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Effect of post-harvest ethylene treatment on degreening of acid lime Cv. Balaji

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Abstract
The objective of the present investigation was to study the effect of post-harvest ethylene treatment on degreening of Acid lime Cv. Balaji. Experiment was designated with two factorial completely randomized design. In the experiment mature green Acid lime fruits were subjected to degreening at four levels of post-harvest ethylene treatments *viz*., 5ppm, 10ppm, 15ppm and 20ppm at four levels of number of pulsings per 24 hrs. *viz*., 6 pulsings, 4 pulsings, 2 pulsings and 1 pulsing. The treated Acid lime fruits were stored for sixteen days at ambient conditions. Acid lime fruits picked randomly from treated lot and evaluated for DA meter reading and hedonic score. The investigation concluded that 15 ppm ethylene with 1, 2, 4 and 6 pulsings in 24 hours and 20 ppm ethylene with 1, 2, 4 and 6 pulsings in 24 hours can be recommended for Acid lime Cv. Balaji for uniform degreening.

Keywords: Degreening, DA meter reading, hedonic score, acid lime, Cv. balaji, ethylene

Introduction
Acid lime (*Citrus aurantifolia* S.) is a subtropical in its climacteric requirements and originated from sub- tropical South- east Asia, which belongs to family Rutaceae. India is the largest producer of acid lime in the world followed by USA, Spain and Israel. Consumers prefer bright colored citrus fruit and are willing to pay a premium for them. Green colored fruits are considered unripe and fetch lower prices. Hence the color of the rind is important for the aesthetic value and as such it is the most important factor determining marketability. In citrus internal edible portion of the fruit (pulp) usually reaches maturity while the external peel is still green, therefore, degreening practices were developed to accelerate fruit color change and render the fruit more acceptable for marketing (Ron, 2008) [(1)]. The development of chloroplast and chromoplast is influenced by endogenous growth regulators. Ethylene causes loss of chlorophyll and produces the minor changes in carotenoids (Grierson and Newhall 1960) [2]. Ethylene do not ripe the immature fruits completely in terms of skin and flesh colour development. It was found that the ethylene has threshold level between 5-15ppm, optimum degreening temperature of 25°C, relative humidity of above 80% which favors both chlorophyll destruction and carotenoid accumulation, (Ron, 2008) [1].

Materials and Methods
Acid lime (*Citrus aurantifolia* S.) Cv. Balaji fruits were collected from citrus orchard Nakrekal, Nalgonda district, Telangana State, India. Matured fruits (DA reading-2.02) are harvested manually and immediately transported to degreeening chamber and subjected to the ethylene treatments. Ethylene release canisters named Ripylene, manufactured by chemtron science laboratories, Mumbai were used in the research. 15 grams of ethylene gas was filled in a cylinder with adjustable gauge. This was approved by National Horticultural Board (NHB), Food Safety and Standards Authority of India (FSSAI) and Food and Drug Administration (FDA) as a gas for ripening. The low-cost ripening chamber consists of 40mm PUF (Polyurethane Foam) insulated panels with PPGI Sheets (Pre painted Galvanised Iron) lamination on either side, with flashings and swing Door - 300 x 600 mm with the capacity of 5 crates (100kg). The fruits of Acid lime (*Citrus aurantifolia* S.) Cv. Balaji were treated with 5 ppm, 10 ppm, 15 ppm and 20 ppm of ethylene. Ethylene gas as per the required concentration was released in to the low-cost ripening chamber. The treated Acid lime fruits were stored in a room openly in a well-ventilated room with free flow of air for sixteen days and evaluated at four days interval. The experiment was laid out in Factorial experiment of Completely Randomized Design (FCRD) with sixteen treatments with three replications and two factors (1) Ethylene doses and (2) Number of pulsings per 24 hrs. Mature green Acid lime fruits were subjected to degreening at four levels of post-harvest ethylene treatments *viz*., 5ppm, 10ppm, 15ppm and 20ppm at four levels of number of pulsings per 24 hrs. *viz*., 6 pulsings, 4 pulsings,

