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Periodical evaluation of enzymes and pigments in mango varieties during storage in card board carton by post harvest application of salicylic acid

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Abstract
Four varieties of mango namely Dashahari, Langra, Chausa and Safeda had been taken for the experiment. At maturity condition, mango fruits were harvested and stored in cardboard carton by post harvest application of salicylic acid at 0.1, 0.5 and 1.0 g/l doses. The analysis of pectin methyl esterase, cellulase, polyphenol oxidase, peroxidase, total chlorophyll and beta carotene were done at five days intervals after storage. Highest pectin methyl esterase enzyme activity (0.91 units/g) was reported in variety Dashahari at five days ambient storage period by 1.0 g/l salicylic acid application. Maximum cellulase and polyphenol oxidase enzyme activity (12.84 and 56.38 units/g) was reported in the variety Dashahari at five days ambient storage period by 0.1 g/l salicylic acid application. Highest peroxidase enzyme activity (463.69 units/g) was reported in variety Dashahari at 20 days ambient storage period after the application of 0.5 g/l salicylic acid treatment. Maximum total chlorophyll content (39.86 mg/g) was reported in the variety Dashahari at five days storage by 1.0 g/l salicylic acid application. Highest beta carotene content (3495.93 µg/100g) was reported in the variety Dashahari at 20 days ambient storage period by 0.5 g/l salicylic acid treatment. The present study indicated that after salicylic acid application pectin methyl esterase, cellulase, polyphenol oxidase and chlorophyll content decreased but the peroxidase and beta carotene content increased during storage in cardboard carton

Keywords: enzymes, pigments in mango, card board carton, salicylic acid

Introduction
The total production of fruits and vegetables in the world is around 370 million tonnes. India ranks first in the world with an annual output of 32 million tonnes fruits; accounts for about 8 per cent the world fruits production (N.H.B. database, 2011) [20]. The total production of mango fruit in India is 15188 million tonnes with an area of about 2297 hectare. The biochemical composition of mango fruit differs among the cultivars and the stage of maturity. Mango fruit is rich in carbohydrates, carotenoids, organic acids, polyphenols, vitamins and minerals etc. A comprehensive report has been made on the biochemical composition after analysis of more than 25 varieties of mango is moisture 73.0 - 86.7 per cent, carbohydrate 11.6 – 24.3 per cent, protein 0.5 – 1.0 per cent, minerals 0.3 – 0.7 per cent, beta carotene 6375 – 20750 µg/ 100 g, vitamin C 6.8 – 38.8 mg/ 100 g (Anonymous, 1994) [2]. According to the Ministry of Food Processing Industry statement '' the lack of processing and storage of fruits and vegetables results in huge wastages estimated at about 35 per cent the value of which is approximately Rs. 33000 crores for perishable and Rs. 15000 crores for non-perishable annually.''

A worldwide increase in the demand of fresh mango fruit is being observed, increasing the prospect for producing countries (Amin *et al*., 2007) [1]. However, like all other fresh commodities, its market potential is also linked with the fruit quality and market access (Anwar and Malik, 2007) [3], whereas certain postharvest factors, like insect-pest and disease management, are important from production point of view, proper post-harvest treatments and packaging are required for maintaining better quality, extended shelf life and having access to international markets (Anwar and Malik, 2007) [3].

Salicylic acid (SA) is a colourless, crystalline organic carboxylic acid which is found in plants, especially in fruits, in the form of methyl salicylate. It is also known as 2-hydroxybenzoic acid, with functions as a plant hormone. Most investigators have focused on the role of

salicylic acid in induction of disease resistance. Exogenous supply of salicylic acid has been reported to delay the ripening of apples (Yan *et al.*, 1998) [27], peach (Han *et al*., 2002) [8], persimmon (Han and Li, 2000) [9], mango and tomato fruits (Rong *et al.*, 2001) [23] probably through inhibition of ethylene biosynthesis (Fan and He, 1998) [6]. Salicylic acid (SA) has been shown to inhibit the conversion of 1-amino cyclopropane 1-carboxylic acid (ACC) into ethylene (Lesilie and Romani, 1988) [15] by suppressing ACC oxidase activity (Fan *et al.*, 1996) [7]. Furthermore, Li *et al.* (1992) [17] demonstrated an inhibitory effects of SA on accumulation of wound induced ACC synthase transcrypt in tomato fruit. Very less literature is available on the application of salicylic acid during ripening and storage of mango varieties regarding enzymes and pigments concentration. Therefore present research work has been undertaken to evaluate enzymes and pigments.

