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Improving phytochemical constituents of pomegranate (*Punica granatum* L.) *cv*. Bhagwa by pre-harvest treatments

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Abstract
Pomegranate is rich source of bioactive compounds and a potent pharmacological important crop, it has gained more importance in human health. Pre-harvest treatment with calcium chloride, Gibberellic acid, Benzyl adenine (BA), Salicylic acid, Methyl Jasmonate and Potassium Nitrate sprays was carried to enhance the phytochemical constituents and to improve the table quality of pomegranate fruit. Selected plants were sprayed at 30 day's interval till commercial harvest and harvested fruits were analysed for biochemical constituents and physical parameters. Analysed fruits had exhibited a higher ascorbic acid (13.80 mg $100ml^{-1}$), antioxidants (168.68 mg AEE $100g^{-1}$), anthocyanins (45.69 mg $100ml^{-1}$), total phenolic (544.78 mg GAE $100g^{-1}$) and total tannins (7.80 mg $100g^{-1}$) as compared to control which signifies the importance of pre-treatments. Fruits sprayed with potassium nitrate and methyl jasmoate had induced more accumulation of phytochemicals as compared to other chemicals.

Keywords: pre-harvest, pomegranate, calcium chloride, gibberellic acid, benzyl adenine, salicylic acid, methyl jasmonate and potassium nitrate

Introduction
Pomegranate (*Punica granatum* L.) belongs to family Lythraceae and it is a native of Persia (Iran), which is widely cultivated in the Mediterranean region (Holland *et al*., 2009). It is classified as non-climacteric fruits and therefore cannot continue the ripening process once detached from the plant (Kader, 2006) [10]. In India, pomegranate is considered as a crop of the arid and semi-arid regions because it withstands different soil and climate stresses. (Dhumal *et al*., 2014) [5]. It is an important source of anthocyanins, phenolic compounds, vitamins and minerals (Gundogdu and Yilmaz, 2012; Melgarejo *et al*., 2000; O'Grady *et al*., 2014) [11, 19, 16]. As the greatest importance among all phenolic compounds presents in pomegranate juice is consider to be due to ellagic acid derivatives. Scientific evidence has linked increasing consumption of pomegranate fruit to improved human health as a result of active phenolic compounds which have potent pharmacological activities, including, antioxidant, anti-mutagenic, anti-hypertension, anti- inflammatory activities (Gil *et al*., 2000; Kaur *et al*., 2006; Duman *et al*., 2009; Viuda *et al*., 2010; Fawole *et al*., 2012) [10, 6, 15, 26, 7]. Cultivar and variety selection, environmental factors, mineral nutrition and chemical and biochemical treatments are among the factors discussed.
Literature on quality attributes, physiological response of various pomegranate cultivars is voluminous. However, there is limited understanding of the effects of pretreatments on surface quality attributes and biochemical constituents of pomegranate fruit (Johnston *et al*., 2001) [12]. Presence of active phenoloc compounds lead to a potent pharmacological importance and with this there is a need to improve the phytochemical compounds in pomegranate for industrial application.

Material and Methods
Ph.D. research work pre-harvest application was carried out in at farmer's field, Babbur village, Hiriyur (T), Chitradurga (D), Karnataka located at an altitude of 693 meters above mean sea level and has latitude of 13° 58'10.7" North and longitude of 76° 38' 02.9" East,

harvested fruits were analysed for biochemical constituents was carried out in the Department Postharvest Technology, College of Horticulture, Bengaluru during the period 2015 to 2017.

Treatment details

T_1	Calcium chloride (0.5%)
T_2	Calcium chloride (1.0%)
T_3	Gibberellic acid (GA_3 at 100ppm)
T_4	Benzyl adenine(BA at 75ppm)
T_5	GA_3 (100ppm) + BA (75ppm)
T_6	Salicylic acid (1mM)
T_7	Salicylic acid (2mM)
T_8	Methyl Jasmonate (0.25mM)
T_9	Methyl Jasmonate (0.5mM)
T_{10}	Potassium Nitrate (250mg)
T_{11}	Potassium Nitrate (350mg)
T_{12}	Control (No treatment)

Selected pomegranate cultivar 'Bhagwa' plants were pre-harvest five spray were carried out at monthly interval till harvest.To increase the surface adhesiveness of the fruit, about 10 ml of surfactant (Triton-X @ 1%) was added to each liter of spray solution. For control fruits, distilled water with surfactant was used as a spray solution. On each tree, about 35-40 fruits were selected and sprayed with respective spray solutions. The spraying was done in a 30 day's interval before the commercial harvest and it was done on all sides of the plants and fruits as well as to the foliage surrounding the fruit. Sampling of fruits for various physical, physiological and biochemical and/or quality attributes was done after harvest.