2 pulsings and 1 pulsing. The combination of 2 factors gives 16 treatments *viz.*, T_1- 5ppm @ 6pulsings in 24 hrs; T_2- 5ppm @ 4pulsings in 24 hrs; T_3- 5ppm @ 2pulsings in 24 hrs; T_4- 5ppm @ 1pulsing in 24 hrs; T_5- 10ppm @ 6pulsings in 24 hrs; T_6- 10ppm @ 4pulsings in 24 hrs; T_7- 10ppm @ 2pulsings in 24 hrs; T_8- 10ppm @ 1pulsing in 24 hrs; T_9- 15ppm @ 6pulsings in 24 hrs; T_{10}- 15ppm @ 4pulsings in 24 hrs; T_{11}- 15ppm @ 2pulsings in 24 hrs; T_{12}- 15ppm @ 1pulsing in 24 hrs; T_{13}- 20ppm @ 6pulsings in 24 hrs; T_{14}- 20ppm @ 4pulsings in 24 hrs; T_{15}- 20ppm @ 2pulsings in 24 hrs; T_{16}- 20ppm @ 1pulsing in 24 hrs. The treated Acid lime fruits were stored for sixteen days at ambient conditions. Acid lime fruits picked randomly from treated lot and evaluated for DA meter readings & hedonic score.

DA Meter Reading: The DA meter was developed by Prof. Costa's team from the university of Bologna. It is the instrument that, by means of its absorbency properties, allows measuring the chlorophyll content in the fruit. The DA index, measured with DA-meter, is useful at many stages in production and consumption cycle of the fruit. The DA is the index of the chlorophyll in the fruit and, as a consequence, of its ripeness state. This index decreases the value during ripening process of the fruit, until it reaches very low value when ripening is complete.

10 fruits from each replication were evaluated by DA meter at 4 days intervals (1st, 4th, 8th, 12th and 16th days). The fruits were exposed to DA meter and readings were recorded. A DA meter of each fruit was taken at three equidistant places on fruit body, stalk end, and distal end of the fruit by DA meter. The average of the three readings were recorded.

Hedonic score: Ten fruits randomly selected were used for studying the peel colour of citrus fruits, different colours were given a scale ranging from 1-5 as follows: Dark Green – 1, Light Green – 2, Yellowish Green – 3, Light Yellow – 4, Yellow – 5.

Results and Discussion

DA Reading: The data pertaining to DA reading of Acid lime Cv. Balaji at ambient temperature as influenced by ethylene concentrations and number of pulsings in tables (1 to 4) and illustrations (1 and 2). It is evident from the data that, the DA reading showed significantly decreased as the ripening advanced during storage at ambient conditions. DA meter measures the chlorophyll content in a fruit and, as a consequence, its state of ripeness. The index of absorbance difference (IAD) decreases in value during ripening by absorbency properties of the fruit, until it reaches very low value (0.00), when ripening was complete. Each kind of fruit and cultivar has specific DA values according to the different phases of maturation. (Ziosi *et al*., 2008; Noferini *et al*., 2008) [3, 4].

On 1st day (Table 1.) among the ethylene concentrations significantly maximum DA reading was observed with ethylene @ 5 ppm (1.96) followed by ethylene @10 ppm (1.89) while, minimum with 20 ppm (0.97). Among the number of pulsings significantly maximum DA reading was observed with 1 pulsing in 24 hrs @ 24 hrs interval (1.50) while, lowest with 6 pulsings in 24 hrs @ 4 hrs interval (1.40). On 4th day (Table 2.) among the ethylene concentrations significantly maximum DA reading was observed with ethylene @ 5 ppm (1.59) followed by ethylene @10 ppm (1.14) while, minimum with 20 ppm (0.85). Among the number of pulsings significantly maximum DA reading was observed with 1 pulsing in 24 hrs @ 24 hrs interval (1.24) followed by 2 pulsings in 24 hrs @ 12 hrs interval (1.18) while, minimum with 6 pulsings in 24 hrs @ 4 hrs interval (1.03). On 8th day (Table 3.) among the ethylene concentrations significantly maximum DA reading was observed with ethylene @ 5 ppm (1.40) followed by ethylene @10 ppm (1.00) while, minimum with 20 ppm (0.73mm). Among the number of pulsings significantly maximum DA reading was observed with 1 pulsing in 24 hrs @ 24 hrs interval (1.08) followed by 2 pulsings in 24 hrs @ 12 hrs interval (0.98) while, minimum with 6 pulsings in 24 hrs @ 4 hrs interval (0.90). Among the storage days 4th and 8th day interaction between ethylene concentration and number of pulsings were significantly differed. But, on 1st day interaction results were non-significant. The mean values recorded in DA reading of Acid lime Cv. Balaji fruits at 12th and 16th day during storage tabulated at Table 4 and 5 respectively. A similar decreasing trend in respect of DA reading was observed.

