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Synergistic Effect of Preharvest Spray Application of Natural Elicitors on Storage Life and Bioactive Compounds of Date Palm (*Phoenix dactylifera* L., cv. Khesab)

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Abstract: Despite the immense capabilities of the date palm, maintaining the fruit's quality, marketability, and shelf life is still a challenge. This study aimed to assess the synergistic effect of a preharvest spray application of a natural elicitor chitosan, (Ch) 1% alone and in combination with salicylic acid (SA) 2 mM and calcium chloride (Ca) 3%; (Ch,SA, Ca,Ch+Ca, Ch+SA, Ch+SA+Ca), on the quality parameters, storage life, and bioactive compounds content of date fruit from 'Khasab' cultivar during cold storage for 60 days. The obtained results revealed that all treatments significantly retard senescence/decay of the fruit compared to the control. Ch+SA treated fruit followed by Ch, and Ch+SA+Ca had the lowest weight loss, color change, and the least decay after 60 days of storage. Ch+Ca, SA, Ca treated fruit had significantly lower levels of total soluble solids and highest total phenolic, tannins, and flavonoids contents compared to the control fruit. Antioxidant activities were found in all treatments, with significantly higher effect in Ch+SA+Ca and Ch+SA compared to the control. Our results provide an evidence for a synergistic effect of elicitors combination to extend the shelf life of date fruit during cold storage by preserving its quality and decreasing senescence/decay and recommend it as a promising strategy.

Keywords: date fruit; shelf life; salicylic acid; chitosan; quality

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1. Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit trees grown in arid and semi-arid regions. The tree thrives in the hot and dry regions of Middle Eastern and North African countries and is highly valued as a 'blessed tree' known since ancient times. It is an essential part of the cultural heritage, traditional books, and poems of Arabian countries. The crops of this tree substantially contribute to the economic development of these countries [1,2].

Apart from its agricultural and commercial importance in the Arabic Gulf region, the fruit has high nutritional value including dietary fibers, carbohydrates, proteins, vitamins, minerals, phenolic, tannins, and antioxidants compounds [3–6]. Based on Arabic terminology, the fruit's maturation and developmental stages were classified as Hababouk, Kimri, Bisr or Khalal, Rutab, and Tamer [7]. These stages characterize the cell division, immature green (cell elongation), the mature hard full colored, the soft brown, and the firm raisin-like fruit, respectively [8]. In general, the harvesting and marketability of the date fruit depends on the cultivar in the context of the level of astringency, physiological conditions, and public demands; hence they are delivered to the market at three stages; Bisr, Rutab, and Tamer (Mohamed et al. 2014). These three ripening stages can be described as

follows: Bisr (full-size, crunchy, colored and mostly edible), Rutab (the fruit apex becomes soft and brown, most cultivars consumed fresh at this stage), and Tamer (hazel to dark brown, wrinkled, raisin-like dry fruit).

Khesab is a very common cultivar in the UAE, and it is generally consumed at the Rutab stage (half soft fruit). However, upon harvesting, the fruit starts losing its firmness, color, taste, and overall physiology which eventually lowers its market value [7]. These changes are outcomes of the postharvest activity of various ripening related enzymes and hormones that soften the fruit tissue, decrease the firmness and actual integrity of the fruit, and ultimately lead to the fruit's senescence [9]. Therefore, slowing down the ripening process and senescence is critical in terms of commercial advantages [10].