Biochemical analysis

pH of extracted pomegranate juice was measurement using pH meter (Make: Trans instruments Model: BO 3001) standardized with buffer solutions of 4.0 and 7.0 according to the method outlined in AOAC (2000) [1]. Acidity was determined by titration method (AOAC, 2000) [1]. Total soluble solids were determined by Digital hand refractometer (Make: Erma Optical Works Ltd., Tokyo, Japan, 0-43°B range) and the values were corrected at 20 °C and expressed as °Brix (Anon., 1984). Anthocyanin estimation was made as per the procedure explained by Fuleki and Francis (1968) [8]. Ascorbic acid content of pomegranate samples was determined modified method using 2, 6-dichlorophenol indophenol sodium salt described by AOAC, 2006. Total phenols and Total tannins were estimated according to procedure given by Singleton & Rossi (1965) [24] and Schandert (1970) [23] respectively. The total antioxidants present in pomegranate juice was estimated using FRAP method given by Benzie and Strain (1996) [4]. Visual quality/appearance of fruits treated with different preharvest treatments is scored using a 9-point hedonic scale at the end of shelf life as 0-1 Not attractive, 2-3 Poor appearance, 4-5-moderately attractive, 6-7-Good and 8-9 Excellent, based on the criteria fruits were analysed for visual quality. All the data was statistical analyzed in according to the Completely Randomised Design (CRD) suggested by Sunderraj *et al*., 1972 [25].

Results and Discussion

The results of this study have indicated that the pre-treatments have a significant effect on phytochemical present in pomegranate while the TSS, ascorbic acid, specific gravity and rind moisture were found non-significant is presented in Table 1 and Table 2**.** TSS varied between 14.49 to 15.85 °B which without showing any important difference between pretreatments on TSS, highest (15.49 °B) TSS was recorded in T_1(calcium chloride @ 0.5%) was followed by T_6 and T_8 (Methyl Jasmonate @ 0.25mM) which might be due to the influence of calcium chloride and Salicylic acid and Methyl jasmonate which induces stress and leads to increase in TSS of pomegranate fruits and lowest TSS was recorded in T_{11}. pH of pomegranate Bhagwa differed significantly each other with a maximum pH of 4.74 and 4.72 in T_7 and T_2 respectively, it might be due to cultivar types, agro-climatic regions and fruit maturity at harvest (Kader *et al*., 1984) [13]. Acidity pomegranate fruit was recorded significant with highest acidity of 0.76 (%) in T_8 (Methyl jasmonate @ 0.25mM) and the lowest was recorded in T_2, variation in acidity might be due to agro-climatic regions and fruit maturity at harvest (Kader *et al*., 1984) [14] and biochemical conversion of sugars to organic acids as indicated by Pool *et al*. (1972) [25].

Table 1. Effect of pre-harvest treatments on biochemical constituents of pomegranate *cv* Bhagwa

	TSS	**pH**	**Acidity (%)**	**Ascorbic acid (mg 100ml^{-1})**	**Antioxidants (mg AEE 100g^{-1})**	**Anthocyanins (mg 100ml^{-1})**	**Tannins (mg 100g^{-1})**	**Phenols (mg GAE 100g^{-1})**
T_1	15.49	4.60	0.72	13.80	156.51	43.79	7.63	483.66
T_2	15.29	4.72	0.41	13.65	158.22	44.25	7.33	409.33
T_3	14.96	4.31	0.69	13.35	164.73	42.55	6.40	354.99
T_4	15.19	4.28	0.72	13.80	157.80	35.69	7.23	354.99
T_5	14.52	4.48	0.70	13.35	168.68	28.48	5.85	385.66
T_6	15.36	4.65	0.66	13.65	156.94	31.99	7.53	421.33
T_7	15.06	4.74	0.69	13.20	162.16	43.33	6.97	406.33
T_8	15.36	4.56	0.76	12.90	157.16	43.61	7.23	376.00
T_9	14.91	4.47	0.62	13.80	164.91	42.16	7.80	483.67
T_{10}	15.05	4.55	0.70	12.90	161.71	42.76	6.29	544.78
T_{11}	14.49	4.44	0.62	13.65	168.76	45.69	6.65	409.33
T_{12}	15.05	4.23	0.65	12.75	152.08	26.00	5.84	349.76
SEm±	0.34	0.10	0.01	0.29	2.60	0.88	0.15	8.62
CD@5%	0.98	0.29	0.03	0.85	7.60	2.55	0.43	25.15
F-test	NS	*	*	NS	*	*	*	*