Table 1: DA Meter Reading of Acid lime Cv. Balaji as influenced by ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature on 1st day

Ethylene concentrations	Number of pulsings per 24 hrs.				
	6 pulsings	4 pulsings	2 pulsings	1 pulsing	Mean
Ethylene @ 5ppm	1.93	1.95	1.97	1.98	1.96^{A}
Ethylene @ 10ppm	1.84	1.88	1.91	1.92	1.89^{B}
Ethylene @ 15ppm	0.94	0.95	1.03	1.07	1.00^{C}
Ethylene @ 20ppm	0.91	0.92	1.01	1.04	0.97^{D}
Mean	1.40^{b}	1.43^{b}	1.48^{a}	1.50^{a}	
Factors	**CD (5%)**		**SEm ±**		
Ethylene concentrations (A)	0.04		0.01		
Number of pulsings (B)	0.04		0.01		
Factor A × B	N.S.		0.03		

Table 2: DA Meter Reading of Acid lime Cv. Balaji as influenced by ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature on 4th day

Ethylene concentrations	Number of pulsings per 24 hrs.				
	6 pulsings	4 pulsings	2 pulsings	1 pulsing	Mean
Ethylene @ 5ppm	1.44	1.56	1.65	1.72	1.59^{A}
Ethylene @ 10ppm	0.93	0.98	1.26	1.37	1.14^{B}
Ethylene @ 15ppm	0.91	0.92	0.96	0.97	0.94^{C}
Ethylene @ 20ppm	0.82	0.84	0.86	0.88	0.85^{D}
Mean	1.03^{c}	1.08^{c}	1.18^{b}	1.24^{a}	
Factors	**CD (5%)**		**SEm ±**		
Ethylene concentrations (A)	0.05		0.02		
Number of pulsings (B)	0.05		0.02		
Factor A × B	0.11		0.04		

Table 3: DA Meter Reading of Acid lime Cv. Balaji as influenced by ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature on 8th day

Ethylene concentrations	Number of pulsings per 24 hrs.				
	6 pulsings	4 pulsings	2 pulsings	1 pulsing	Mean
Ethylene @ 5ppm	1.28	1.32	1.38	1.58	1.40^{A}
Ethylene @ 10ppm	0.88	0.92	1.01	1.19	1.00^{B}
Ethylene @ 15ppm	0.74	0.75	0.78	0.81	0.78^{C}
Ethylene @ 20ppm	0.70	0.72	0.74	0.76	0.73^{D}
Mean	0.90^{c}	0.93^{c}	0.98^{b}	1.08^{a}	
Factors	**CD (5%)**		**SEm ±**		
Ethylene concentrations (A)	0.04		0.01		
Number of pulsings (B)	0.04		0.01		
Factor A × B	0.08		0.03		

Table 4: DA Meter Reading of Acid lime Cv. Balaji as influenced by ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature on 12th day

Ethylene concentrations	Number of pulsings per 24 hrs.			
	6 pulsings	4 pulsings	2 pulsings	1 pulsing
Ethylene @ 5ppm	-	-	-	-
Ethylene @ 10ppm	0.82	0.84	-	-
Ethylene @ 15ppm	0.70	0.72	0.76	0.78
Ethylene @ 20ppm	-	-	0.70	0.70

Table 5: DA Meter Reading and Hedonic Score of Acid lime Cv. Balaji as influenced by ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature on 16th day

Ethylene concentrations	Number of pulsings per 24 hrs.			
	6 pulsings	4 pulsings	2 pulsings	1 pulsing
Ethylene @ 5ppm	-	-	-	-
Ethylene @ 10ppm	-	-	-	-
Ethylene @ 15ppm	-	0.70	0.70	-
Ethylene @ 20ppm	-	-	-	-