Materials and methods

Four varieties of mango namely Dashahari (v_1), Langra (v_2), Chausa (v_3) and Safeda (v_4) were collected from main Experimental Station, Horticulture, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad. Salicylic acid was applied (S_0, S_1, S_2, S_3) to the mango fruits for better quality and shelf life enhancement at ambient room temperature. The packaging materials cardboard carton was used to keep fruits. After the packaging of mango fruits the analysis was carried out at five days intervals. Pectin methyl esterase activity was measured by the method given by Line Weaber and Janson (1951) [16]. Pectin methyl esterase or pectinase deestrifies pectin in to galacturonic acid or its low molecular weight polymers. Cellulase enzyme activity was measured by the methods of Mandal *et al.* (1976) [18]. Cellulases comprises an enzyme system which is capable of breaking down highly condensed cellulose complex in to soluble sugars. Polyphenol enzyme activity was measured by the methods of Herewitz *et al.*, (1960) [10]. This enzyme acts on different phenolic compounds in the presence of oxygen and converts them in to complex condensation products, brown in colour. Peroxidase enzyme activity was measured calorimetrically by the method of McCune and Galston (1959) [19]. Total chlorophyll content was estimated by the method given by Arnon (1939) [4]. The β- carotene content was estimated in fresh fruit sample using the method developed by Rangana (1991) [22].

Results and Discussion

The data regarding to pectin methyl esterase enzyme activity in varrious mango varieties by salicylic acid application stored in cardboard carton was presented in Table-1. In control treatment maximum pectin methyl esterase activity (0.93 units/g) was recorded in variety Dashahari at five days storage period. Highest pectin methyl esterase activity (0.91 units/g) was reported in variety Dashahari at five days storage period by 1.0 g/l salicylic acid treatment. Statistical analysis of data revealed that salicylic acid treatments significantly effected the pectin methyl esterase enzyme activity of the mango varieties during entire storage period stored in cardboard carton. In control treatments all the fruits were destroyed due to rotting and microbial infection after five days ambient storage in all the mango varieties. The activity of this enzyme decreases during entire storage period may be due to increasing content of pectins from maturity to ripening. This enzyme is actively utilized in ripening process, so its activity due to application of salicylic acid decreased. The main role of salicylic acid in ripening process may be delayed by reducing the ethylene synthesis with the use of antisense gene for ethylene biosynthetic enzyme namely pectin methyl esterase and polygalacturonase which involved in the ripening process itself. The decreased activity of this enzyme in the present investigation may be due to less activity of these enzymes during storage as given by Chaesworth *et al.* (1998) [5]. The similar finding was also observed by Zhixi and Yuxing (2001) [29].

The data regarding to cellulase enzyme activity in mango varieties by salicylic acid application stored in cardboard carton was given Table -2. Maximum cellulase enzyme activity (13.89 units/g) was found in the variety Dashahari at five days ambient storage period in the control treatment. After salicylic acid application, maximum cellulase enzyme activity (12.84 units/g) was reported in the variety Dashahari at five days ambient storage period by 0.1 g/l salicylic acid application. A non-significant correlation was noticed between salicylic acid treatments and cellulase enzyme activity of the mango varieties during entire storage in cardboard carton. In control treatment after five days storage period all the fruits were destroyed due to rotting and microbial infection in all mango varieties Cellulase is a cell wall degrading enzymes. The levels of this cell wall degrading enzyme was found to be decreased, in a concentration dependent manner, suggesting that salicylic acid delay banana ripening (Srivastava and Dwivedi, 2000) [24].