NS- Non Significant * - significant at 5%

T_1	Calcium chloride – 0.5%	**T_7**	Salicylic acid- 2mM
T_2	Calcium chloride - 1.0%	**T_8**	Methyl Jasmonate - 0.25mM
T_3	Gibberellic acid (GA_3)– 100ppm	**T_9**	Methyl Jasmonate - 0.5mM
T_4	Benzyl adenine (BA) – 75ppm	**T_{10}**	Potassium Nitrate - 250ppm
T_5	GA_3 (100ppm) + BA (75ppm)	**T_{11}**	Potassium Nitrate - 350ppm
T_6	Salicylic acid- 1mM	**T_{12}**	Control (Distilled Water)

Table 2: Effect of pre-harvest treatments on physical parameters of pomegranate cv Bhagwa

Treatments	Specific Gravity	Rind Moisture (%)	Visual Quality
T_1 :Calcium chloride – 0.5%	1.24	72.22	7.83
T_2: Calcium chloride - 1.0%	1.36	73.47	7.96
T_3: Gibberellic acid (GA_3)– 100ppm	1.17	72.46	8.14
T_4: Benzyl adenine (BA) – 75ppm	1.15	72.61	8.00
T_5: GA_3 (100ppm) with BA (75ppm)	1.20	74.86	8.33
T_6: Salicylic acid (1mM)	1.23	72.06	7.86
T_7: Salicylic acid (2mM)	1.21	72.87	8.20
T_8: Methyl Jasmonate - 0.25mM	1.15	73.50	7.89
T_9: Methyl Jasmonate - 0.50mM	1.29	75.79	8.21
T_{10}: Potassium Nitrate - 250ppm	1.22	72.69	8.10
T_{11}: Potassium Nitrate - 350ppm	1.18	73.31	8.65
T_{12}: Control (Distilled Water)	1.25	72.01	7.56
SEm	0.07	1.60	0.14
CD @ 5%	0.19	4.69	0.42
F-test	NS	NS	*

NS- Non Significant * - significant at 5%

Although pomegranate does not distinguish by large quantity of ascorbic acid as compared to other fruits, pretreatments has significant influence on the quantity of ascorbic acid, with highest ascorbic acid (13.80 mg 100ml^{-1}) content was recorded in T_2, T_9 and T_4 whereas the lowest (12.75) ascorbic acid content was recorded in untreated fruit's (control), similar observation were reported by While Mirdehghan *et al.* (2006) [17] reported a significant increase in organic acids for 'Mollar de Elche' and Bayram *et al.* (2009) [3] in 'Hicaznar' fruit. Total Antioxidant capacity in pomegranate differed significantly on pre-harvest treatments, maximum antioxidant capacity was recorded in Potassium Nitrate (350ppm) sprayed fruits with 168.76, which was followed by Salicylic acid (1mM) sprayed fruits with 168.68 (mg AEE 100g^{-1}), while the minimum was recorded in untreated fruits with 152.08(mg AEE 100g^{-1}), higher amount of antioxidants activity might be due to the influence of potassium nitrate which enhanced the biochemical constituents of fruits and the results are in line agreement with Ghatge *et al*. (2005) [9] in pomegranate fruits when treated with different chemical treatments. Total anthocyanin content of pomegranate fruits was found significantly differed on pre-harvest sprays with higher anthocyanin content was recorded in in Potassium Nitrate (350ppm) sprayed fruits with 45.69 (mg 100ml^{-1}) and lower was in control with 26.00 (mg 100ml^{-1}) the results are in line with Nanda *et al.*, 2001 [18] in ganesh pomegranate when pre-harvest sprays are imposed.

Total phenols and tannins were analysed in fruit juice it was found significant with maximum Total phenols and Total tannins were recorded in T_{10} and T_9 (544.78 mg GAE 100g^{-1} and 7.80 mg 100g^{-1}) respectively while the minimum was recorded in control (349.76 mg GAE 100g^{-1} and 5.84 mg 100g^{-1} respectively). Higher phenols and tannins might be due to influence of potassium nitrate. Specific gravity (SG) and rind moisture (RM) per cent content were found non-significant on pre harvest sprays with maximum SG and RM was recorded in T_2 and T_9 (1.35 and 75.79 %) while the lowest was recorded in T_8 and T_4. Visual appearance of fresh produce is an important attribute a consumer considers while buying and so do wholesalers and retailers. Fruits were significantly different on treatments with highest (8.65) visual quality score was recorded in Potassium Nitrate (350ppm) sprayed fruits while the lowest (7.56) was recorded in control. This resulted in lower visual skin quality as excessive water loss and shrivelling gave fruit skin a leathery appearance. The results are in agreement with Omayma *et al*. (2014) [20] and Kader *et al.* (1984) [13] in pomegranate fruit.

Conclusion

It is concluded that pre-harvest application of growth regulators, secondary metabolites and mineral nutrients during fruit development stage in pomegranate trees had positive response on improving phytochemical constituents, quality of pomegranate and higher table quality for consumption and greater scope for industrial application.

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