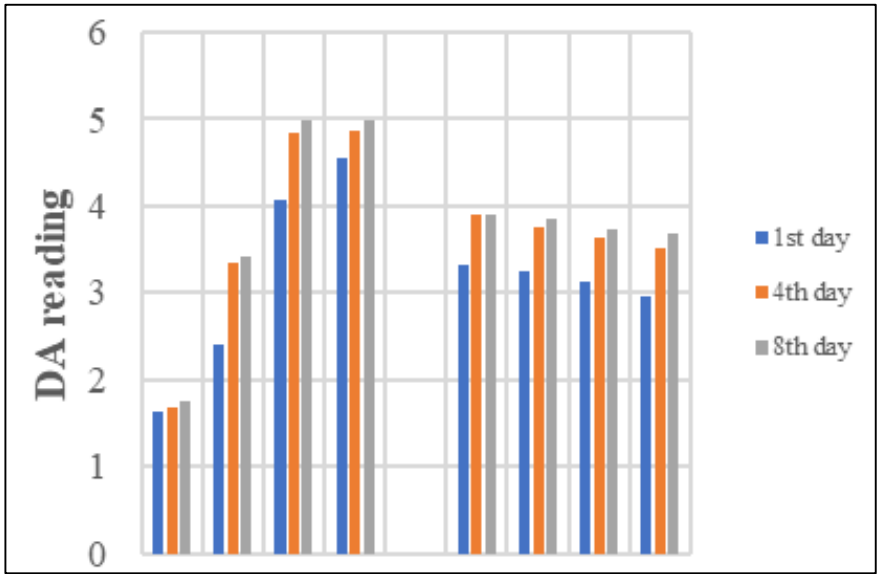

Fig 1: DA reading of Acid lime Cv. Balaji as influenced by different ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature.

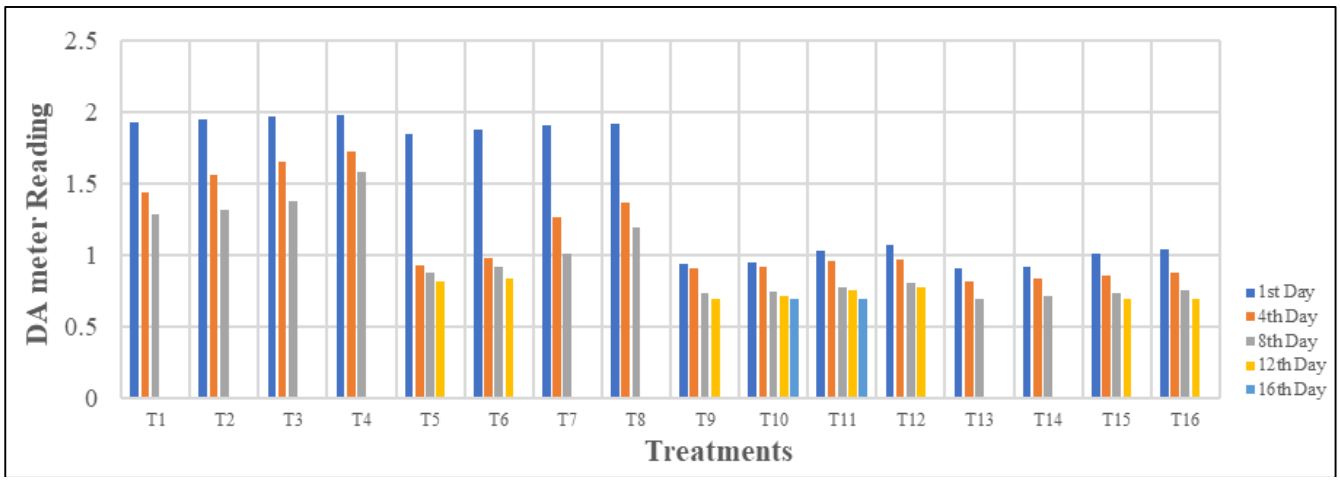

Fig 2: DA reading of Acid lime Cv. Balaji as influenced by interaction between ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature on 1st, 4th, 8th, 12th and 16th day.