The data containing polyphenol oxidase enzyme activity in mango varieties by application of salicylic acid stored in cardboard carton was shown in Table-3. Maximum polyphenol oxidase enzyme activity (59.64 units/g) in mango varieties was noticed in the variety Dashahari at five days ambient storage period in the control treatment. After the salicylic acid application the highest polyphenol oxidase enzyme activity (56.38 units/g) was noticed in the variety Dashahari at five days ambient storage period after the application of 0.1 g/l salicylic treatment. Statistical analysis of the data revealed that salicylic acid treatment significantly effected the polyphenol oxidase enzyme activity of mango fruits stored in card board carton. In control treatment all the fruits were destroyed due to rotting and microbial infection after five days storage in all the mango varieties. The activity of polyphenol oxidase enzyme tends to decrease in salicylic acid treated fruits during ambient storage. Phenolics are secondary plant metabolites, are synthesized by all plants. Salicylic acid treated ‘’Cara Cara’’ navel orange had increased total phenolics content and higher salicylic acid concentration was observed with further profound effect in this respect (Huang *et al.*, 2008) [11]. Similar findings were also reported by Tareen *et al.*, (2012) [25].

The data regarding to peroxidase enzyme activity in mango varieties by salicylic acid application stored in cardboard carton was given in Table-4. Maximum peroxidase activity (356.23 units/g) was reported in the variety Dashahari at five days ambient storage period in the control treatment. Highest peroxidase activity (463.69 units/g) was reported in Dashahari variety at 20 days ambient storage period by 0.5 g/l salicylic acid application. Salicylic acid treatment significantly effected the peroxidase enzyme activity of mango fruits stored in cardboard carton of mango varieties during entire period of storage. In control treatment all the fruits were destroyed due to rotting and microbial infection after five days storage period in all the varieties of mango fruits. The activity of peroxidase enzyme in various varieties of mango fruits by the

application of salicylic acid during storage was found in increasing pattern, it may be due to salicylic acid mediated hypersensitive and systemic acquired resistance against pathogenic attack and proposed to be mediated fruits through inhibition of catalase, which subsequently rases extracellular hydrogen peroxide. Peroxidases increased intracellular hydrogen peroxide concentration is converted into hydrogen peroxide and oxygen by increased activity of peroxidase enzyme. Lamikanra and Watson (2001, 2002) [13-14] indicated that the ascorbate dependency of peroxidase enzyme in a number of commonly fresh cut processed fruits, whose activity appears to be related to the level of oxidative stress in cut fruit. The result was also supported by Kazemi *et al.* (2011) [12].

The data regarding to total chlorophyll content in mango varieties by salicylic acid application stored in cardboard carton was given in Table-5. In control treatment, the maximum total chlorophyll content (24.83 mg/g) was reported in the variety Dashahari at five days ambient storage period. After the treatments, highest total chlorophyll content (39.87 mg/g) was recorded in the variety Dashahari at five days ambient storage period by 1.0 g/l salicylic acid application. A non significant correlation was found between salicylic acid treatments and the total chlorophyll of mango varieties stored in cardboard carton. In control treatments all the fruits were destroyed due to rotting and microbial infection after five days entire period of storage in all the mango varieties In the present investigation total chlorophyll content in various of mango fruits by the application of salicylic acid during storage has been noticed in decreasing pattern which may be due to its active conversion in to coloured pigment carotene. The findings are supported by Thompson (2003) [26]. The lower rate of the decrease in chlorophyll content of salicylic acid treated fruit could be attributed to be slower ripening. Ozer *et al.* (1997) [21] pointed out that storage of modified atmospheric packaging and control atmosphere packaging conditions, chlorophyll content of kiwifruit decreased with storage time.

