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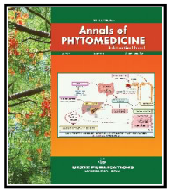

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Exogenous application of calcium chloride on biochemical characteristics of Dragon fruit (*Selenicereus undatus*)

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Abstract

The study reported the effects of different concentrations of $CaCl_2$, applied as spray and soaked treatments on the biochemical traits for anthracnose-infected and non-infected dragon fruits. The findings revealed that the soak treatment outperformed the spray treatments in enhancing the biochemical traits of both infected and non-infected dragon fruit besides improving its storage life. Additionally, the application of 4% $CaCl_2$ concentration with both the application methods significantly improved the fruit's biochemical parameters. However, the soaked treatment demonstrated superior results, in comparison to sprayed treatments thereby, extending the fruit storage life. Therefore, the study highlights the importance of calcium application towards extending fruit shelf-life and also emphasizes upon the potential for enhancing fruit quality.

1. Introduction

Post-harvest loss refers to the decrease in both the amount and quality of food produce from the time of harvest to consumption. Quality losses refer to the deterioration in the nutrient content, acceptability and overall edibility of the product, which is typically more prevalent in the developed countries. While, quantity losses pertain to the reduction in the amount of product which is more commonly observed in developing nations around the globe (Guru *et al.*, 2017). Fruits and vegetables exhibit high perishability, with the most significant losses occurring during the period between harvest and consumption. Approximately, 45-55% of the total global production is wasted or lost due to post-harvest factors. These losses stem from various issues, including physical, physiological, mechanical, and unhygienic conditions. Challenges such as elevated metabolic activities, substantial losses during harvesting, pests and diseases caused by non-infectious pathogens, as well as pathological rots contribute to the substantial % age of post-harvest losses in fruits and vegetables (Yahaya *et al.*, 2019; Lufu *et al.*, 2020).

Physiological losses in dragon fruit primarily result from a heightened presence of disease-causing pathogens, leading to the degradation of the fruit's quality. Anthracnose, a global disease caused by the *Colletotrichum genus*, impacts numerous tropical and subtropical fruits worldwide (Cannon *et al.*, 2012). It is also responsible for significant losses in the crop yield of dragon fruit during both pre- and post-harvesting of crop which can be identified by the presence of dark brown or black sunken lesions on the surface of infected fruits, which contains conidial masses (Dean *et al.*, 2012). These symptoms are highly visible and unappealing to consumers that significantly decreases the market value of the affected fruits, thereby increasing the financial risk to dragon fruit growers (Bordoh *et al.*, 2020; Zakaria *et al.*, 2021).

To overcome such loses that may occur due to the incidence of anthracnose, calcium chloride ($CaCl_2$) can play a vital role (Gayed *et al.*, 2017). Calcium is known to improve the fruit quality by increasing the production of phytoalexin and phenolic compounds in fruit which helps in inhibiting the enzymes released by the fungus. Further, calcium also helps in inhibition of the release of polygalacturonase (PG), pectin-n-methylesterase (PME), and β-galactosidase enzymes which are responsible for cell wall disintegration, hence helps in delaying ageing and ripening processes by reducing the postharvest decay and reducing the loss of major biochemical properties such as total phenols, total flavonoids content, ascorbic acid, titratable acidity besides, an overall increase in the calcium content of the fruit (Serrano *et al.*, 2002; Awang *et al.*, 2017; Razali *et al.*, 2021) . Therefore, taking an account of all the above-mentioned facts the present study was planned to determine the effect of $CaCl_2$ on disease resistance and biochemical traits of dragon fruit.

2. Materials and Methods

2.1 Study site

The research was carried out at the research facility of ICAR-National Institute of Abiotic Stress Management (NIASM) in Baramati (Pune), Maharashtra. The location is positioned at 18° 09' 30.62''N latitude and 74° 30' 03.08''E longitude, with an elevation of 570 meters

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above mean sea level. Baramati experiences an average annual precipitation of 659 mm, distributed from June to September, and maintains an average temperature ranging from a minimum of 13.0°C to a maximum of 33.7°C.

2.2 Experimental detail

To assess disease resistance and biochemical parameters in dragon fruit, a randomized block design with three factors was employed in the experiment. The layout consisted of three replications, incorporating four distinct pre- and post-harvest treatments of $CaCl_2$, specifically 1.0, 2.0, 3.0, and 4.0 g/l in comparison to control. These treatments were applied through spraying and soaking methods on both anthracnose-infected and non-infected white-pulped dragon fruit. Various biochemical parameters, including TSS, total sugar, reducing sugar, non-reducing sugar, phenol, flavonoids, etc., were measured to assess the dragon fruit's response to different concentrations of $CaCl_2$.