Hedonic score

The data pertaining hedonic score of Acid lime Cv. Balaji at ambient temperature as influenced by ethylene concentrations and number of pulsings in tables (6 to 10) and illustrations (3 and 4). It is evident from the data that as the degreening advanced, hedonic score increased progressively at ambient conditions and significantly differed among the treatments. Present results were agreement with results of Ladaniya and Singh (2001) [5] degreening of citrus fruits continued after removing from degreening chamber and resulted in elimination of original treatment differences. It has been reported that once the colour development started with ethylene, it remains unaffected by the break in ethylene supply.

On 1st day (Table 6.) among the ethylene concentrations significantly maximum value recorded with ethylene @ 20ppm (4.55) followed by 15ppm (4.07) while, minimum with 5ppm (1.63). Among the number of pulsings maximum hedonic score was recorded with 6 pulsings in 24 hrs @ 4 hrs interval (3.33), followed by 4 pulsings in 24 hrs @ 6 hrs interval (3.24) while, minimum with 1 pulsing in 24 hrs @ 24 hrs interval (2.96). On 4th day (Table 7.) among the ethylene concentrations significantly maximum value recorded with ethylene @ 20ppm (4.87) followed by 10ppm (3.35). 15ppm (4.85) was at par with ethylene @ 20ppm (4.87) while, minimum with 5ppm (1.69). Among the number of pulsings maximum hedonic score was recorded with 6 pulsings in 24 hrs @ 4 hrs interval (3.89), followed by 4 pulsings in 24 hrs

@ 6 hrs interval (3.76) while, minimum with 1 pulsing in 24 hrs @ 24 hrs interval (3.52). On 8th day (Table 8.) among the ethylene concentrations significantly maximum value recorded with ethylene @ 20ppm and 15 ppm (4.99) followed by 10ppm (3.41) while, minimum with 5ppm (1.76). Among the number of pulsings maximum hedonic score was recorded with 6 pulsings in 24 hrs @ 4 hrs interval (3.90), followed by 2 pulsings in 24 hrs @ 12 hrs interval (3.73). 4 pulsings in 24 hrs @ 6 hrs interval (3.85) was at par with 6 pulsings in 24 hrs @ 4 hrs interval (3.90) while, minimum with 1 pulsing in 24 hrs @ 24 hrs interval (3.68). Among the storage days 1st, 4th and 8th day interaction between ethylene concentration and number of pulsings were significantly differed. The mean values recorded among the hedonic score reading of Acid lime Cv. Balaji fruits at 12th day and 16th day during storage tabulated at Table 9. and 10. respectively. A similar increasing trend in respect of hedonic score was observed.

Table 6: Hedonic Score of Acid lime Cv. Balaji as influenced by ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature on 1st day

Ethylene concentrations	Number of pulsings per 24 hrs.				
	6 pulsings	4 pulsings	2 pulsings	1 pulsing	Mean
Ethylene @ 5ppm	1.72	1.68	1.64	1.46	1.63^{A}
Ethylene @ 10ppm	2.56	2.44	2.32	2.27	2.40^{B}
Ethylene @ 15ppm	4.43	4.26	3.98	3.63	4.07^{C}
Ethylene @ 20ppm	4.62	4.58	4.54	4.46	4.55^{D}
Mean	3.33^{a}	3.24^{b}	3.12^{c}	2.96^{d}	
Factors	**CD (5%)**		**SEm ±**		
Ethylene concentrations (A)	0.07		0.02		
Number of pulsings (B)	0.07		0.02		
Factor A × B	0.14		0.05		

Table 7: Hedonic Score of Acid lime Cv. Balaji as influenced by ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature on 4th day

Ethylene concentrations	Number of pulsings per 24 hrs.				
	6 pulsings	4 pulsings	2 pulsings	1 pulsing	Mean
Ethylene @ 5ppm	1.92	1.81	1.71	1.61	1.76^{C}
Ethylene @ 10ppm	3.71	3.61	3.21	3.11	3.41^{B}
Ethylene @ 15ppm	4.99	4.99	4.99	4.99	4.99^{A}
Ethylene @ 20ppm	4.99	4.99	4.99	4.99	4.99^{A}
Mean	3.90^{a}	3.85^{a}	3.73^{b}	3.68^{b}	
Factors	**CD (5%)**		**SEm ±**		
Ethylene concentrations (A)	0.06		0.02		
Number of pulsings (B)	0.06		0.02		
Factor A × B	0.12		0.04		

