plant short but strong with gathered trunk; Make the leaves darker and thicker and increase the ability to resist collapse, drought, cold and alkaline. The reasons for the irregular flowering behaviour are not yet fully understood and, therefore, attempts were made to study the problem of irregular flowering from several angles. The studies conducted at the National Research Centre for Citrus on the use of growth

regulators like Chlormequat chloride up to 4000 ppm (Dass et al. 1991)

Materials and Methods

This study was conducted at the Institute's experimental farm in Nagpur mandarin block no. 44. The experiment was laid out in randomized block design with seven treatments replicated four times. Four trees were used for each replication. The treatments were imposed four months before flowering (June). A total of eight years old 112 trees of Nagpur mandarin budded on rough lemon with 6x6 m spacing with similar vigour were selected to investigate the effect of chlormequat chloride (CCC) and paclobutrazol (PBZ) on monsoon flowering of Nagpur mandarin. The treatments tried were T1- Chlormequat chloride 2000 ppm as foliar spray, T2- Chlormequat chloride 3000 ppm as foliar spray, T3- Chlormequat chloride 4000 ppm as foliar spray, T4 - Paclobutrazol 6g as soil application, T5- Paclobutrazol 9g as soil application, T6- Paclobutrazol 12g as soil application, T7- Control. Six years old trees of Nagpur mandarin were used as experimental plants. The trees were uniform in age and size and trained to single stem. The trees were grown under drip irrigation following common cultural practices during the study period. These trees had set fruits by the end of February 2015 (spring flowering crop, *Ambia bahar*) which were defruited one month after fruit set in the first week of April when the fruits were of marble size (30 to 50 mm diameter).

Soil moisture stress was imposed from 16.5.2015 and intermittent irrigation @ 90 litres/plant to all experimental plants along with foliar application of chlormequat chloride

(2000, 3000 and 4000 ppm) and soil application of paclobutrazol (6, 9 and 12 g/plant) was given on 21.5.2015. Second intermittent irrigation was given on 30.5.2015 @90 litres/ plant. Leaf sampling for hormone analysis was done before and after the imposition of water stress and growth retardants on 15th May and 15th June, 2015

respectively. Observations of soil moisture content at weekly interval, Leaf water potential (LWP), Soil moisture content (%) during at weekly interval, till July 2015. Weather data was recorded in May-July 2015 (Table 1). Orchard microclimate: temperature, humidity and rainfall of each treatment were recorded.

Table 1. Weather data at CCRI and IMD, Nagpur during experimental period-2015

Experimental site at ICAR-CCRI						Nagpur airport (IMD)			
MW	Date	Temperature (°C)		RH (%)	Rainfall (mm)	Temperature (°C)		RH (%)	Rainfall (mm)
		Max.	Min.			Max.	Min.		
21	21-27 May	44.91	27.89	54.79	0	39.66	25.01	34.14	0.00
22	28 - 3 June	43.40	26.10	62.42	0	44.77	27.53	44.57	2.00
23	4 -10 June	34.17	26.08	58.00	0	29.34	19.34	40.71	6.00
24	11-17 June	37.36	25.28	65.9	47.3	32.40	22.23	67.71	146.00
25	18-24 June	32.43	23.81	76.00	113	27.53	20.27	77.86	241.00
26	25 June - 1 July	33.53	23.63	61.75	8.5	34.77	24.71	81.29	10.00
27	2 - 8 July	33.48	23.52	61.75	13.4	35.13	24.80	69.43	2.00
28	9 – 15 July	33.96	24.32	71.3	0	29.29	21.57	69.86	9.00
29	16 – 22 July	32.08	24.16	70.4	0	22.99	18.10	60.57	5.00
30	23 – 29 July	30.96	23.66	76.25	69.2	26.70	20.03	75.43	34.00

Chlormequat chloride (50% SL)

Chlormequat chloride (50%) (2-chloroethyltrimethylammonium chloride), a plant growth retardant (an anti-gibberellin compound) locally available in the market was used as a source of CCC.

Paclobutrazol (2RS, 3RS)-1-(4-

chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol) a plant growth retardant (an anti-gibberellin compound) locally available in the market was used as a source of paclobutrazol.

Paclobutrazol (28% W/W SC 30% W/V)

Table 2. Details of treatments combinations and application methods

Treatments	Plant growth Retardants	Concentration	Quantity used of the commercially available formulation per plant	Application method
T ₁	Chlormequat chloride	2000 ppm	4ml	Foliar spray
T ₂	Chlormequat chloride	3000 ppm	6 ml	Foliar spray
T ₃	Chlormequat chloride	4000 ppm	8 ml	Foliar spray
T ₄	Paclobutrazol	6 g	21.43 g	Soil application

T ₅	Paclobutrazol	9 g	32.14 g	Soil application
T ₆	Paclobutrazol	12 g	42.85 g	Soil application
T ₇	Control	-	-	-

Leaf water potential

The leaf water potential (LWP) was measured using a pressure chamber (PSYPRO Water Potential System., WESCOR, Logan, USA). Two fully expanded leaves at the fourth position around the canopy of each experimental tree were sampled. LWP was measured during at weekly interval of experiment till experiment completion.

