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Effect of pre-harvest application of elicitors and bio-formulations on biochemical characteristics and shelf life of papaya (*Carica papaya* L.) Fruits

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Abstract
The study was conducted to assess the influence of pre-harvest application of elicitors and bio-formulations (Brassinosteriod @ 0.05% and Brassinosteriod @ 0.1%, Chitosan @ 2%, *Pseudomonas fluorescens* @ 1x10^8cells/ml, *Bacillus subtilis* @ 1x10^8cells/ml, Salicylic acid @ 300ppm, Clove oil @ 0.2%, Eucalyptus oil @ 0.2%) on papaya fruits at different developmental stages. The first spray was taken up 2 months before harvest, 2nd and 3rd spray 15 days after 1st and 2nd spray respectively. The results revealed that treatments, Brassinosteriod @ 0.1%, *Pseudomonas fluorescens* @ 1x10^8cells/ml and Chitosan @ 2% have highly influence on biochemical parameters like total soluble solids (14.85 ^{0}B), carotene content (1.19 mg/100 g) and titratable acidity (0.23%) respectively. Their by improve the fruit quality and shelf life (ranges from 8.33 days to 9.00 days) during postharvest life of papaya cv. Red lady.

Keywords: Elicitors, bio-formulations, TSS, titratable acidity, caroteniod, shelf life, sensory evaluation

Introduction

Papaya (*Carica papaya* L.) also known as pawpaw belongs to the family Caricaceae. It is considered as one of the most popular fruits among the millions of people due to it taste, nutritive value and medicinal importance. It is regarded as the wonder fruit of the tropical and sub-tropical regions. It is originated in Mexico and was introduced to India during 16th century from Malaca (Kumar and Abraham, 1943) [12]. India is the main papaya producing country, whereas, Mexico occupies the sixth place and provides 37.10 per cent and 5.90 per cent of the total production, respectively (Anonymous, 2014) [4]. India has approximately 1.38 lakh hectares of land under papaya cultivation and produces around 59.89 million metric tons per year (Anonymous, 2018) [5]. Andhra Pradesh is the leader in papaya production among the Indian states followed by Karnataka, Gujarat and Maharashtra.

Papaya is rich source of antioxidants (Anonymous, 2012) [3]. Each 100 g of fruit contains 88.80 per cent moisture, 42.28 per cent starch, 15.50 per cent sugar but low levels of fat. Papaya fruit also contains high levels of vitamin C (61.8 mg), vitamin A precursors including β-carotene (276 µg) and β-cryptoxanthin (594.3 µg), as well as magnesium (10 mg) (Anonymous, 2004) [2].

Papaya fruit is a highly perishable and has a very short storage life at ambient condition. Physiologically, papaya fruits exhibit a climacteric behaviour. Post harvest losses of approximately 11 to 25 per cent have been generally reported in papaya in developing countries (Anonymous, 2014) [4]. The major constraint that hinder the expansion of export of papaya fruit are short storage life, susceptibility to postharvest diseases, high shipment cost and pesticide residues that is harmful to the human consumption. Post harvest fruit decay is a major constraint in post harvest handlings causing decreases in both quantity and quality of produce in general.

Elicitors simulate the different biotic and abiotic stresses conditions and that trigger the plant biochemical system towards the increasing of secondary metabolites (Zhao *et al.*, 2005) [23]. They suggest that, treating the plants with elicitors could be a strategy of low risk to enhance the presence of these compounds in the plant. Hence elicitors are effective in preserve the post-harvest quality of papaya fruits.

Materials and methods
Experimental details
The experiment was carried out at farmer field, Kaladagi hobli, Bagalkot district and Department of Post-harvest Technology, College of Horticulture, University of Horticultural Sciences Campus, Bagalkot, Karnataka during the year 2017-2018 with 9 treatments and 3 replications. The statistical design applied was completely randomized design (CRD). The treatment Details are T_1 - Control (water spray), T_2 -Brassinosteroid @ 0.05%, T_3 - Brassinosteroid @ 0.1%, T_4- Chitosan @ 2%, T_5- *Pseudomonas fluorescens* @ $1x10^8$ cells/ml, T_6 -*Bacillus subtilis* @ $1x10^8$ cells/ml, T_7 - Salicylic acid @ 300 ppm, T_8 - Clove oil @ 0.2%, T_9 - Eucalyptus oil @ 0.2%.

Elicitors/bio-formulations were sprayed as per the treatments at different fruit developmental stages *i.e.*, 1st spray- 2 months before harvest, 2nd and 3rd spray - 15 days after 1st and 2nd spray, respectively. Mature fruits were harvested at 1 or 2 yellow streaks on the fruit and were wrapped with paper to prevent mechanical injury during transportation to laboratory. Then removed the covered papers from fruits, the pre harvest treated as well as control fruits were stored under ambient conditions for further observations. Five fruits were used in each replication for analysis of physiological and biochemical parameters.

