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Effect of pre-harvest sprays on biochemical parameters in red pulp tamarind variety Anantha Rudhira

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Abstract
Tamarind is a tropical tree (*Tamarindus indica* L.) known for its pod-like fruits, which contain a sour pulp. This pulp, often used in cooking, has a acidic sweet and tangy taste. The research was conducted at Dr. Y.S.R Horticultural University-Horticulture Research Station (AICRP on Arid Zone Fruits), situated in Rekulakunta, Anantapuramu district, Andhra Pradesh, during the period of 2022-2023.The research was centred on assessing the impact of pre-harvest (October sprays) on retaining anthocyanin pigment levels in the red pulp tamarind variety Anantha Rudhira, considering various biochemical parameters. The experiment employed a randomized block design comprising eight distinct treatments, each of which was replicated four times. The details of treatments used in experiments were T_1 (Methyl Jasmonate @ 5mM); T_2 (Phenylalanine @ 500µM); T_3 (Ethephon @ 72ppm); T_4 (Potassium dihydrogen orthophosphate @ 0.5%); T_5 (*Rhodopseudomonas* @ 5ml/l); T_6 (Salicylic acid @ 200µM); T_7 (Potassium nitrate @ 2%) and T_8 (Control-No spraying). The results of the experiment revealed that the treatment T_1(Methyl Jasmonate @ 5 mM) showed best performance for biochemical parameters *viz*., TSS (14.84, 47.94 °Brix), total anthocyanin (5.49, 2.42 mg/100 g), reducing sugars (3.50, 36.53%), non-reducing sugars (2.09, 1.68%) total sugars (3.72, 38.3%) at 7 and months of flower initiation for October spray respectively.

Keywords: *Tamarindus indica,* pre-harvest spray, TSS, Anthocyanins

Introduction
Tamarind, scientifically known as *Tamarindus indica* L., is a tropical fruit crop belonging to the family Leguminosae (Fabaceae) family and the sub-family Caesalpinioideae. Originating from tropical Africa, tamarind has spread to over 50 countries worldwide, as documented by Vuyyala *et al.* (2021) [1]. The tree of the tamarind, also called the Assam tree, is commonly referred to as 'Imli' or 'Indian date'. In India, tamarind cultivation spans across 46,000 hectares, yielding a production of 174 thousand tonnes as per the data from the National Horticulture Board (NHB) for 2022. The major states contributing to tamarind cultivation are Tamil Nadu, Karnataka, Kerala, Andhra Pradesh, Telangana, Maharashtra, Madhya Pradesh, Nagaland, and Mizoram. Specifically focusing on Andhra Pradesh, tamarind is cultivated in about 4,300 hectares, resulting in a yield of 14.32 thousand tonnes according to the NHB report for 2021-2022. It serves as a traditional medicine, a culinary ingredient, and finds application in confectionery, pharmaceuticals, textile production, grain, timber, and fuel industries. According to Vuyyala *et al*., (2021) [1], every part of the tamarind tree is utilized in some capacity. Anthocyanins, among the most visually striking plant pigments, have garnered increasing interest for their broad spectrum of colours, ranging from orange and red to purple and blue. Found in flowers, fruits, and vegetables, these pigments hold potential as food colorants that are both harmless and beneficial to health. Given the food industry's preference for natural pigments, particularly red hues, anthocyanins emerge as promising options for these colorants. Consequently, there's a current emphasis on exploring anthocyanins from new sources for application in food and cosmetic products. Anantha Rudhira is a new tamarind variety that was identified and released in 2018 by scientists at the AICRP for Arid Zone fruits in Aanantapuramu district of Andhra Pradesh. Its pulp vacuoles contain the non-toxic red pigment anthocyanin, which is water soluble. This tamarind variety has a great yielding potential and is a consistent bearer. Pods are born in clusters and are long and straight. However, it's essential to note that isolated anthocyanins are quite sensitive and prone to degradation, as highlighted by Giusti and Wrolstad, (2003) [3].