Soil moisture content

Soil moisture was determined using a gravimetric method and soil samples were taken on the same dates as the LWP measurements at weekly interval. Clean and dry the weighing tin+lid and weigh (W1). Select a representative quantity of moist soil in the amount specified by a test. Place the sample in the weighing tin+lid, the tin and contents (W2). place the tin with contents and lid in the oven and dry to constant weight between 105 °C and 110 °C for 24 hr. Remove the tin with contents from the oven, and place the whole in the desiccator to cool. Weigh the tin+lid and contents to (W3).

Calculate the moisture content of the soil as a percentage of the dry soil weight.

$$MC\% = \frac{W2-W3}{W3-W1} \times 100$$

Where:

W1 = Weight of tin (g)

W2 = Weight of moist soil + tin (g)

W3 = Weight of dried soil + tin (g)

Results and Discussion

Soil physical properties and nutrient status

The soil physico-chemical properties of the experimental plants were determined for various important parameters as detailed in table 3. The soil physical properties were estimated as pH (7.88 to 8.28), electrical conductivity (0.230 dS/m to 0.324 dS/m), organic carbon (0.59 % to 1.43%) and CaCO₃ (1.54 to 4.03). The soil available nutrients were analysed as nitrogen (from 137.98 kg/ha to 200.7 kg/ha), phosphorus (14.49 kg/ha to 79.00 Kg/ha) and potassium (519.4 kg/ha to 991.1 kg/ha) in various treatments in experimental orchard. The status of total soil micronutrients varied as Fe (9.56 ppm to 17.84 ppm), Mn (54.37 ppm to 74.56 ppm), Cu (4.40 ppm to 8.75 ppm) and Zn (0.84 to 1.74 ppm).

Table 3. Leaf water potential (MPa) and soil moisture content (%) during experimentation

Date	27-05-2015		03-06-2015		10-06-2015		17-06-2015	
Parameters	MC (%)	LWP (MPa)	MC (%)	LWP (MPa)	MC (%)	LWP (MPa)	MC (%)	LWP (MPa)
CCC 2000ppm	6.99	-3.19	7.17	-3.01	7.07	-3.00	10.12	-2.81
CCC 3000ppm	7.18	-3.01	6.75	-3.17	7.00	-3.00	10.49	-2.84
CCC 4000ppm	7.02	-3.00	6.30	-3.13	7.42	-3.04	9.01	-2.80
PBZ 6g/tree	7.45	-3.04	6.53	-3.15	7.65	-3.06	10.54	-2.85
PBZ 9g/tree	6.43	-3.14	6.11	-3.11	7.76	-3.07	9.04	-2.80
PBZ 12g/tree	6.39	-3.13	6.40	-3.14	7.54	-3.05	9.95	-2.89
Control	7.41	-3.04	6.89	-3.18	7.77	-3.07	10.12	-2.81
CD at 5%	0.23	0.12	0.22	0.11	0.25	0.10	0.41	0.10

Table 3. Cont....

Date	24-06-2015		01-07-15		08-07-2015		15-07-15	
Parameters	MC (%)	LWP (MPa)	MC (%)	LWP (MPa)	MC (%)	LWP (MPa)	MC (%)	LWP (MPa)
CCC 2000ppm	14.96	-2.49	9.37	-2.83	7.81	-3.08	12.23	-2.62
CCC 3000ppm	14.83	-2.48	9.40	-2.84	8.62	-2.96	12.57	-2.65
CCC 4000ppm	15.59	-2.45	9.96	-2.89	8.57	-2.95	12.24	-2.62
PBZ 6g/tree	14.72	-2.47	9.40	-2.84	8.33	-2.93	10.90	-2.89
PBZ 9g/tree	13.78	-2.57	8.98	-2.99	7.98	-3.09	10.03	-2.80
PBZ 12g/tree	13.04	-2.50	8.30	-2.93	7.34	-3.03	12.34	-2.63
Control	14.80	-2.48	8.66	-2.96	8.43	-2.94	13.79	-2.57
CD at 5%	0.32	0.10	0.38	0.10	0.29	0.13	0.40	0.11

Leaf nutrient status of experimental plants before imposition of growth retardant treatment

The leaf nutrient status of the experimental plants was determined before imposition of treatments for various important parameters as detailed in table 4. The leaf available nutrients were analysed as nitrogen (0.685 % to 2.155

(%), phosphorus (0.016 % to 0.054 %) and potassium (0.73 % to 1.75 %) in various treatments in experimental orchard. The status of total leaf micronutrients varied as Fe (221.65 ppm to 690.55 ppm) Mn (25.01 ppm to 55.45 ppm) Cu (6.6 ppm to 13.65 ppm) and Zn (36.3 to 41.1 ppm).

