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Influence of pre-harvest foliar spray on the biochemical and physiological properties of strawberry (*Fragaria* × *ananassa* Duch.)

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Abstract
Foliar application of salicylic acid (100 ppm and 200ppm), methyl jasmonate (25 ppm and 50 ppm) and calcium nitrate (0.25% and 0.5%) and its implications on the fruit biochemical and physiological properties *viz*., TSS, titratable acidity, TSS: acid ratio, fruit firmness, fruit decay, PLW and shelf-life were evaluated. Fruits harvested from the treatment T_5 (methyl jasmonate 50 ppm along with RDF) showed the highest total soluble solids content of 7.24 °B, lowest titratable acidity of 0.92% and highest sugar-acid ratio of 7.87 whereas preharvest sprays of salicylic acid at the rate of 200 ppm along with RDF (T_3) resulted in higher fruit firmness (2.30 kg/cm^2), lower incidence of fruit decay (61.33%), minimum physiological loss in weight (1.20%) and shelf-life (3.60 days).

Keywords: Strawberry, pre-harvest spray, biochemical, shelf-life

Introduction
Strawberry (*Fragaria* × *ananassa* Duchesne) is one of the most attractive soft fruits with a pleasant aroma and flavor being native to America (Galletta *et al.*, 1990) [5]. Most of the present day cultivated strawberries are the resultant of hybrid of two American octoploid species *Fragaria chiloensis* (South America) and *Fragaria virginiana* (North East America) developed in France during seventeenth century (Mitra, 1991) [10]. Strawberries have about 98% of edible portion with high anti-oxidant properties. The fruits for distant market are usually picked at three-fourth colour development stage which turns red or pink colour while for local market at full colour development stage (Hancock, 1999) [7]. The crop is more profitable due to its good export potential with high economic returns in the quickest possible period.

Strawberry shows non-climacteric ripening behavior. The rate of ethylene evolution is low, but due to its characteristic high respiration rate (50-100 ml CO_2 per kg of fruits per hour at 20 °C), it has short shelf-life (Nunes *et al*., 2006) [11]. Due to its soft nature, it is easily susceptible to mechanical injuries leading to postharvest losses of about 20-50 per cent (Mingchi and Kojimo, 2005) [9]. Thus, handling without damage is a risk in the marketing of fruits. From this perspective, extending the shelf-life and maintaining the fruit firmness without affecting the marketing of fruits is a much needed solution.

Hence, pre-harvest sprays or postharvest techniques to extend the postharvest life and quality of these soft fruits are being focussed in the recent past. Salicylic acid, a natural phenolic compound, is known for inducing the defense resistance systems against postharvest diseases and this would be a promising measure for controlling postharvest decay on a commercial scale. Methyl jasmonate, a methyl ester of jasmonic acid, is a fragrant and naturally occurring growth regulator. It has a major role in defense resistance and fruit ripening and also known to affect the ripening processes of both climacteric and non-climacteric fruits (Zapata *et al*., 2014) [14]. Calcium (Ca^{2+}) is well known for the development of membrane structure and integrity, modulation of cell structure, providing stability and cell wall regidity (Sun, 2009) [13]. In this background, this study was planned with an objective to determine the influence of pre-harvest sprays of salicylic acid, methyl jasmonate and calcium nitrate on the biochemical and physiological characteristics of strawberry cv. 'Nabila'.

Materials and methods
A field trial was conducted at farmer's field in Thambatty village, Nilgiris Dt. to find out the influence of pre-harvest foliar sprays of salicylic acid (100 ppm and 200ppm), methyl jasmonate (25 ppm and 50 ppm) and calcium nitrate (0.25% and 0.5%) to improve the quality and shelf-life of strawberry cv. 'Nabila' cultivated under polyhouse conditions.