Exogenous application of phenylpropanoids and biosynthetic intermediates of secondary metabolites has therefore been one of the most effective strategies to induce/enhance biosynthesis of anthocyanins and flavonoids in plants (Edahiro *et al.*, 2005) [6]. The anthocyanin content in Anantha Rudhira pods get decreases with increase in maturity. The blood red colour pulp with high anthocyanins turns into reddish brown and finally to dark brown with low anthocyanins. If anthocyanins are retained in the pulp till maturity, the fully ripen pulp will be rosy, red in colour which fetches premium price in the market. Several value-added products can be prepared out of the coloured tamarind pulp. Keeping in view of all the above and in order to retain the anthocyanin pigment the present research work was carried with an aim to study the effect of pre-harvest sprays on retention of anthocyanin pigment and study of biochemical properties of red pulp tamarind variety Anantha Rudhira.

Materials and Methods

The research was conducted at Dr. Y.S.R. Horticultural University -Horticulture Research Station (AICRP on Arid Zone Fruits), situated in Rekulakunta, Anantapuramu, Andhra Pradesh, during the period of 2022-2023. Laboratory analyses were performed at the Central Laboratory of Dr. Y.S.R. Horticultural University, housed within the College of Horticulture in Anantharajupeta, Annamayya district of Andhra Pradesh. The experimental site was located at Dr. Y.S.R. Horticultural University -Horticulture Research Station (AICRP Centre on Arid Zone Fruits) in Rekulakunta, Anantapuramu, Andhra Pradesh. This area falls within a region characterized by limited rainfall, averaging around 521 mm annually. The site rests at an altitude of approximately 350 meters above mean sea level. Its geographical coordinates are approximately 14.41°N latitude and 77.40°E longitude. The research was centred on assessing the impact of pre-harvest October sprays on retention of anthocyanin pigment levels in the red pulp tamarind variety Anantha Rudhira, considering various biochemical parameters. The experiment employed a randomized block design comprising eight distinct treatments, each of which was replicated four times. This design allowed for a systematic and controlled evaluation of the effects of these treatments on the biochemical aspects studied in the red tamarind variety Anantha Rudhira. The detail of treatments used in experiments were T_1 (Methyl Jasmonate @ 5mM); T_2 (Phenylalanine @ 500µM); T_3 (Ethephon @ 72ppm); T_4 (Potassium dihydrogen orthophosphate @ 0.5%); T_5 (*Rhodopseudomonas* @ 5ml/l); T_6 (Salicylic acid @ 200µM); T_7 (Potassium nitrate @ 2%) and T_8 (Control-No spraying).

TSS

The percentage of total soluble solids was determined by using Atago RX 1000 hand refractometer and the reading expressed as ° Brix.

Anthocyanin content

Anthocyanin content was determined by using spectrophotometric method (Ranganna, 2007) [5]. By using spectrophotometer, the absorbance was recorded at 535nm. Anthocyanin content was expressed in mg/100 g.

Reducing sugars

The reducing sugars was calculated using DNS method, absorbance was recorded at 540 nm. The recorded absorbance values can be compared against the standard curve to determine the glucose concentration.

Non-Reducing sugars

The content of non- reducing sugars was calculated using the following formula (Ranganna, 1986) [5].

Non-reducing sugars (%) = (Total sugars (%) – Reducing sugars (%)) x 95%

Total sugars

The total sugar content was estimated by Anthrone reagent method. The absorbance was read using spectrophotometer at 630 nm absorbance. A graph was drawn by plotting concentration of the standard on the x-axis and absorbance on the Y-axis. From the graph, the amount of total sugars present in the sample was calculated. The values obtained were expressed as percent. Total sugars and of non- reducing sugars was calculated using the spectrophotometric method. (Ranganna, 1986) [4].

Results and Discussions

TSS [°Brix]