2.3 Biochemical parameter

2.3.1 Total soluble solids (°Brix)

Total soluble solids (TSS) in dragon fruit with different treatments of $CaCl_2$ was estimated with help of a digital refractometer (Milwaukee Electronics, US) according to the protocol standardized by AOAC (AOAC, 1965).

2.3.2 Total, reducing and non-reducing sugar (%)

To determine the total sugars in dragon fruit, the methodology developed by Lane and Eynon (1923) was employed. In the analysis, 10 ml of dragon fruit juice was combined with 100 ml of distilled water and 2 ml of 45% lead acetate in a conical flask. The solution was left undisturbed for 2 days, and then 1.9 ml of potassium oxalate (22%) was added to the solution, bringing the final volume to 250 ml with distilled water. From this final volume, a 50 ml sample was extracted and mixed with 5 ml of concentrated HCl, allowing it to stand for 24 h. Subsequently, the sample was neutralized with 40% NaOH until reaching a light pink color, and the final volume was adjusted to 100 ml.

In a distinct conical flask, 2.5 ml of both Fehling's solution A and B were combined and then diluted with 50 ml of distilled water. The resulting mixture was heated to boiling on a hot plate, and a few drops of methylene blue indicator were added. Thereafter, the titration was carried out on the prepared solution using already prepared juice sample in the burette. The attainment of a brick red color was identified as the endpoint, and the %age of total sugar and reducing sugar (%)

2.3.3 Ascorbic acid (mg/100 g)

To determine the concentration of ascorbic acid in dragon fruit, the 2,6-dichlorophenol indophenols visual titration method was employed. For sample preparation, 10 g of pulp were ground using a mortar and pestle, and then mixed with a 3% metaphosphoric acid (HPO_3) solution. The resulting solution was titrated against the dye (2,6-dichlorophenol-indophenol) until a pink color appeared and remained stable for 15 sec. The volume of titrant used was recorded, and the concentration of ascorbic acid was calculated as milligrams per 100 g of pulp (Rangana *et al.*, 1977).

2.3.4 Total phenols (mg/GAE/100 g)

The determination of the total phenolic content in the edible portion of the fruit was conducted utilizing the Folin-Ciocalteu reagent method (Singleton *et al.*, 1965). To 100 µl of the sample extract (dissolved in 80% ethanol), 2.9 ml of distilled water, 0.5 ml of Folin-Ciocalteu reagent, and 2.0 ml of 20% Na_2CO_3 solution were added. The mixture was allowed to stand for 90 min, and subsequently, the absorbance was recorded at a wavelength of 760 nm using a spectrophotometer. The total phenolic content was expressed as micrograms of gallic acid equivalent per gram of fresh weight.

2.3.5 Total flavonoid (mg catechin/100 g)

The aluminum chloride ($AlCl_3$) method, as outlined by Zhishen *et al.* (1999), was employed to assess the total flavonoid content. In this procedure, 1 ml of dragon fruit extract in methanol was combined with 4 ml of distilled water, 0.3 ml of 5% sodium nitrite ($NaNO_2$), and 0.3 ml of 10% $AlCl_3 \cdot 6H_2O$. The mixture was allowed to stand for 6 min at room temperature. Subsequently, 2 ml of 1 N NaOH was added, and the solution was diluted to a final volume of 10 ml with distilled water. The absorbance of the resulting solution was measured at 510 nm using a spectrophotometer against a reagent blank. The total flavonoid content was expressed as micrograms of catechin equivalent per 100g of fresh weight (Jain and Chaudhary, 2023).

2.3.6 Antioxidant (DPPH) (%)

The antioxidant activity of fruit juice was determined using the DPPH (2,2-Diphenyl-1-picrylhydrazyl)free radical scavenging assay as described by Brand-Williams *et al.* (1995). The sample and DPPH reagent were prepared in 70% methanol. A 100 µl sample was mixed with 3.9 ml of DPPH reagent, and the absorbance was measured at 517 nm using a Nanodrop machine (ND2000CLAPTOP). The DPPH solution was allowed to stabilize for 2 h in the dark. For the fruit sample, 10 g was homogenized with 20 ml of 80% ethanol, and the supernatant was used for analysis. To 200 µl of the sample, 4.5 ml of ethanol, 800 µl of Tris-HCl, and 1 ml of DPPH solution were added, and the mixture was kept in the dark for 30 min. The absorbance was measured at 517 nm.

2.3.7 Titratable acidity

To create the sample, 10 g of fruit pulp were pulverized using a mortar and pestle. The resulting blend was filtered, and the juice obtained was gathered in a 100 ml volumetric flask. Distilled water was incorporated to achieve the final volume. A 10 ml portion of this solution was employed for titration following the specified materials and methods. Titratable acidity was assessed by titrating the pulp extract with N/10 NaOH, with phenolphthalein serving as the indicator. The acidity level was expressed in % age (AOAC, 1965).