Many studies have been conducted to retard postharvest changes in the quality of date fruit using various approaches such as cold storage, Modified Atmosphere Packaging [11], and Controlled Atmosphere Storage (CA) [11–14]. An alternative approach is the application of natural elicitors to enhance physiological adaptations and accelerate the plant's defense system [15,16]. Various studies on pre and postharvest applications have gained success in different fruit crops by using eco-friendly elicitors such as chitosan [9,17–20], salicylic acid [10,21–25], and calcium chloride [26–28], retarding tissue softening, delaying ripening, enhancing quality, extending shelf life, and preventing fruit deterioration. Chitosan is one of the most recognized natural elicitors known for its antimicrobial, eliciting, and film forming characteristics [16,19]. Pre- and post-harvest studies in various fruit crops have shown the positive effects of chitosan on the quality and the shelf and storage life of fruit and vegetables [17–20]. Besides chitosan, calcium chloride is another natural elicitor that has a well-known role in the physiology of plant tissue. Calcium has been reported to play an important role in retarding tissue softening and delaying ripening [26–28]. The use of calcium chloride after the fruit has been harvested maintains cell turgor, membrane integrity, tissue firmness, and delays membrane lipid catabolism, thus extending storage life of fresh fruits [26]. Another natural elicitor is salicylic acid, which is a simple phenolic phytohormone with various roles in plant growth and developmental processes. Pre/post-harvest treatments with salicylic acid can dramatically enhance the quality and shelf life, and prevent deterioration in many fruits [10,21–25]. These elicitors exert their effects by employing different mechanisms such as triggering the synthesis of phytochemicals, enhancing the production of particular antioxidant enzymes, and reducing ethylene production in vegetables and fruit [15,19,29].

To the best of our knowledge, limited research is available on the 'Khasab' cultivar and no report is yet available on the synergistic effects of natural elicitors on 'Khasab' fruit, as preharvest spray application. Therefore, this study aims to investigate the combined effects of chitosan with salicylic acid and $CaCl_2$ on 'Khasab' fruit quality at harvest and during storage time for 60 days, as well as to examine the impact of these elicitors at different maturity stages on the chemical properties and phytochemical content after harvesting and during cold storage.

2. Materials and Methods

2.1. Plant Material and Sampling

During the 2020 season, uniform date palm trees (*Phoenix dactylifera* L., cv. Khesab) were randomly selected in the experimental farm of the College of Food and Agriculture located in Al Foah region, Al Ain, UAE, located in the co-ordinate latitude and longitude of 24.2191° N and 55.7146° E. The trees were pruned to maintain a leaf to bunch ratio of 8: 1 and the number of female spathes per palm was adjusted to 8. The design of the experiment was a randomized complete block design with 6 palms (replicate) from each 'Khasab' palm receiving 7 different treatments (one treatment for each bunch). Three stages of development (5 and 15 weeks from pollination, and two weeks before harvest) were selected for spraying treatments with different elicitors: chitosan, SA, and $CaCl_2$ alone and in combinations (7 treatments): Control (Water), Ch, SA, Ca, Ch+Ca, Ch+SA, Ch+SA+Ca (Table 1). The control date palm trees were sprayed with only deionized

water. Some date palm trees were sprayed with only deionized water for control. The harvesting of bunches were done at the commercial stage (when roughly about 50% of dates have reached the Rutab stage (half Bisr)). After washing, fruits were carefully separated according to their maturity level (Bisr and 50% Rutab (so-called Rutab)). Then Bisr fruit were used for further analysis. Randomly, 100 fruits were collected from each treatment for initial physiochemical, phytochemical and bioactive properties and microbial analysis at harvest time (day 0) [30]. Another batch of 500 fruit from each treatment was collected and stored in punched plastic bags (100 fruit in each bag) and stored at 2 °C and 90–92% relative humidity (RH) for a period of 60 days. For each time interval (15 days) a bag was withdrawn randomly for analysis.

Table 1. Preharvest spry treatments of 'Khesab' date fruit with different elicitors.