The collected data pertaining to the impact of various pre-harvest sprays during October on Total soluble solids (ºBrix) of red pulp tamarind variety Anantha Rudhira at 7 months of flower initiation, indicated that the observed effect was determined to be statistically significant. It was observed that the effect of treatment T_1 (Methyl Jasmonate @ 5 mM) recorded the maximum Total soluble solids (ºBrix) i.e., 14.84ºBrix whereas, minimum Total soluble solids (ºBrix) i.e., 13.01ºBrix was recorded in T_8 (Control). It was also found that effect of treatments T_3 (Ethephon @ 72 ppm) and T_6 (Salicylic acid @ 200 µM) were found at par with T_1 (Methyl Jasmonate @ 5 mM) whereas effect of treatment T_5 (*Rhodopseudomonas* @ 5 ml/l) was found at par with treatment T_8 (Control). The data collected regarding the influence of pre-harvest sprays during October on Total soluble solids (ºBrix) of red pulp tamarind variety Anantha Rudhira at 10 months of flower initiation demonstrated a statistically significant impact. The effect of treatment T_1 (Methyl Jasmonate @ 5 mM) recorded the maximum Total soluble solids (ºBrix) i.e., 47.94ºBrix where-as minimum Total soluble solids (ºBrix) i.e., 39.94ºBrix was recorded in T_8 (Control). MJ application might have increased several catabolic processes that resulted into breakdown of cell wall and pectin into sugars and had been contributed to improve the soluble solid content of Tamarind (Taiz and Zeiger 2010) [7]. Also found similar results in flame seedless grapes.

Total Anthocyanins

The impact of various pre-harvest sprays during October on Total Anthocyanins (mg/100 g) of red pulp tamarind variety Anantha Rudhira at 7 months of flower initiation, indicated that the observed effect was determined to be statistically significant. It was observed that the effect of treatment T_1(Methyl Jasmonate @ 5 mM) recorded the maximum Total Anthocyanins (mg/100 g) i.e., 5.49 mg/100 g whereas, minimum Total Anthocyanins (mg/100 g) i.e., 4.07 mg/100 g was recorded in T_8 (Control).The data collected with respect to the influence of pre-harvest sprays during October on Total Anthocyanins (mg/100 g) of red pulp tamarind variety

Anantha Rudhira at 10 months of flower initiation recorded a statistically significant impact. The effect of treatment T_1 (Methyl jasmonate @ 5 mM) resulted in maximum Total Anthocyanins (mg/100 g) i.e., 2.42 mg/100 g whereas, minimum Total Anthocyanins (mg/100 g)i.e., 1.94 mg/100 g was recorded in T_8 (Control). Similar results were reported by Tzortzakis and Economakis (2007) [8]. Flavonoids represent a class of defence chemicals that are synthesised by plants. According to research reports, the application of methyl jasmonate has indicated the ability to enhance the production of defence flavonoids, such as anthocyanin, in tamarind. According to Pérez-Balibrea *et al.* (2011) [9], the augmentation of the mevalonic acid pathways, which serve as the primary origin of plant flavones and isoflavones, is responsible for this accomplishment.

Sugar content (Reducing, non-reducing and total sugars)

The collected data pertaining to the impact of various pre-harvest sprays during October on reducing, non-reducing and total sugars (%) of red pulp tamarind variety Anantha Rudhira at 7 months of flower initiation, indicated that the observed effect was found to be statistically significant. It was observed that the effect of treatment T_1(Methyl jasmonate @ 5 mM) recorded the maximum reducing, non-reducing and total sugars respectively (%) i.e.,3.50; 0.209 and 3.72% where, as minimum reducing, non-reducing and total sugars (%) i.e., 3.12; 0.181 and 3.31% was recorded in T_8 (Control).It was also found that effect of treatment T_3 (Ethephon @ 72 ppm) and T_6 (Salicylic acid @ 200 µM) were found at par with treatment T_1 (Methyl jasmonate @ 5 mM) whereas effect of treatment T_4 (Potassium dihydrogen orthophosphate @ 0.5%), T_5 (*Rhodopseudomonas* @ 5 ml/l) and T_7 (Potassium nitrate @ 2%) were found at par with T_8 (Control).The data collected regarding the influence of pre-harvest sprays during October on reducing, non-reducing and total sugars (g/100 gm) of red pulp tamarind variety Anantha Rudhira at 10 months of flower initiation showed a statistically significant impact. The effect of treatment T_1(Methyl jasmonate @ 5 mM) recorded maximum reducing, non-reducing and total sugars (%) i.e., 36.53, 1.68 and 38.3% whereas, minimum reducing, non-reducing and total sugars (%) i.e., 33.90, 1.24 and 35.21% was recorded in T_8 (Control).It was also found that effect of treatment T_6(Salicylic acid @ 200 µM) was found at par with treatment T_1(Methyl jasmonate @ 5 mM).The utilization of MJ resulted in an elevation of the sugar concentration in tamarind. The observed phenomenon can be ascribed to the capacity of methyl jasmonate to reinstate the levels of chlorophyll and carotenoids, thereby enhancing the functionality of the photosynthetic machinery in plants. The potential stimulatory effects of MJ on photosynthetic pigments and the photosynthetic machinery may be attributed to an observed increase in the absorption of vital nutrients, specifically iron (Fe), calcium (Ca), potassium (K), manganese (Mn), and magnesium (Mg), in biochemical processes related to the synthesis of chlorophyll. Additionally, the activity of enzymes associated with the Calvin cycle may be enhanced (Mostofa *et al.*, 2015) [10]. The previous studies conducted by Salavati *et al.* (2021) [11] has documented the advantageous impacts of MJ on photosynthetic pigments.