2.3.8 Residual effect of calcium

Fresh weight samples, each weighing 5 g, underwent drying in a hot air oven. After the drying process, the samples were ground, and 0.5 g of the resulting powder were placed into digestion tubes. To these tubes, 10 ml of a diacid solution (composed of nitric acid and perchloric acid in a ratio of 4:1) was added. The tubes were left for autodigestion overnight. The next day, the tubes were positioned on a digestor and gently heated until dense brown fumes were observed. Subsequently, gentle heating continued until dense white fumes appeared. Any excess solution (approximately 1-2 ml) was removed

upon condensation of the fumes at the neck of the flask. After cooling, the solution's volume was adjusted to 100 ml using double distilled water. The solution was then filtered through Whatman's filter paper No.1, and the filtrate was stored in polyethylene bottles. Calcium analysis was conducted using an Atomic Absorption Spectrophotometer (Analyst 200, Perkin Elmer) following the methodology outlined by Bisergaeva *et al.* (2020).

3. Results

3.1 Total soluble solids (TSS)

The data in Table 1 illustrates the impact of $CaCl_2$ treatments, applied through spraying and soaking, on total soluble solids (TSS) in both anthracnose-infected and non-infected dragon fruit. The study revealed a significant influence of $CaCl_2$ treatment on increasing overall TSS in both categories of dragon fruits. However, soaked treatments were found to be more effective in enhancing TSS in both infected and non-infected dragon fruit compared to sprayed treatments.

In non-infected dragon fruit, soaked treatments with a 4% dose of $CaCl_2$ significantly increased TSS (11.32%) compared to the control (no $CaCl_2$ application). Similarly, sprayed treatment on non-infected dragon fruits showed a significant variation in TSS, with the maximum content (10.56%) observed at a 4% dose of $CaCl_2$ compared to 9.98% in the control.

For anthracnose-infected dragon fruit, the highest TSS (10.64%) was found in fruits soaked with a 4% concentration of $CaCl_2$, while the lowest values (9.75%) were recorded for fruits that were not soaked with any $CaCl_2$ concentration (Control). Similarly, in sprayed treatments, the maximum TSS (10.21%) was reported with a 4% dose of $CaCl_2$ compared to the control. Furthermore, the results indicate that non-infected fruits exhibited higher total soluble solids under both soaked (11.17%) and sprayed (10.31%) treatments compared to anthracnose-infected fruits. This suggests that $CaCl_2$ treatment can help maintain TSS in dragon fruits under both infected and non-infected conditions.

3.2 Titratable acidity

The data presented in Table 1 outlines the observations recorded for spray and soaked treatments of $CaCl_2$ on both infected and non-infected dragon fruit concerning titratable acidity (TA). In both types of dragon fruit, whether infected or non-infected, there was minimal variation observed for different concentrations of $CaCl_2$ applied through spray and soak treatments. The data revealed that for non-infected fruit, the highest % age of TA was observed for those treated with a 4% concentration of $CaCl_2$, measuring 0.46 and 0.60%, respectively. Similarly, for anthracnose-infected fruits, the application of 4% $CaCl_2$ reported the maximum TA, *i.e.*, 0.53 and 0.56%, respectively. However, the data indicates that all the treatments were comparable, suggesting that different treatments did not significantly impact the modification of TA in dragon fruit.

3.3 Ascorbic acid

The data presented in Table 1 outlines the impact of sprayed and soaked $CaCl_2$ treatments on ascorbic acid content in both anthracnose-infected and non-infected dragon fruit. In both infected and non-infected types of dragon fruit, minimal variation was observed for different concentrations of $CaCl_2$ applied through spray and soak treatments. For non-infected fruit, the maximum %age was observed for those treated with a 4% concentration of $CaCl_2$, measuring 9.46 and 9.99%, respectively. Similarly, for anthracnose-infected fruits, the application of 4% $CaCl_2$ reported the highest ascorbic acid content, *i.e.*, 9.91 and 10.26%, respectively. However, the data indicates that all the treatments were comparable, suggesting that different treatments did not significantly modify the ascorbic acid content in dragon fruit.

Table 1: Effect of spray and soaked application of $CaCl_2$ on infected and non-infected dragon fruit for titratable acidity, ascorbic acid and total soluble solids