Table 8: Hedonic Score of Acid lime Cv. Balaji as influenced by ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature on 8th day

Ethylene concentrations	Number of pulsings per 24 hrs.				
	6 pulsings	4 pulsings	2 pulsings	1 pulsing	Mean
Ethylene @ 5ppm	1.88	1.72	1.65	1.53	1.69^{C}
Ethylene @ 10ppm	3.72	3.32	3.24	3.13	3.35^{B}
Ethylene @ 15ppm	4.96	4.90	4.82	4.70	4.85^{A}
Ethylene @ 20ppm	4.98	4.92	4.86	4.73	4.87^{A}
Mean	3.89^{a}	3.76^{b}	3.64^{c}	3.52^{d}	
Factors	**CD (5%)**		**SEm ±**		
Ethylene concentrations (A)	0.06		0.02		
Number of pulsings (B)	0.06		0.02		
Factor A × B	0.11		0.04		

Table 9: Hedonic Score of Acid lime Cv. Balaji as influenced by ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature on 12th day

Ethylene concentrations	Number of pulsings per 24 hrs.			
	6 pulsings	4 pulsings	2 pulsings	1 pulsing
Ethylene @ 5ppm	-	-	-	-
Ethylene @ 10ppm	3.20	3.40	-	-
Ethylene @ 15ppm	4.99	4.99	4.99	4.99
Ethylene @ 20ppm	-	-	4.99	4.99

Table 10: Hedonic Score of Acid lime Cv. Balaji as influenced by ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature on 16th day

Ethylene concentrations	Number of pulsings per 24 hrs.			
	6 pulsings	4 pulsings	2 pulsings	1 pulsing
Ethylene @ 5ppm	-	-	-	-
Ethylene @ 10ppm	-	-	-	-
Ethylene @ 15ppm	-	4.99	4.99	-
Ethylene @ 20ppm	-	-	-	-

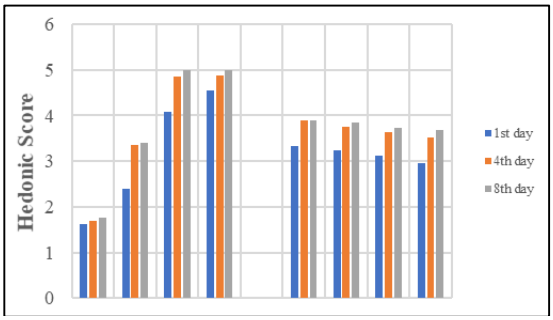

Fig 3: Hedonic Score of Acid lime Cv. Balaji as influenced by different ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature

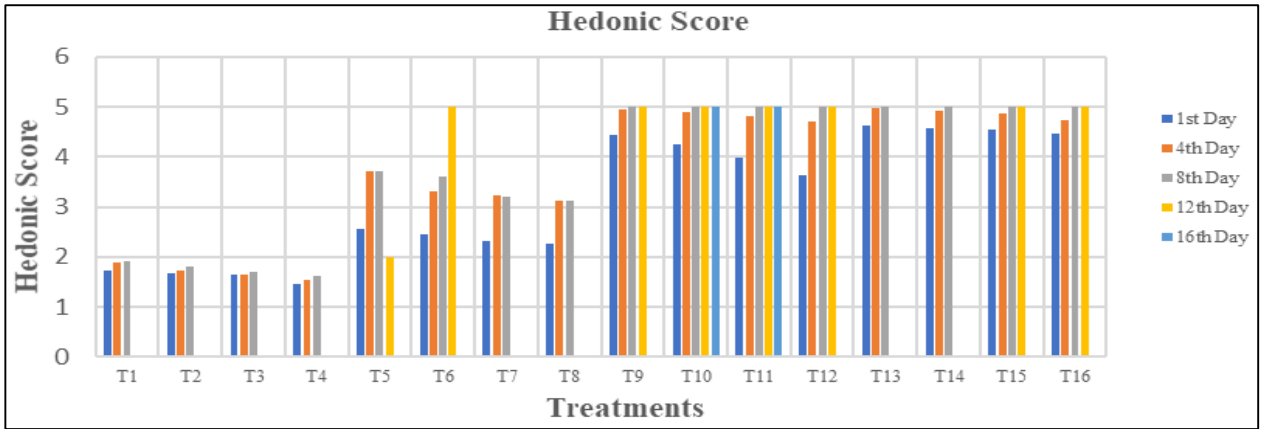

Fig 4: Hedonic Score of Acid lime Cv. Balaji as influenced by interaction between ethylene concentrations and number of pulsings per 24 hrs. at ambient temperature on 1st, 4th, 8th, 12th and 16th day.