Treatment	Chemical	Application
Control	Water	Water
Ch	Chitosan	1%
Ca	Calcium chloride	2 mM
SA	Salicylic acid	3%
Ch+SA	Chitosan+Salicylic acid	1:1, *v/v*
Ch+Ca	Chitosan+Calcium chloride	1:1, *v/v*
Ch+Ca+SA	Chitosan+Calcium chloride+Salicylic acid	1:1:1, *v/v/v*

2.2. Physiochemical Analysis

2.2.1. Fruit Characteristics

The fruit dimensional studies were performed after harvesting according to Rastegar et al. [31]. The loss of fruit weight during storage was recorded for each treatment once every two weeks and reported as a percentage of weight loss against the original weight before the cold storage, utilizing the following equation:

$$\text{Fruit weight loss\%} = (\text{Initial weight} - \text{Weight at specific interval})/(\text{Initial weight}) \times 100$$

2.2.2. Fruit Ripening and Decay Percentage

A visual evaluation of fruits was done to record the ripening burst (fully Rutab) and spoilage every other week until a complete decay of fruit occurred during storage for 60 days. The ripening and decay percentages were calculated using the following equation [32]:

$$\text{Decay or ripe fruit (\%)} = \text{"Number of ripe or number of decay fruit"}/\text{"Total fruit number"} \times 100$$

2.2.3. Total Soluble Solids (TSS)

Ten grams of pitted date fruits were mashed and blended with 10 mL of distilled water using mixer blender to prepare a slurry which was filtered to get the clear juice. The Brix value was determined in the juice using a digital refractometer (DR 6000, A. Kruss Optronic GmbH, Hamburg, Germany).

2.2.4. Fruit Surface Color

The surface color of fruits was determined by using a Hunter Lab colorimeter (Hunter Lab Inc., Reston, VA, USA). The values came out were represented as L^* (brightness), a^* (blue/yellow), and b^* (red/green) [33]. These values were further used to calculate the Hue angle ((h^o), chroma (C^*), and total color difference (ΔE^*) as follows; $h^o = 180° + \arctan(b^*/a^*)$; $(C^*) = (a^*2 + b^*2)^{1/2}$.

$\Delta E = [(L^* - L^*_0) + (a^* - a^*_0) + (b^* - b^*_0)]^{1/2}$ [34], where L^*_0, a^*_0, and b^*_0 values were from control fruit at harvest time (day zero) [35].

2.3. Determination of Total Microbial Load

The total fungal/mold and bacterial count on fruit samples was determined upon harvest (day 0) and at the end of storage (60 days). One gram of mashed fruit tissue was vortexed vigorously in 9 mL autoclaved buffered peptone water under sterile conditions and serially diluted to prepare several different dilutions. Employing the pour plating technique, a 1 mL sample from each dilution was plated with plate count agar (PCA) which was incubated at 37 °C for 48 h and potato dextrose agar (PDA) incubated at 27 °C for 5 d. The number of colonies was recorded at the end of incubation time and calculations were performed using Log_{10} colony forming unit per g of fresh weight (Log_{10} CFU g^{-1}).

2.4. Extraction of Bioactive Compounds

The extraction of phenolic compounds was performed via homogenizing two grams of fresh fruit samples in 20 mL of 80% methanol with water bath shaker at 150 rpm for 24 h, 45 °C. Whatman #1 filter paper used to filter the slurry. The filtrate (date extract) was used for further analysis.

2.4.1. Phytochemical Analysis

Total Phenolic Content (TPC)

The total phenolic content was measured according to Velioglu et al. [36] with some modifications. Date extract (100 µL each) was added into clean and dry test tubes, followed by 50 µL of Folin Ciocalteu reagent, and then vortexed. All the tubes were incubated at room temperature for 2 min. Then, 2 mL of NaOH (6%) was added to each tube and incubated in the dark for 45 min. UV-visible spectrophotometer was used to measure absorbance at 750 nm. The results obtained were expressed as milligram gallic acid equivalents (GAE) per 100 g of fresh weight (mg 100 g^{-1} GAE) according to the standard curve obtained by measuring the absorbance of known concentrations of gallic acid [36].

Total Tannin Content (TTC)

The amount of tannin in the date extracts was determined using the colorimetric method described by Bentebba et al. [37], with some modifications. First, 1 mL of 4% vanillin solution prepared in absolute ethanol and 0.2 mL of HCl (37%) were added to 0.4 mL of extract or catechin as standard. The mixture was then shaken and allowed to react in dark at room temperature for 15 min before measuring the absorbance at 500 nm using a spectrophotometer. The total tannin content was expressed in milligram of catechin equivalents per 100 g of fresh weight (mg 100 g^{-1} CE).