$CaCl_2$(g/l)	Titratable acidity (%)				Ascorbic acid (%)				TSS (^{0}Brix)			
	Non-infected		Infected		Non-infected		Infected		Non-infected		Infected	
	Spray	Soak	Spray	Soak	Spray	Soak	Spray	Soak	Spray	Soak	Spray	Soak
0.0	0.45	0.54	0.50	0.52	9.10	9.71	9.25	9.91	9.98	10.88	9.95	9.75
1.0	0.45	0.56	0.51	0.55	9.26	9.77	9.56	10.10	10.26	11.07	9.97	10.04
2.0	0.45	0.59	0.52	0.55	9.30	9.81	9.60	10.12	10.29	11.24	9.99	10.28
3.0	0.46	0.59	0.55	0.60	9.32	9.81	9.62	10.17	10.43	11.31	10.13	10.31
4.0	0.46	0.60	0.56	0.61	9.46	9.99	9.91	10.26	10.56	11.32	10.21	10.64
Mean	0.45	0.58	0.53	0.56	9.29	9.82	9.59	10.11	10.31	11.17	10.05	10.20
CD (0.05)												
Factor A	0.028				0.189				0.243			
Factor B	0.028				0.189				0.243			
Factor C	0.03				0.267				0.02			
A × B	0.02				NS				0.343			
B × C	NS				NS				NS			
A × C	NS				NS				0.01			
A × B × C	NS				NS				NS			

3.4 Antioxidant activity (DPPH)

The data presented in Table 2 illustrates the impact of sprayed and soaked $CaCl_2$ treatments on antioxidant activity (DPPH%) in both anthracnose-infected and non-infected dragon fruit. The results revealed a significant influence of $CaCl_2$ treatment on enhancing the antioxidant activity of both types of dragon fruits. Notably, soaked treatments were found to be more effective in boosting the antioxidant activity of both infected and non-infected dragon fruits compared to sprayed treatments.

For anthracnose-infected dragon fruit, soaked treatments with a 4% dose of $CaCl_2$ significantly increased antioxidant activity (67.14 DPPH%) compared to the control (no $CaCl_2$ application). Similarly, sprayed treatment on anthracnose-infected dragon fruits showed a significant difference in antioxidant activity, with the maximum observed (63.18 DPPH%) at a 4% dose of $CaCl_2$ compared to 59.20 DPPH per cent in the control.

Conversely, for non-infected dragon fruit, maximum antioxidant activity (60.88 DPPH%) was found in fruits soaked with a 4% concentration of $CaCl_2$. Similarly, in sprayed treatments, the highest antioxidant activity (59.01 DPPH%) was recorded for fruits that were not sprayed with any concentration of $CaCl_2$ (Control). Additionally, the results illustrate that anthracnose-infected fruits exhibited higher antioxidant activity under both soaked and sprayed treatments (64.51 and 61.12 DPPH%, respectively) compared to non-infected fruits.

3.5 Total phenol content

The data presented in Table 2 highlights the impact of sprayed and soaked $CaCl_2$ treatments on total phenol content in both anthracnose-infected and non-infected dragon fruit. The results indicate a significant effect of $CaCl_2$ treatment in increasing the overall total phenol content in both types of dragon fruits. Notably, soaked treatments were found to be more effective in enhancing the total phenol content in both infected and non-infected dragon fruit compared to sprayed treatments.

For anthracnose-infected dragon fruit, soaked treatments with a 4% dose of $CaCl_2$ significantly elevated the total phenol content (18.16 GAE/100 g) compared to the control (no $CaCl_2$ application). Similarly, sprayed treatment on anthracnose-infected dragon fruits showed a significant variation in total phenol content, with the maximum observed (179.10 GAE/100 g) at a 4% dose of $CaCl_2$ compared to 169.91 GAE/100 g in the control.

Conversely, for non-infected dragon fruit, the maximum total phenol content (177.94 GAE/100 g) was observed in fruits soaked with a 4% concentration of $CaCl_2$. Similarly, in sprayed treatments, the highest total phenol content (173.54 GAE/100 g) was recorded for fruits that were not sprayed with any concentration of $CaCl_2$ (Control). Additionally, the results indicate that anthracnose-infected fruits exhibited higher total phenol content under both soaked and sprayed treatments (178.82 and 174.37 GAE/100 g, respectively) compared to non-infected fruits.

3.6 Total flavonoid content

Table 2 illustrates the impact of $CaCl_2$ application through spray and soaked treatment on the flavonoid content on anthracnose infected and non-infected dragon fruit. Based on the results obtained from the experiment, it was observed that the $CaCl_2$ treatment had a significant effect on increasing the overall flavonoid content both infected and non-infected dragon fruits. However, it was found that soaked treatments were reported to be increased flavonoid content in both infected and non-infected dragon fruit in comparison to sprayed treatments. A significant increase in flavonoid content (84.16 mg catechin/100 g) was observed in anthracnose-infected dragon fruit treated with 4% $CaCl_2$ through the soaked application in comparison to the control (no application of $CaCl_2$). Similarly, a significant variation in flavonoid content was observed with sprayed treatment on anthracnose-infected dragon fruits, with the maximum flavonoid content (79.10 mg catechin /100 g) with 4% dose of $CaCl_2$ was obtained in compassion to 69.91 mg catechin /100 g in control (no application of $CaCl_2$).