The data related to beta carotene content in mango varieties by salicylic acid application stored in cardboard carton was given in Table-6. Highest beta carotene content (2434.00 µg/100 g) was recorded in variety Dashahari at five days ambient storage period in the control treatment. After salicylic acid treatment, maximum beta carotene content (3495.93 µg/100 g) was reported in the variety Dashahari at 20 days ambient storage period by 0.5 g/l salicylic acid application. Statistical data showed that salicylic acid treatment significantly effected the beta carotene content of mango varieties during storage in cardboard carton. In control treatment all the fruits were destroyed due to rotting and microbial infection after five days in all the mango varieties. The increasing content of beta carotene during ripening and storage may be due to degradation of chlorophyll and increased synthesis and accumulation of carotenoids in the plastids which are formed from simple five carbon isoprenoid units followed by cyclisation of terminal bond finally converted into 40 carbon beta carotene. Beta carotene pigment was increased during storage after salicylic acid treatment. This result was well supported by Zheng *et al.* (2007) [28].

Table 1: Pectin methyl esterase enzyme activity (units/g) of mango fruits at different intervals during storage in cardboard carton after salicylic acid application

Treatments \ Varieties		V_1	V_2	V_3	V_4	CD at 5 %	
S_0	5 d	0.93	0.84	0.91	0.78		
	10 d	0.00	0.00	0.00	0.00	V	0.013
	15 d	0.00	0.00	0.00	0.00		
	20 d	0.00	0.00	0.00	0.00	S	0.013
S_1	5 d	0.88	0.79	0.86	0.62		
	10d	0.82	0.64	0.81	0.56	T	0.013
	15 d	0.73	0.56	0.72	0.51		
	20 d	0.64	0.42	0.61	0.39	V X S	0.025
S_2	5 d	0.78	0.67	0.76	0.58		
	10 d	0.71	0.60	0.69	0.53	V X T	0.025
	15 d	0.67	0.57	0.62	0.45		
	20 d	0.51	0.43	0.47	0.39	S X T	0.025
S_3	5 d	0.91	0.81	0.89	0.65		
	10 d	0.83	0.67	0.84	0.60	V X S X T	0.050
	15 d	0.76	0.59	0.75	0.54		
	20 d	0.67	0.45	0.65	0.44		

Table 2: Cellulase enzyme activity (units/g) of mango fruits at different intervals during storage in card board carton after salicylic acid application

Treatments \ Varieties		V_1	V_2	V_3	V_4	CD at 5 %	
S_0	5 d	13.89	12.58	13.54	11.84		
	10 d	0.00	0.00	0.00	0.00	V	0.199
	15 d	0.00	0.00	0.00	0.00		
	20 d	0.00	0.00	0.00	0.00	S	0.199
S_1	5 d	12.84	11.53	12.62	10.92		
	10d	12.13	11.09	12.02	10.54	T	0.199
	15 d	11.73	10.84	11.68	10.12		
	20 d	11.22	10.52	11.34	9.87	V X S	0.399
S_2	5 d	11.72	10.48	11.36	9.89		
	10 d	10.53	9.43	10.32	8.79	V X T	0.399
	15 d	9.41	8.45	9.15	7.33		
	20 d	8.34	7.38	8.12	6.79	S X T	0.399
S_3	5 d	12.73	11.46	12.56	10.87		
	10 d	12.08	11.05	12.01	10.48	V X S X T	0.798
	15 d	11.68	10.73	11.57	10.07		
	20 d	11.15	10.47	11.28	9.74		

Table 3: Polyphenol oxidase enzyme activity (units/g) of mango fruits at different intervals during storage in card board carton after salicylic acid application

Treatments \ Varieties		V_1	V_2	V_3	V_4	CD at 5 %	
S_0	5 d	59.64	54.78	57.73	51.74		
	10 d	0.00	0.00	0.00	0.00	V	0.961
	15 d	0.00	0.00	0.00	0.00		
	20 d	0.00	0.00	0.00	0.00	S	0.961
S_1	5 d	56.38	51.47	53.84	48.49		
	10d	55.47	50.39	52.79	47.52	T	0.961
	15 d	54.58	49.68	51.65	46.59		
	20 d	53.12	48.32	50.19	45.31	V X S	1.923
S_2	5 d	54.76	50.38	51.76	47.78		
	10 d	53.48	49.93	50.39	46.52	V X T	1.923
	15 d	52.31	48.79	49.72	45.32		
	20 d	51.08	47.38	48.19	44.08	S X T	1.923
S_3	5 d	56.12	51.38	53.56	48.23		
	10 d	55.31	50.17	52.47	47.31		
	15 d	54.34	49.42	51.36	46.27	V X S X T	3.846
	20 d	53.02	48.13	50.07	45.07		