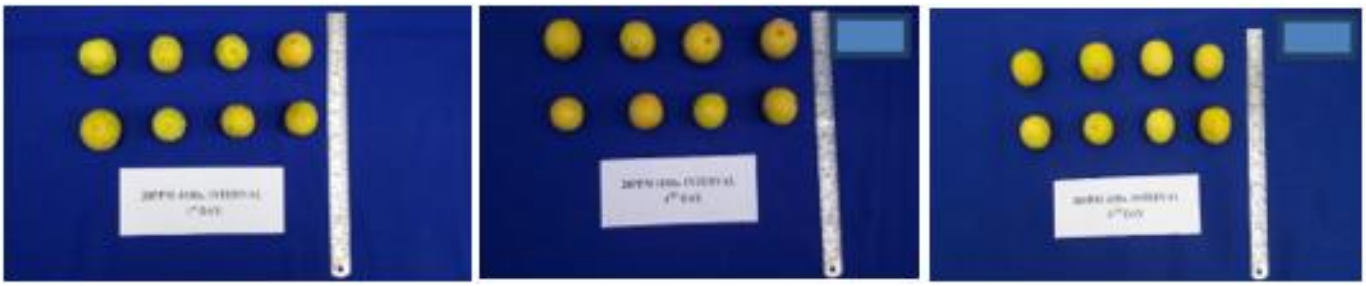

Plate 1: Acid Lime Cv. Balaji subjected to 20ppm ethylene with 6 pulsings in 24 hrs @ 4 hrs. interval on 1st, 4th and 8th day at ambient temperature

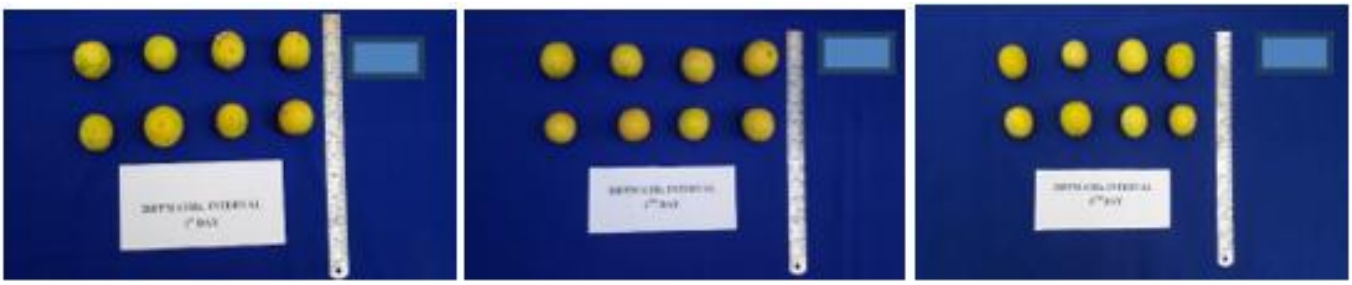

Plate 2: Acid Lime Cv. Balaji subjected to 20ppm ethylene with 4 pulsings in 24 hrs @ 6 hrs. interval on 1st, 4th and 8th day at ambient temperature

Conclusion

The study revealed that significant difference was noticed in Acid lime Cv. Balaji for DA reading and Hedonic Score. Among different ethylene concentrations (5ppm, 10 ppm, 15ppm and 20ppm) there was decreasing trend in DA meter readings and an increasing trend during ripening in hedonic score. Ethylene @ 20 ppm and 15ppm showed better results for both the parameters. 20 ppm ethylene with 6 pulsings in 24 hours @ 4 hrs. interval followed by 20 ppm ethylene with 4 pulsings in 24 hours @ 6 hrs. interval can be recommended for Acid lime Cv. Balaji for uniform degreening in normal room temperature.

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