Concomitantly, maximum flavonoid content (77.94 mg catechin / 100g) was found in fruits soaked with 4% concentration of $CaCl_2$ in non-infected dragon fruits. However, for the sprayed treatments maximum flavonoid content (73.54 mg catechin /100 g) were recorded for the fruit which were not sprayed with any concentration of $CaCl_2$ (Control). Further, the results also indicate that the anthracnose-infected fruits exhibited higher flavonoid content under both soaked and sprayed treatments, *i.e.*, 78.8 and 74.3 mg catechin /100 g, respectively as compared to the non-infected fruits.

3.7 Total sugar content

The data in Table 3 illustrates the impact of sprayed and soaked $CaCl_2$ treatments on total sugar content in both anthracnose-infected and non-infected dragon fruit. The $CaCl_2$ treatment was observed to significantly increase the overall total sugar content in both types of dragon fruits. However, soaked treatments proved to be more effective in enhancing the total sugar content in both infected and non-infected dragon fruit compared to sprayed treatments.

For non-infected dragon fruit, soaked treatments with a 4% dose of $CaCl_2$ significantly raised the total sugar content (8.79%), followed by 8.67% with a 3% treatment of $CaCl_2$, in comparison to the control (no $CaCl_2$ application). Similarly, in sprayed treatment on non-infected dragon fruits, a significant variation in total sugar content was observed, with the maximum (8.15%) recorded at a 4% dose of $CaCl_2$ compared to 7.62% in the control.

Concurrently, for anthracnose-infected dragon fruit, soaked treatments with a 4% concentration of $CaCl_2$ reported the maximum total sugar content (8.98%), followed closely by 3% (8.96%) and 2% (8.92%) concentrations of $CaCl_2$. Similarly, in sprayed treatments, the highest total sugar content (8.60%) was reported with a 4% application of $CaCl_2$, whereas the minimum values (7.74%) were recorded for fruits not sprayed with any concentration of $CaCl_2$ (Control). Furthermore, the results indicate that anthracnose-infected fruits exhibited higher total sugar content under both soaked and sprayed treatments (8.79 and 8.11%, respectively) compared to non-infected fruits.

3.8 Reducing sugar content

Table 3 highlights the impact of sprayed and soaked $CaCl_2$ treatments on the total sugar content of anthracnose-infected and non-infected dragon fruit. The findings reveal a significant effect of $CaCl_2$ treatment in elevating the reducing sugar content in both types of dragon fruits. However, soaked treatments prove to be more effective in increasing the reducing sugar content in comparison to sprayed treatments for both infected and non-infected dragon fruit.

For non-infected dragon fruit, soaked treatments with a 4% dose of $CaCl_2$ significantly increased the reducing sugar content (4.63%), followed by 4.46% with a 3% treatment of $CaCl_2$, compared to the control (no $CaCl_2$ application). Similarly, in sprayed treatment on non-infected dragon fruits, a significant difference in reducing sugar content was reported, with the maximum (4.45%) recorded at a 4% dose of $CaCl_2$ compared to 4.28% in the control.

Table 2: Effect of spray and soaked application of $CaCl_2$ on infected and non-infected dragon fruit for total phenol content, flavonoid content and antioxidant activity

$CaCl_2$(g/l)	Total Phenol GAE/100 g				Flavonoid mg catechin/100 g				DPPH%			
	Non-infected		Infected		Non-infected		Infected		Non-infected		Infected	
	Spray	Soak	Spray	Soak	Spray	Soak	Spray	Soak	Spray	Soak	Spray	Soak
0.0	167.31	168.09	169.91	176.43	65.91	68.09	69.91	74.44	56.54	57.90	59.20	61.79
1.0	169.30	171.01	172.26	178.43	67.31	71.01	72.70	76.47	57.25	58.79	60.78	64.35
2.0	169.30	173.28	174.26	178.50	69.30	73.28	74.26	78.43	57.80	59.19	61.10	64.07
3.0	170.79	174.30	175.90	180.58	70.79	74.30	75.90	80.58	58.09	59.73	61.35	65.19
4.0	173.54	177.94	179.10	184.16	73.54	77.94	79.10	84.16	59.01	60.88	63.18	67.14
Mean	169.37	172.92	174.37	178.82	69.30	72.90	74.30	78.80	57.75	59.30	61.12	64.51
CD (0.05)												
Factor A	3.962				3.962				2.229			
Factor B	3.962				3.962				2.229			
Factor C	0.01				1.26				0.06			
A × B	NS				NS				NS			
B × C	NS				132				NS			
A × C	NS				NS				NS			
A × B × C	NS				NS				NS			

Table 3: Effect of spray and soaked application of $CaCl_2$ on infected and non-infected dragon fruit for total sugar, reducing sugar and non-reducing sugar