The experimental plot was located at an altitude of 6510 ft above mean sea level and with a latitude of 11°35' N and longitude 76°69' E. The soil status was sandy clayey loam with pH and EC of 6.6 and 0.41 dS/m respectively. The initial status of available N, P, K in the experimental field was 384 kg/ha, 102 kg/ha and 423 kg/ha respectively. The study was carried out during the month of February, 2019 with a set of seven treatments *viz*., T_1 - Recommended dose of fertilizers (150: 100: 120 kg/ha), T_2- T_1 + salicylic acid @100 ppm, T_3 - T_1 + salicylic acid @ 200 ppm, T_4 -T_1 + methyl jasmonate @ 25 ppm, T_5 -T_1 + methyl jasmonate @ 50 ppm, T_6 -T_1 + calcium nitrate @ 0.25% and T_7 - T_1 + calcium nitrate @ 0.5% with three replications in randomized block design.

Strawberry runner plants were purchased from Channarayapatna region of Mysore. Beds were prepared inside the polyhouse with a height of 45 cm from ground level with a width of 60 cm and bed to bed spacing of 30 cm. Mulching of beds was done with 50 mm thickness sheet and drip system was installed at 30 cm intervals. Strawberry plants were planted in two rows alternatively with a spacing of 30 X 30 cm in each bed. Regular cultural operations were followed as per the farmer's practices. RDF was given through fertigation as per the nutrient schedule at weekly intervals at each growth stages as given in the Table 1.

Table 1: Nutrient scheduling (%)

Nutrients	Vegetative stage (5 weeks) (%)	Flowering stage (4 weeks) (%)	Fruiting stage (20 weeks) (%)	Total (%)
N	30	20	50	100
P	20	30	50	100
K	10	20	70	100

Foliar spraying of plant growth regulators (salicylic acid and methyl jasmonate) and nutrient (calcium nitrate) was given at three stages *viz*., at the time of emergence of inflorescence, one week after the emergence of inflorescence and two weeks after the emergence of inflorescence. The fruits were taken from the first harvest at 75% maturity and biochemical analysis was carried out at Post Graduate Laboratory at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

Data were recorded on the following quality and physiological attributes:

- **Total Soluble Solids (°B):** Total Soluble Solids in the fruits was determined using an 'ERMA' Hand Refractometer, make ERMA®, Japan. Readings were recorded in °Brix.
- **Titratable acidity (%):** Titratable acidity (%) was determined as per the method suggested by AOAC (1960) [1].
- **TSS to acid ratio:** The ratio was determined between total soluble solids and acidity.
- **Fruit firmness (kg/cm^2):** Firmness was measured using a Digital Fruit Penetrometer (Model: GY-4, Sundoo industries co., ltd China) with a cylindrical probe with a size of 7.9 mm. Readings were taken at the proximal, distal and middle portions and mean values expressed as kilogram per centimetre square.
- **Percentage of fruit decay (%):** Fruit decay was visually inspected during room storage. Percentage of fruit decay was calculated as per the following formula:

$$\text{Percentage of fruit decay (\%)} = \frac{\text{Decayed fruits}}{\text{Total number of fruits}} \text{ X } 100$$

- **Physiological loss in weight (%):** The weight of individually numbered fruits was recorded using electronic balance at the initiation of storage. Thereafter, weights were recorded periodically at one day interval and the physiological loss in weight (PLW) was calculated under room storage with the following formula:

$$\text{PLW (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \text{ X } 100$$

- **Shelf-life (days):** Fruits were visually observed until the consumption stage under room storage. The number of days was recorded as shelf-life.

Statistical analysis

Data analysis was carried out using STAR package (Statistical tools for Agricultural Research developed by IRRI, Phillipines) under the statistical design *i.e*., Randomised Block Design (RBD). The data taken are the average of three replications and tested for significance at 5% probability level.

Results and Discussion

Pre-harvest treatments significantly influenced the biochemical and physiological parameters. Total soluble solids were found to be the highest in fruits sprayed with methyl jasmonate at the rate of 50 ppm along with RDF (T_5) with the value of 7.24 °B and the lowest was noticed in the treatments T_3, T_2 and T_7 (salicylic acid @ 200 ppm along with RDF, salicylic acid @ 100 ppm along with RDF and calcium nitrate @ 0.5% along with RDF respectively) with the values of 6.21 °B, 6.27°B and 6.25°B respectively (Table 2). The highest TSS observed in T_5 may be due to the role of methyl jasmonate contributing to the increase in the ethylene biosynthesis. This might have probably led to increase in TSS content by promoting carbohydrate production resulting in conversion of glucose, fructose and sucrose contents at a faster rate as reported by Kucuker *et al*. (2014).