Table 4: Peroxidase enzyme activity (units/g) of mango fruits at different intervals during storage in card board carton after salicylic acid application

Treatments \ Varieties		V_1	V_2	V_3	V_4	CD at 5 %	
S_0	5 d	356.23	348.63	351.46	342.37		
	10 d	0.00	0.00	0.00	0.00	V	7.812
	15 d	0.00	0.00	0.00	0.00		
	20 d	0.00	0.00	0.00	0.00	S	7.812
S_1	5 d	376.75	367.75	372.76	357.78		
	10d	389.72	378.65	386.67	363.93	T	7.812
	15 d	412.56	392.93	402.39	387.34		
	20 d	425.37	406.71	419.59	405.56	V X S	15.624
S_2	5 d	397.75	388.89	392.94	376.72		
	10 d	423.36	412.13	418.83	393.94	V X T	15.624
	15 d	446.72	433.69	439.39	411.14		
	20 d	463.69	451.58	460.64	433.63	S X T	15.624
S_3	5 d	375.72	365.67	370.72	355.46		
	10 d	387.64	376.79	383.84	361.67		
	15 d	409.58	390.78	398.42	385.56	V X S X T	31.248
	20 d	423.31	402.96	416.79	400.98		

Table 5: Total chlorophyll content (mg/g dry weight) of mango peel at different intervals during storage in card board carton after salicylic acid application

Treatments \ Varieties		V_1	V_2	V_3	V_4	CD at 5 %	
S_0	5 d	24.83	20.97	18.54	15.52		
	10 d	0.00	0.00	0.00	0.00	V	0.609
	15 d	0.00	0.00	0.00	0.00		
	20 d	0.00	0.00	0.00	0.00	S	0.609
S_1	5 d	39.37	36.63	34.59	28.91		
	10d	38.46	35.69	33.67	27.87	T	0.609
	15 d	37.38	34.53	32.58	26.78		
	20 d	33.26	30.59	28.69	22.34	V X S	1.218
S_2	5 d	38.68	36.25	34.17	28.39		
	10 d	36.78	35.11	32.95	27.32	V X T	1.218
	15 d	34.38	34.07	30.62	26.12		
	20 d	28.56	30.31	27.13	24.57	S X T	1.218
S_3	5 d	39.87	36.97	34.99	29.21		
	10 d	38.96	36.12	33.97	28.25		
	15 d	37.81	34.92	32.94	27.18	V X S X T	2.435
	20 d	33.85	30.98	29.03	23.16		

Table 6: Beta carotene content (µg/100g) of mango fruits at different intervals during storage in card board carton after salicylic acid application

Treatments \ Varieties		V_1	V_2	V_3	V_4	CD at 5 %	
S_0	5 d	2434.00	2218.35	2374.67	2064.84		
	10 d	0.00	0.00	0.00	0.00	V	46.760
	15 d	0.00	0.00	0.00	0.00		
	20 d	0.00	0.00	0.00	0.00	S	46.760
S_1	5 d	2664.32	2228.31	2576.74	2271.86		
	10d	2694.54	2435.52	2658.79	2312.33	T	46.760
	15 d	2768.68	2512.32	2712.32	2430.39		
	20 d	2859.35	2665.47	2832.57	2495.98	V X S	93.520
S_2	5 d	2715.58	2351.49	2667.64	2352.84		
	10 d	2897.95	2465.44	2758.46	2447.76	V X T	93.520
	15 d	3085.65	2595.13	2968.95	2594.81		
	20 d	3495.93	2778.59	3264.97	2691.89	S X T	93.520
S_3	5 d	2612.58	2218.35	2556.83	2257.59		
	10 d	2681.55	2415.37	2622.80	2297.84		
	15 d	2748.32	2498.85	2702.67	2415.68	V X S X T	187.039
	20 d	2812.22	2627.95	2812.84	2485.47		

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