Table 4. Soil physical properties and nutrient status in experimental block

Treatments	pH	EC	OC (%)	CaCO ₃	N (kg/ha)	P (kg/ha)	K (kg/ha)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)
T ₁ - CCC 2000ppm	8.28	0.280	1.40	4.03	163.07	71.53	519.4	9.56	54.37	4.65	1.747
T ₂ - CCC 3000ppm	8.14	0.303	1.43	3.32	150.53	79.00	754.6	14.43	68.87	6.09	2.11
T ₃ - CCC 4000ppm	7.88	0.324	1.23	3.91	200.70	67.80	975.6	8.59	74.56	5.79	1.68
T ₄ - PBZ 6 g/tree	7.93	0.243	0.606	1.54	181.88	26.66	991.1	13.55	57.187	4.59	0.84
T ₅ - PBZ 9 g/tree	8.13	0.262	0.686	3.43	163.07	14.49	522.2	15.47	68.93	4.4	1.17
T ₆ - PBZ 12 g/tree	7.96	0.291	0.829	3.32	181.88	33.60	775.6	17.84	69.93	5.51	1.56
T ₇ -Control	7.89	0.230	0.59	3.08	137.98	67.87	931.0	15.36	74.125	8.75	1.33
CD at 5 %	0.203	0.013	0.063	0.125	2.56	7.75	4.86	0.213	1.039	0.098	0.120

Leaf nutrient status of experimental plants after imposition of growth retardant treatment

The leaf nutrient status of the experimental plants was determined after imposition of treatments for various important parameters as detailed in table 4. The leaf available nutrients were analysed as nitrogen (1.2 % to 2.28 %), phosphorus (0.0158 % to 0.0104 %) and

potassium (1.315 % to 1.725 %). The status of total leaf micronutrients varied as Fe (311.6 ppm to 702.75 ppm), Mn (29.25 ppm to 44.1 ppm), Cu (14.8 ppm to 33.8 ppm), and Zn (33.45 to 38.4 ppm) in various treatments in experimental orchard. The quantum of rains received in June was 160 mm (normal 163 mm) with maximum temperatures of around 32 to 37 °C, minimum temperatures of around

23 to 26 °C and RH of 58-76 % in the month of June whereas in the month of July only 90 mm rains were received as against a normal of 304 mm. Only vegetative growth flushes were observed and no flowering was observed till the end of July, 2015. This could be due to continuous dry spell with scanty rains in the month of July.

Flower expression in *Mrig* crop is more dependent on the prevailing weather conditions as described above in the absence of which there is risk of total crop failure leading to uncertainties in taking sustainable returns from the orchard. Many times, it happens that there are pre-monsoon rains or untimely rains of significant magnitude to the extent of 20-30 mm or more due to cyclonic disturbances in the Arabian sea or Bay of Bengal which result in to untimely breakage of water deficit stress followed by normal summer temperatures with low humidity. Such plants fail to initiate flowering in June-July. Therefore, to offset the ill effects of these untimely rains it is necessary to reimpose the physiological (abiotic) stress with growth retardant (anti-gibberellin) induced stress. With this concept in mind this study entitled “Effect of growth retardants on intermittent irrigation during water deficit stress period in *Mrig* (Monsoon flowering) crop of Nagpur mandarin” was initiated at the Institute’s experimental farm in Nagpur mandarin block no. 44. These trees had set fruits by the end of February 2015 (spring flowering crop, *Ambia bahar*) which were defruited one month after fruit set in the first week of April when the fruits were of marble size (30 to 50 mm diameter). Soil moisture stress, intermittent irrigation with imposed

treatment at this period. The quantum of rains received in June was 160 mm (normal 163 mm) with maximum temperatures of around 32 to 37 °C, minimum temperatures of around 23 to 26 °C and RH of 58-76 % in the month of June whereas in the month of July only 90 mm rains were received as against a normal of 304 mm. Only vegetative growth flushes were observed and no flowering was observed till the end of July, 2015. This could be due to continuous dry spell with scanty rains in the month of July and due to less soil available phosphorus (0.55 kg/ha to 3.3 kg/ha) as against a normal of (13 to 22 kg/ha) and sub optimal leaf phosphorus status (0.0158 % to 0.0104 %) as against a normal of (0.07 to 0.09 %).

Conclusion

The leaf GA₃ levels in all the growth retardant treatments decreased significantly one month after imposition of CCC and paclobutrazol. It was least in paclobutrazol 12g/tree (185.2 ng/g) and CCC 4000 ppm (157.9 ng/g) whereas in control trees it remained about the same (351.70 ng/g) as before application of treatments (341.92 ng/g). Only vegetative growth flushes were observed and no flowering was observed till the end of July month. This could be due to continuous dry spell with scanty rains in the month of July (90 mm as against a normal of 304 mm) and less soil available and leaf phosphorus status.

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