Table 1: TSS and Anthocyanin content as influenced by pre-harvest sprays during October on red tamarind variety Anantha Rudhira

Treatment Details		TSS [ºBrix]		Total Anthocyanins (mg/100 g)	
		At 7 months of flower initiation	At 10 months of flower initiation	At 7 months of flower initiation	At 10 months of flower initiation
T_1	Methyl jasmonate @ 5 mM	14.84	47.94	5.49	2.42
T_2	Phenyl alanine @ 500 µM	14.18	45.91	5.20	2.20
T_3	Ethephon @ 72 ppm	14.31	46.37	5.26	2.27
T_4	Potassium dihydrogen orthophosphate @ 0.5%	13.74	44.15	4.97	2.12
T_5	*Rhodopseudomonas*@ 5 ml/L	13.14	43.05	4.84	2.01
T_6	Salicylic acid @ 200 µM	14.36	47.00	5.39	2.34
T_7	Potassium nitrate @ 2%	14.19	45.65	5.11	2.20
T_8	Control	13.01	39.94	4.07	1.94
S.Em (±)		0.14	0.46	0.56	0.23
CD at 5%		0.41	1.34	1.67	0.70

Table 2: Reducing sugars, Non-reducing sugars and Total sugars as influenced by pre-harvest sprays during October on red tamarind variety Anantha Rudhira

Treatment Details		Reducing sugars (%)		Non-reducing sugars (%)		Total sugars (%)	
		At 7 months of flower initiation	At 10 months of flower initiation	At 7 months of flower initiation	At 10 months of flower initiation	At 7 months of flower initiation	At 10 months of flower initiation
T_1	Methyl Jasmonate @ 5 mM	3.50	36.53	0.20	1.68	3.72	38.30
T_2	Phenyl alanine @ 500 µM	3.23	35.90	0.19	1.44	3.43	37.42
T_3	Ethephon @ 72 ppm	3.32	36.01	0.20	1.51	3.54	37.60
T_4	Potassium dihydrogen orthophosphate @ 0.5%	3.22	35.30	0.18	1.18	3.41	36.54
T_5	*Rhodopseudomonas*@ 5 ml/L	3.21	35.21	0.18	1.11	3.40	36.38
T_6	Salicylic acid @ 200 µM	3.42	36.05	0.20	1.62	3.64	37.75
T_7	Potassium nitrate @ 2%	3.22	35.69	0.19	1.36	3.42	37.12
T_8	Control	3.12	33.90	0.18	1.24	3.31	35.21
S.Em (±)		0.04	0.17	0.003	0.03	0.03	0.20
CD at 5%		0.11	0.51	0.009	0.09	0.09	0.60

Conclusion
From the above experimental findings, it was concluded that treatment T_1 (Methyl Jasmonate @ 5 mM) performed the best for different biochemical parameters like TSS. Anthocyanin content, sugar content (total, reducing and non-reducing).

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Author's contribution
Pallavi. T.A formulated the theory and conducted the calculations. B. Srinivasulu (co-author) validated the analytical techniques. Under the guidance of B.Vimala, V. N. P. Siva Rama Krishna and V.V. Padmaja, T.A. Pallavi explored and oversaw the outcomes of this research. The results were collectively deliberated by all authors, and each played a role in shaping the final manuscript.

Declarations
The corresponding author is responsible for ensuring that the descriptions are accurate and agreed by all authors.

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