Total soluble solids (TSS) were estimated by using digital Refractrometer and the results were expressed as degree brix (^{0}B). Titratable acidity (%) and Beta Carotene (mg 100 g $^{-1}$) contents were determined by titrating the fruit extract against 0.1 N NAOH, using phenolphthalein as an indicator for titratable acidity and absorbance spectrophotometer at 452nm using petroleum ether as a blank for Beta Carotene respectively as described by Ranganna (1986) [14]. The number of days of the ripe fruits were in edible condition was taken as the shelf life of the fruits. Sensory evaluation was carried out on 9th day of storage for all the treatments for the characters like flesh colour, visual appearance, flavour and taste, texture and over all acceptability by a panel of 10 judges using 9 point hedonic rating scale *i.e.*, 9 - like extremely, 8-like very much 7-like moderately, 6- like slightly, 5- neither like nor dislike, 4-dislike slightly, 3-dislike moderately, 2-dislike very much, 1-dislike extremely.

Table 1: Effect of pre harvest application of elicitors and bio-formulations on total soluble solids (^{0}B), titratable acidity (%), caroteniod (mg 100 g $^{-1}$) and shelf life (days) under ambient storage of papaya fruit

Treatments	Total soluble solids (^{0}B)				Titratable acidity (%)				Carotenoid (mg 100 g $^{-1}$)	Shelf life (days)
	Days of storage				Days of storage				9th Day of storage	
	3	5	7	9	3	5	7	9		
T_1 - Control (water spray)	9.28	11.03	12.64	13.47	0.09	0.23	0.19	0.11	1.07	7.00
T_2 - Brassinosteroid @ 0.05%	8.76	11.14	13.21	14.30	0.22	0.27	0.34	0.19	1.10	8.33
T_3 - Brassinosteroid @ 0.1%	8.90	11.65	13.99	14.85	0.24	0.33	0.37	0.20	1.16	9.00
T_4 - Chitosan @ 2%	7.52	9.11	10.10	11.68	0.27	0.36	0.42	0.23	0.93	8.66
T_5 - *Pseudomonas fluorescens* @ 1 x 10^8 cells/ml	8.81	11.26	13.48	14.66	0.25	0.34	0.39	0.21	1.19	8.33
T_6 - *Bacillus subtilis* @ 1 x 10^8 cells/ml	8.6	10.41	12.84	13.85	0.25	0.27	0.34	0.19	0.98	8.00
T_7 - Salicylic acid @ 300 ppm	8.41	10.85	13.01	14.03	0.19	0.24	0.30	0.14	1.01	9.00
T_8 - Clove oil @ 0.2%	7.92	9.38	10.49	12.48	0.24	0.25	0.32	0.17	0.96	8.66
T_9 - Eucalyptus oil @ 0.2%	8.39	9.75	10.98	13.00	0.17	0.22	0.27	0.13	0.94	8.66
SEm±	0.09	0.07	0.11	0.15	0.004	0.003	0.003	0.004	0.005	0.18
CD at 1%	0.37	0.29	0.47	0.61	0.01	0.01	0.01	0.01	0.02	0.74

Table 2: Effect of pre harvest application of elicitors and bio-formulations on sensory evaluation under ambient storage of papaya fruit

Treatments	Sensory evaluation (9th day of storage)				
	Flesh colour	Visual appearance	Flavour and Taste	Texture	Overall Acceptability
T_1 - Control (water spray)	6.00	5.66	6.75	6.33	6.00
T_2 - Brassinosteroid @ 0.05%	7.33	7.00	7.16	7.35	7.25
T_3 - Brassinosteroid @ 0.1%	7.66	7.75	8.06	7.91	7.80
T_4 - Chitosan @ 2%	7.23	7.50	7.66	7.28	7.60
T_5 - *Pseudomonas fluorescens* @ 1 x 10^8 cells/ml suspension	8.50	7.23	7.83	6.75	7.40
T_6 - *Bacillus subtilis* @ 1 x 10^8 cells/ml suspension	6.75	6.66	6.83	6.83	6.76
T_7 - Salicylic acid @ 300 ppm	7.58	8.00	8.46	7.58	8.26
T_8 - Clove oil @ 0.2%	7.08	7.63	7.76	7.41	7.31
T_9 - Eucalyptus oil @ 0.2%	6.81	7.41	7.58	7.48	6.90
SEm±	0.11	0.06	0.1	0.09	0.09
CD at 1%	0.46	0.26	0.40	0.39	0.38