$CaCl_2$(g/l)	Total sugar (%)				Reducing sugar (%)				Non-reducing (%)			
	Non-infected		Infected		Non-infected		Infected		Non-infected		Infected	
	Spray	Soak	Spray	Soak	Spray	Soak	Spray	Soak	Spray	Soak	Spray	Soak
0.0	7.62	8.46	7.74	8.79	4.28	4.38	4.39	4.52	3.34	4.08	3.35	4.22
1.0	7.72	8.54	7.87	8.84	4.32	4.38	4.46	4.55	3.40	4.16	3.41	4.27
2.0	7.80	8.63	8.14	8.92	4.37	4.45	4.47	4.61	3.43	4.18	3.67	4.29
3.0	7.98	8.67	8.23	8.96	4.40	4.46	4.52	4.61	3.58	4.21	3.71	4.31
4.0	8.15	8.79	8.60	8.98	4.45	4.63	4.63	4.78	3.70	4.16	3.97	4.35
Mean	7.85	8.62	8.11	8.79	4.37	4.46	4.49	4.54	3.48	4.15	3.62	4.28
CD (0.05)												
Factor A	0.219				0.094				NS			
Factor B	0.219				0.094				0.184			
Factor C	0.309				NS				0.01			
A × B	NS				NS				NS			
B × C	NS				NS				NS			
A × C	NS				NS				NS			
A × B × C	NS				NS				NS			

Similarly, for anthracnose-infected dragon fruit, soaked treatments with a 4% concentration of $CaCl_2$ reported the maximum reducing sugar content (4.78%), followed closely by 3% (4.61%) and 2% (4.61%) concentrations of $CaCl_2$. In the sprayed treatments, the highest reducing sugar content (4.63%) was reported with a 4% application of $CaCl_2$, whereas the minimum values (4.39%) were recorded for fruits not sprayed with any concentration of $CaCl_2$ (Control). Furthermore, the results indicate that anthracnose-infected fruits exhibited higher reducing sugar content under both soaked and sprayed treatments (4.54 and 4.49%, respectively) compared to non-infected fruits.

3.9 Non-reducing sugar

Table 3 outlines the impact of sprayed and soaked $CaCl_2$ treatments on total sugar content in anthracnose-infected and non-infected dragon fruit. The results indicate that the $CaCl_2$ treatment had no significant effect on non-reducing sugar content in both types of dragon fruits. However, soaked treatments were found to be more effective in preserving non-reducing sugar content in both infected and non-infected dragon fruit compared to sprayed treatments.

In anthracnose-infected dragon fruit, soaked treatments with a 4% dose of $CaCl_2$ significantly increased the non-reducing sugar content (4.35%) compared to the control group with no $CaCl_2$ application. Similarly, in sprayed treatment on anthracnose-infected dragon fruits, a significant variation in non-reducing sugar content was observed, with the maximum (3.97%) recorded at a 4% dose of $CaCl_2$ compared to 3.35% in the control.

For non-infected dragon fruit, soaked treatments with a 4% concentration of $CaCl_2$ resulted in the highest non-reducing sugar content (4.16%). Similarly, in sprayed treatments, the maximum non-reducing sugar content (3.7%) was reported with a 4% application of $CaCl_2$, whereas the minimum values (3.34%) were recorded for fruits not sprayed with any concentration of $CaCl_2$ (Control). Furthermore, the results also indicate that anthracnose-infected fruits exhibited higher non-reducing sugar content under both soaked and sprayed treatments (4.28 and 3.62%, respectively) compared to non-infected fruits.

3.10 Residual effect of $CaCl_2$ on dragon fruit

Table 4 outlines the impact of sprayed and soaked $CaCl_2$ treatments on calcium content in anthracnose-infected and non-infected dragon fruit. The results indicate that the $CaCl_2$ treatment had a significant effect on increasing the calcium content in both types of dragon fruits. However, soaked treatments were found to be more effective in raising the calcium content in both infected and non-infected dragon fruit compared to sprayed treatments.

In non-infected dragon fruit, soaked treatments with a 4% dose of $CaCl_2$ significantly increased the calcium content (16.89 mg/100 g) compared to the control group with no $CaCl_2$ application. Similarly, in sprayed treatment on non-infected dragon fruits, a significant difference in calcium content was reported, with the maximum (15.20 mg/100 g) recorded at a 4% dose of $CaCl_2$ compared to 12.76 mg/100 g in the control group.

For anthracnose-infected dragon fruit, soaked treatments with a 4% concentration of $CaCl_2$ resulted in the highest calcium content (16.42 mg/100 g), followed by 3% (14.09 mg/100 g) and 2% (13.17 mg/100 g) concentrations of $CaCl_2$. Similarly, for the sprayed treatments, the maximum calcium content (14.14 mg/100 g) was reported with a 4% application of $CaCl_2$, whereas the minimum values (10.58 mg/100 g) were recorded for fruits not sprayed with $CaCl_2$ (Control).