The titratable acidity was registered lowest in fruits sprayed with methyl jasmonate at the rate of 50 ppm (T_5) and 25 ppm (T_4) along with RDF with the values of 0.92% and 0.94% respectively (Table 2). The pre-harvest sprays of salicylic acid @ 200 ppm along with RDF (T_3) and calcium nitrate @ 0.5% along with RDF (T_7) registered the highest titratable acidity (1.27% and 1.21% respectively). This might be due to increased TSS level that decreased the acidity level in fruits by the methyl jasmonate treated fruits which is in accordance with Kucuker *et al*. (2014).

TSS to acid ratio was significantly influenced by the pre-harvest treatments (Table 2) with the highest ratio (7.87) registered in treatment T_5 (methyl jasmonate at the rate of 50 ppm along with RDF). The lowest TSS to acid ratio was registered in the treatment T_3 (salicylic acid @ 200 ppm along with RDF) and T_7 (calcium nitrate @ 0.5% along with RDF) with the values of 4.89 and 5.17 respectively. The highest

TSS/ acid ratio was due to the increased TSS and decreased TA levels in the treatment (Ghasemnezhad and Javaherdasti, 2008) [6].

The treatmental effect was significant for fruit firmness ranging from 0.83 kg/cm^2 to 2.30 kg/cm^2(Table 3). The highest firmness of 2.30 kg/cm^2(T_3) and 2.07 kg/cm^2(T_7) were registered in fruits sprayed with salicylic acid @ 200 ppm along with RDF and calcium nitrate @ 0.5% along with RDF. The lowest firmness of 0.83 kg/cm^2 was registered in control fruits (T_1). Salicylic acid and calcium application were found to express beneficial effect with increase in fruit firmness. This might be due to minimal loss in moisture, thickened cell walls and improved membrane integrity which helped in retaining the fruit firmness (Abassi *et al*., 2009).

Fruit decay was significantly influenced by the pre-harvest sprays (Table 3). The fruit decay was found to be the lowest (61.33%) in treatment T_3 (salicylic acid @ 200 ppm along with RDF) and the highest decay (96.33%) was registered in control fruits (T_1). It might be due to the involvement of salicylic acid as an active signal molecule in the activation of plant defense mechanisms. Because of this, there might be an increase of H_2O_2 production in plants and H_2O_2, as a signal molecule, which might have activated the plant's systemic resistance against pathogens (Cai & Zheng, 1999) [4].

Physiological loss in weight was recorded minimum (1.20%) in the treatment T_3 (salicylic acid @ 200 ppm along with RDF) whereas the maximum physiological loss in weight (4.77%) was recorded in control (T_1) (Fig.1). Regarding shelf-life, the fruits sprayed with salicylic acid @ 200 ppm along with RDF (T_3) and calcium nitrate @ 0.5% along with RDF (T_7) had a shelf-life of 3.60 days and 3.20 days respectively whereas the lowest shelf-life was noticed in control fruits (T_1) and methyl jasmonate at the rate of 50 ppm along with RDF (T_5) (2.00 days and 2.10 days respectively) (Fig.2).

Pre-harvest sprays of salicylic acid @ 200 ppm along with RDF (T_3) and calcium nitrate @ 0.5% along with RDF (T_7) had an impact in reducing the physiological loss in weight and increase in shelf-life. A plausible explanantion for the effect of salicylic acid and calcium nitrate could be the decrease in ethylene synthesis. It might be due to the influence of salicylic acid involved in reducing the production of ACS (aminocyclopropane carboxylate synthase) and ACO (aminocyclopropane carboxylate oxidase) production thereby reducing the respiration rate and in turn delaying the senesence, preserving cellular structure and maintaining the shelf-life of fruits (Asghari and Aghdam, 2010) [3]. Calcium is also known to retard respiration rate and ethylene production and increasing the rigidity of fruits by decreasing the activity of cell wall degrading enzymes (pectin methyl esterase and polygalacturonase) in fruits (Lara *et al*., 2004; Shafiee *et al*., 2010) [8, 12].