Results and discussion
Total soluble solids (^{0}B)
Total soluble solids of papaya fruits was found to be increased considerably during the storage in all treatments (Table1.). However, pre-harvest spray of Brassinosteroid at 0.1% (T_3) recorded highest TSS content of 14.85 ^{0}B followed by fruits sprayed with *Pseudomonas fluorescens* @ 1 x 10^8 cells/ml (T_5**)** i.e. 14.66 ^{0}B compared to the control treatment(13.47 ^{0}B) under ambient condition on 9th day of storage. The increase in TSS content of fruits might be attributed to increase in soluble solids, pectin and organic acid etc. Similar kind of works was carried out in sweet cherry (Champa *et al.*, 2015) [8], Orange (Wang *et al.*, 2004) [18], Passion fruit (Gomes *et al.*, 2006) [10] and in Jujube (Zhua *et al.*, 2010) [24].

It is also evident from the study that the TSS of pretreated papaya fruits with Chitosan at 2% showed a slow increase and their by registered lower TSS of 11.68^0 Brix. The effect of Chitosan is reducing the TSS of papaya fruit was probably due to the slowing down of respiration and metabolic activity, hence retarding the ripening process, resulting in lower TSS due to the slower hydrolysis of carbohydrates to sugar. These results are line with the result of Ali *et al.* (2011) [1] and Asgar *et al.* (2011) [6] in papaya.

Titratable acidity (%)

Titratable acidity of papaya fruits was always recorded higher values at early stage and then a decreased by the end of the storage (Table 1) in all treatments.

Papaya fruits sprayed with Chitosan 2% (T_4) had highest Titratable acidity (0.23%) during storage followed by fruits spray with *Pseudomonas fluorescens* @ 1 x 10^8 cells/ml suspension(0.21%) and the lowest was in control treatment (0.11%) at 9th day of storage. This might be due to the reason that, as the ripening advances, increase in acidity can be justified by the formation of organic acids produced by enzymes, such as pectin methyl esterase and poly galacturonase that degrade the cell wall during the ripening of papaya with a drop in pH. Similar results were reported by Oliveira *et al.* (2004) [13]. These results are similar to the findings of Wills *et al.* (1984) [20] reported that the titratable acidity per cent of the fruit increased during ripening of the fruit and then decreased due to the apparent stability observed as an indicator of metabolic stability. This was mainly due to papaya fruits treated with chitosan at 2 per cent which reduced the respiration and transpiration rate that could have slowly decreased in acid content. It was also reported that, acidity decreases with ripening and senescence. Similar results have been reported in papaya (Ali *et al.*, 2011 and Asgar *et al.*, 2011) [1, 6].

Carotenoid (mg 100 g^{-1})

The total carotenoid content of papaya fruits increased with increasing ripening. The disappearance of chlorophyll is often associated with the synthesis and/or the revelation of pigments ranging from yellow to red. Carotenoids are stable compounds and remain intact in the tissue even when extensive senescence has occurred (Wills *et al.*, 2007) [21].

The contents of phenolics were influenced by the degree of maturity at and after harvest, genetic differences (cultivar), pre harvest storage conditions and processing (Zadernowski *et al.*, 2005) [22]. We presumed that, deep coloured fruits and vegetables are good source of phenolics, flavonoids, anthocyanins and carotenoids.

There was a significant changes in carotenoid content of papaya fruits, as influenced by pre harvest spray with elicitors and bio-formulations under ambient storage condition. Papaya fruits sprayed with *Pseudomonas fluorescens* @ 1x10^8 cells/ml (T_5) recorded maximum amount of carotenoid of 1.19 mg/100 g compared to minimum of 0.93 mg/100 g in Chitosan 2%, sprayed fruits at 9th day of storage (Table 1).

Flavonoids are a diverse group of phytonutrients (plant chemicals) found in almost all fruits and vegetables along with carotenoids. They are responsible for the vivid colours in fruits and vegetables. Expression of flavonoid biosynthetic genes with the accumulation of anthocyanins and flavonols in the developing fruits of blackberry. Elicitation of blackberry plants by treatment of roots with *Pseudomonas fluorescens* caused an increased expression of some flavonoid biosynthetic genes (Garcia-Seco *et al.*, 2015) [9].

In the chitosan at 2 per cent treated fruits, the beta carotenoids content was lowest compared to control. However, in elicitors and bio-formulations treated fruits, the beta carotene content increased rapidly due to the carotenoids pigments expressed concurrently with chlorophyll degradation. Whereas, beta carotene content of chitosan treated fruits recorded the lesser chlorophyll degradation of fruits during storage. In addition, the temperature during storage condition affect the pathways involved in biosynthesis of secondary metabolites, lead to higher phenolic metabolism and antioxidant capacity of papaya fruit. Similar results have been reported in papaya (Barrera *et al.*, 2015) [7] and bell pepper (Zoran *et al.*, 2008) [25].