Table 4: Effect of spray and soaked application of $CaCl_2$ on infected and non-infected dragon fruit for calcium content of fruit

$CaCl_2$(g/l)	Ca content mg/100 g			
	Non-infected		Infected	
	Spray	Soak	Spray	Soak
0.0	12.76	13.44	10.58	11.58
1.0	12.76	13.53	12.06	12.35
2.0	13.76	15.26	12.67	13.17
3.0	14.43	16.16	12.74	14.09
4.0	15.20	16.89	14.14	16.42
Mean	14.51	15.06	12.44	13.50
CD (0.05)				
Factor A	1.604			
Factor B	0.09			
Factor C	2.536			
A × B	NS			
B × C	0.52			
A × C	NS			
A × B × C	NS			

4. Discussion

The increase in TSS in fruits treated with $CaCl_2$ may be attributed to a reduction in the respiration rate, leading to sugar accumulation. Additionally, the application of $CaCl_2$ contributes to maintaining the structural integrity of fruit cells, aiding in TSS retention (Akhtar *et al.*, 2010). These findings align with Gol *et al.* (2015), who investigated the effects of edible coatings on the quality and shelf life of carambola fruit during storage. Enhanced TSS levels may also be linked to the hydrolytic breakdown of complex polymers into simpler substances, utilized during respiration in later stages of storage (Tharanathan *et al.*, 2006). Similar results have been observed in mango and custard apple (Wahdan *et al.*, 2011; Karemera *et al.*, 2014; Vidya *et al.*, 2014; Bagul *et al.*, 2017 and Reddy *et al.*, 2022).

The decrease in titratable acidity in fruit over time is often attributed to the conversion of carbohydrates into sugars. This phenomenon is thought to result from fermentation or the breakdown of acids into sugars during fruit respiration (Kuswandi *et al.*, 2013). In the present study, it was observed that calcium treatments did not significantly affect the fermentation process, indicating that they did not play a significant role in altering the breakdown of acids and maintaining the titratable acidity of the fruit (Ball, 1997). Similar methodologies and implications have been observed in red-pulped dragon fruit, sapota, pear, and loquat (Bhanja *et al.*, 1994; Akhtar *et al.*, 2010; Awang *et al.*, 2011; Sajid *et al.*, 2019).

The treatment of fruits with $CaCl_2$ resulted in an increase in total phenol and flavonoid content, contributing to enhanced antioxidant properties and improved defence mechanisms against various stress

conditions (El-Beltagi *et al*., 2022). Calcium's role in reducing respiration, maintaining cellular structure, and preventing the reduction of antioxidants during storage is highlighted (Lurie, 1998). Studies in blackberries by Hager *et al.* (2008) and Wu *et al.* (2010) observed high antioxidant capacity in berries, with the application of $CaCl_2$, that significantly delaying the loss of antioxidant activity. Similar effects of $CaCl_2$ on antioxidant activity have been reported in mango, cherry, and papaya (Aghdam *et al.*, 2013; Karemera *et al*., 2014; Antunes *et al.*, 2014; Madani *et al.*, 2016; Sabir *et al.*, 2019).

The presence of Ca_2+ in fruit treated with $CaCl_2$ was observed to positively impact total phenol concentration and antioxidant activity. This effect could be attributed to the activation of NADPH oxidase by calcium, leading to the synthesis of phenolic compounds and increased activity of antioxidant enzymes such as superoxide dismutase, peroxidase, and catalase, acting as a defence mechanism during plant stress (Gómez-Maldonado *et al.*, 2020). Comparable results were also observed in olives, blueberries, blackberries, and strawberries (Turmanidze *et al.*, 2016; Sabir *et al.*, 2019; Langer *et al.*, 2019; Morales Sillero *et al.*, 2021; Lobos *et al.*, 2021).

Flavonoids in plants serve as defence compounds, exhibiting antimicrobial properties and inhibiting the growth and colonization of fungal pathogens like Colletotrichum (causes anthracnose) (Jiang *et al.*, 2021). The presence of anthracnose infection triggers the synthesis of flavonoids as part of the plant's defence response (Madani, 2016). The application of $CaCl_2$ on fruits was found to increase the concentration of total flavonoids and enhance antioxidant activity, this effect can be attributed to the role of calcium ions in activating NADPH oxidase, leading to the synthesis of phenolic compounds and promoting the activity of antioxidant enzymes such as superoxide dismutase, peroxidase, and catalase. These mechanisms contribute to the fruit's defence against fungal pathogens and oxidative stress (Conceiçao *et al.*, 2000; Jiang *et al.*, 2021; Aswany *et al.,* 2023).