Conclusion

It can be concluded from the results of the experiment that pre-harvest treatments of strawberry fruits with salicylic acid at the rate of 200 ppm along with RDF (T_3) followed by calcium nitrate at the rate of 0.5% along with RDF (T_7) were effectively involved in improving the biochemical properties, thereby extending the shelf-life under room storage condition.

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Table 2: Influence of pre-harvest spray on TSS (°B), Titratable acidity (%) and TSS to acid in strawberry cv. Nabila

Treatments	TSS (°B)	Titratable acidity (%)	TSS to acid ratio
T_1	6.43	1.06	6.07
T_2	6.27	1.19	5.27
T_3	6.21	1.27	4.89
T_4	6.89	0.94	7.33
T_5	7.24	0.92	7.87
T_6	6.37	1.11	5.74
T_7	6.25	1.21	5.17
SE d	0.074	0.035	0.201
C.D (0.05)	0.151	0.069	0.423

Table 3: Influence of pre-harvest spray on fruit firmness (kg/cm^2) and fruit decay (%) in strawberry bcv. Nabila

Treatments	Fruit firmness (kg/cm^2)	Fruit decay (%)
T_1	0.83	96.33
T_2	1.80	74.67
T_3	2.30	61.33
T_4	1.37	80.00
T_5	1.22	88.00
T_6	1.67	77.33
T_7	2.07	71.33
SE d	0.185	2.810
C.D (0.05)	0.371	5.625

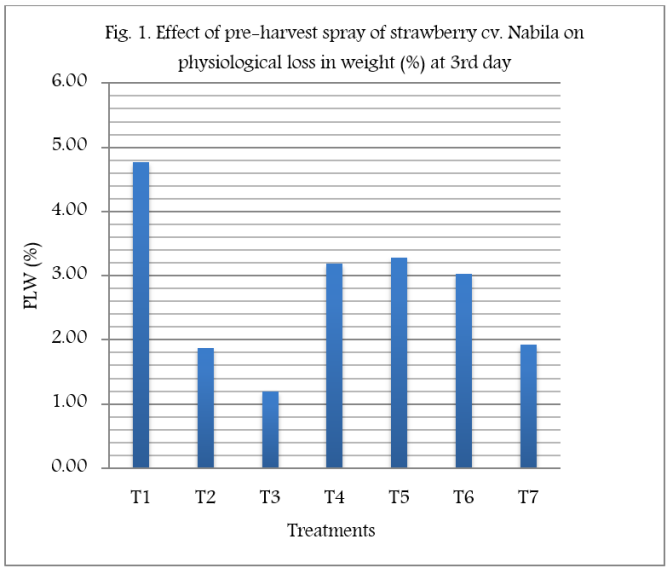

Fig 1: Influence of pre-harvest sprays on physiological loss in weight (%) at 3rd day in strawberry cv. Nabila

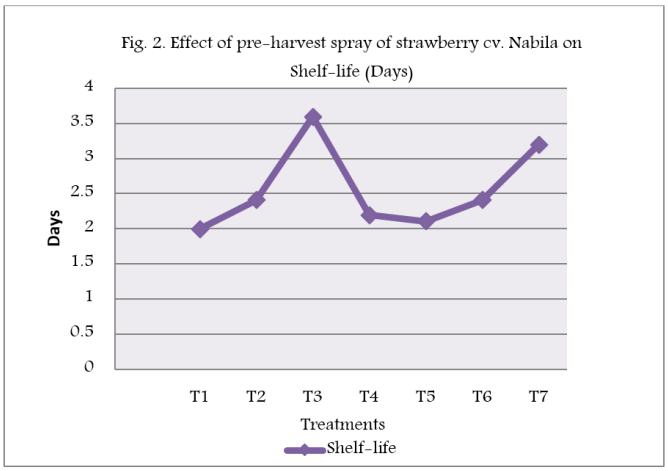

Fig 2: Influence of pre-harvest spray on shelf-life (days) in strawberry cv. Nabila

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