Shelf life (days)

Shelf life of papaya fruit is interconnected with broader physical, physiological, Physico-chemical and microbiological parameter. Each parameter has its own impact on fruit quality and storage life. However, present study was tried to address one factor, in physiology of papaya fruits by pre-harvest application of elicitors and bio-formulations on fruits at different fruit development stages. The first spray was applied before two months of the harvest; second and third spray was taken at 15 days of an interval for positive impact on chemical property.

The papaya fruits sprayed with Salicylic acid at 300 ppm (T_7) and (Brassinosteroid @ 0.1% (T_3) showed an extended storage period up to 9 days under ambient storage when compared to control fruits (7days) is presented in Table1. This extension of storage period was due to the delay in the ripening process caused by elicitors and bio-formulations. Among the elicitors and bio-formulations, SA was effectively reduced respiration in plants and harvested fruits (Han *et al.*, 2003; Srivastava and Dwivedi, 2000) [11, 16]. Pre-storage or pre harvest application of Salicylic acid may provide a useful means of controlling postharvest decay thereby extending the storage life (Wang and Li, 2008) [19].

Similarly, Brassinosteroid significantly delayed the fruit senescence by reducing ethylene production, respiration rate and maintained fruit quality. It is suggested that the effects of Brassinosteroid on reducing decay caused by *Pencillium expansum* may be associated with induction of disease resistance in fruit and delay of senescence leading to enhancement of the shelf life (Zhua *et al.*, 2010) [24].

Sensory evaluation

The data pertaining to the sensory evaluation scores of papaya var. Red Lady as influenced by pre harvest application of elicitors and bio-formulations under ambient storage condition are depicted in Table 2. The sensory traits *viz.*, flesh colour, flavour and taste, texture, visual appearance and overall acceptability of papaya fruits were judged on 9th day of storage. The results are significant among the treatments for all the sensory traits studied.

Among the treatments, maximum score for flesh colour was observed in T_5 (8.50), followed by T_3 (7.66) and T_7 (7.58) and minimum score was found in T_1 (6.00). Regarding flavour and taste, the maximum score was observed in T_7 (8.46) and T_3 (8.06) and a minimum score was recorded in T_1 (6.75). With respect to texture, maximum score was observed in T_3 (7.91) followed by T_7 (7.58), T_9 (7.48) and a minimum score was found in T_1 (6.33). Higher score for visual appearance (freshness) T_7 (8.00) followed by T_3 (7.75), T_8 (7.63) and minimum score was recorded in T_1 (5.66).

The results indicated that, there were significant differences between the treatments with respect to overall acceptability at 9th day of sensory evaluation during ambient storage. Among the treatments, the maximum mean score for overall acceptability was recorded in T_7 (8.26) followed by T_3 (7.80). However, the minimum mean score was noted in T_1 (6.00) at 9th day of ambient storage conditions.

In general, colour increases the attractiveness of fruits. Surface colour is important for choosing fruits in the market and internal colour is also important to influence overall acceptability during consumption. Maximum score for texture, visual attractiveness, flavour and overall acceptability was noted in the treatment T_7 (Salicylic acid @ 300 ppm) and this could be due to treatment with salicylic acid as it maintained greater firmness and delayed membrane lipid peroxidation in fruits. Exogenous application of salicylic acid may also induce the expression of many defense genes during fruit storage. Pre-harvest application of salicylic acid may provide a useful means of controlling postharvest decay thereby extending storage life (Wang and Li, 2008) [19]. Delay in ripening of fruit, which retain the flavour for longer period of time and release pleasant flavour in fruits and maintenance of freshness by delaying the physico-chemical changes in the papaya fruits during the storage. The results are in conformity with Valverde *et al.* (2005) [17] in grapes, Sandooja *et al.* (1987) [15] in tomato.

Conclusion

Results revealed that, pre-harvest application of elicitors and bio-formulations influence on biochemical quality characteristics and shelf life of papaya fruit and there by overall quality improvement during storage. Whereas, chitosan reduced the respiration and transpiration rate that could have slowly decreased in acid content. *Pseudomonas fluorescens* caused an increased expression of some flavonoid biosynthetic genes and salicylic acid treated fruits recorded higher score for overall acceptability. The data presented here showed that, total soluble solids, carotene content and titratable acidity were induced by brassinosteriod, *Psedomonas fluorescens* and Chitosan, respectively and might have increased shelf life of papaya fruits.

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