$CaCl_2$ was found to have no significant effect on the ascorbic acid content of dragon fruit, which may be attributed to the enzymatic oxidation of L-ascorbic acid to dehydroascorbic acid during metabolic processes. This process may contribute to the lack of an effect on fruits (Patel *et al.*, 2017). These findings align with previous studies conducted in apple, sapota, and pear (Sharafi *et al.*, 2011; Ashoori *et al.*, 2013; Prasad *et al.*, 2017; Patel *et al.*, 2017). However, calcium plays a crucial role in regulating enzymatic activities associated with the biosynthesis of ascorbic acid. It has been reported that calcium not only enhances the activity of enzymes like ascorbate peroxidase, responsible for ascorbic acid regeneration, but also increases the overall ascorbic acid content in fruits, particularly when studying abiotic stress tolerance in plants (Akram *et al.,* 2017).

The reason for the difference in sugar content between soaked and sprayed treatments may be attributed to the deeper penetration of calcium ions in fruits soaked in a $CaCl_2$ solution, allowing for more efficient uptake. This can activate enzymes involved in sugar metabolism, leading to an increase in total sugar content. Conversely, when fruits are sprayed, the solution may remain mostly on the surface, penetrating less deeply into the fruit tissue, resulting in lower calcium ion uptake and potentially less activation of sugar metabolism enzymes. Similar findings have been reported in previous studies on red-pulped dragon fruit, suggesting that the application of $CaCl_2$ plays a role in these effects (Manganaris *et al.*, 2007; Awang *et al.*, 2013).

Calcium's role in regulating enzymatic and non-enzymatic processes in sugar metabolism may enhance sugar content. Additionally, calcium has been shown to reduce enzyme levels and increase neutral sugar content in dragon fruit. The initial increase in total sugar content may be attributed to starch conversion into sugar through hydrolysis, but a subsequent decline can be expected once complete hydrolysis occurs. Similar trends have been observed in previous studies on dragon fruit, figs, mangoes, sapotas, and apples (Ali *et al.*, 2004; Islam *et al.*, 2012; Patel *et al.*, 2017; Parmar *et al.*, 2020; Souza *et al.*, 2023).

The application of calcium is known to potentially increase the content of reducing sugars. This increase is believed to occur due to a reduction in sugar utilization for respiration and the conversion of starch into sugars. However, during storage, a subsequent decline in reducing sugar content may occur as the sugars are consumed for respiration (Patel *et al.*, 2017). Similar results have been reported in previous studies conducted on dragon fruit, apples, strawberries, and tamarillos (tree tomatoes) (Awang *et al.*, 2011; Sharafi *et al.*, 2011; Chen *et al.*, 2011; Pinzón-Gómez *et al.*, 2014; Samidha and Ranade, 2022).

The stability and lower reactivity of non-reducing sugars compared to reducing sugars, which lack a free carbonyl group, may explain the observed results. Non-reducing sugars, such as sucrose, do not readily participate in reactions. The increase in non-reducing sugar content during storage can be attributed to the conversion of starch into sugar. However, the decrease in sugar content may be due to the utilization of sugar for respiration during the storage period (Patel *et al.*, 2017). Similar results have been reported in red-pulped dragon fruit, ber, and guava (Inderjit *et al.*, 2009; Islam *et al.*, 2012; Luo *et al.*, 2019; Parmar *et al.*, 2020; Arivalagan *et al.*, 2021; Kaushik *et al.*, 2021; Trong *et al.*, 2022).

The application of calcium on fruit has minimal environmental impact, and its effective treatment aids in retarding fruit ripening and preserving fruit quality (Gao *et al.*, 2019; Gao *et al.*, 2020). Similar results were also reported in grapes, where the development of surface irregularities and cracks during the later stages of fruit growth facilitated calcium penetration (Conway *et al.*, 2001; Shi *et al.*, 2022). This phenomenon was also observed in apples, indicating the mobilization and integration of soluble calcium into the cell wall. Similar effects were demonstrated in mangoes, while Pessoa *et al.* (2022) observed similar effects in pears (Saftner *et al.*, 1998; Singh *et al.*, 1999; Chardonnet *et al.*, 2003; Manganaris *et al.*, 2007).

5. Conclusion

The application of calcium chloride ($CaCl_2$) on dragon fruit has demonstrated a noteworthy enhancement in its biochemical properties. The diverse treatments employed revealed significant variations in the fruits' outcomes. Optimal results, particularly in biochemical parameters such as Total Soluble Solids (TSS), titratable acidity, sugar content, antioxidants, and phenolic compounds, were observed when dragon fruit was subjected to various concentrations of calcium chloride (1.0%, 2.0%, 3.0%, and 4.0%) through both spray and soak applications. This was evident in both anthracnose-infected and non-infected dragon fruit, surpassing the results of the control group (no $CaCl_2$ treatments).In summary, the application of $CaCl_2$ has not only proven to enhance the quality of dragon fruit while extending its shelf life of the fruit as well. Consequently,

incorporating these $CaCl_2$ treatments in dragon fruit cultivation holds the potential for substantial income returns for farmers. Additionally, these treatments are environmentally friendly and deemed safe for consumer intake.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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