Total Flavonoid Content (TFC)

Total flavonoids were determined according to Kim et al. [38] with some modifications. First, 75 µL of NaNO2 (5%) was added to the date extract (250 µL) in a test tube and vortexted followed by incubation for 5 min in the dark. Afterwards, 75 µL of AlCl3 (10%) was added; the mixture was vortexed and kept in the dark for 6 min followed by addition of 500 µL of NaOH (1 M) then complete the volume to 2.5 mL with distilled water. Then, 510 nm wavelength was utilized to measure the absorbance in a spectrophotometer (Shimadzu, Kyoto, Japan). The results were expressed as milligram catechin per 100 g fresh weight basis (mg 100 g^{-1} CE).

2.4.2. Antioxidant Activities

Antioxidant activities were evaluated according to the method reported in Abd Elwahab et al. [39].

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was determined as defined by Wu et al. (2003), with slight modifications. The 1.5 mL samples with concentrations ranging from 0.5 to 10 mg L^{-1} were mixed with 1.5 mL of 0.15 mM 2,2-diphenyl-1-picryl hydrazyl (DPPH) in

95% ethanol. After 30 min incubation at room temperature in dark conditions, readings were taken at 517 nm using a spectrophotometer. Blank was prepared for each concentration in the same manner, except that ethanol was utilized in place of DPPH solution. IC50 values (mg mL^{-1}) were calculated for each sample.

ABTS Radical Scavenging Activity

The ABTS radical scavenging activity was determined according to Arnao et al. (2001). The stock solutions of ABTS (7.4 mM) and potassium persulphate (2.6 mM) were prepared and kept in the refrigerator (4 °C). Equal parts of the two stocks were mixed and allowed to react for 12 h at ambient temperature in dark. Just prior to perform assay, the solution was further diluted by combining 1 mL of ABTS solution with 50 mL of methanol, yielding an absorbance of 1.1 ± 0.02 units at 734 nm as measured with a spectrophotometer. For each assay, a fresh ABTS solution was made. Then, 150 µL of sample with concentrations ranging from 0.5 to 10 mg mL^{-1} was combined with 2850 µL of ABTS solution and left at room temperature for 2 h in the dark. The absorbance was then measured at 734 nm using UV-visible spectrophotometer. Blank was prepared for each concentration in the same way, except that methanol was utilized in place of ABTS solution. A Trolox standard curve ranging from 50 to 600 µM was generated. The activity was recorded as milligram of Trolox equivalents (TE) per 100 g fresh weight basis (mg 100 g^{-1} TE).

2.5. Statistical Analysis

Analysis of variance (ANOVA) was employed for the completely randomized design experiment with six replicates using SAS statistical software (SAS Institute Inc., 2000, Cary, NC, USA). Least significant differences (LSD) at level $p \leq 0.05$ were utilized to compare means between treatments at each time interval for each analysis. Correlation coefficient between the main biochemical and physical characteristics was also done by SAS.

3. Results and Discussion

3.1. Physical Quality Characteristics of Fruit at Harvest

The average weight, width, and length of 'Khasab fruit at harvest are shown in Table 2. Significant differences ($p < 0.05$) in physical characteristics of treated 'Khasab' fruit were observed as affected by preharvest elicitors treatments. The average weight, width, and length of Khesab fruit were significantly ($p < 0.05$) higher in SA and Ch+SA treatments as compared to control and other treatments. These improvements in fruit physical properties might be due to the influence of SA in combination with Ch in maintaining cells integrity and enhancing the strength and improving weight and size of the fruit [40]. Similarly, Mohamed et al. [10] described that preharvest spray application of SA increased date fruit weight, width and length significantly compared to control fruit. The physical characteristics of fresh fruit are important as they directly indicate the physical impact of the applied elicitors. The above results show that using SA alone or in combination with Ch had an influence on the physical characteristics of fresh 'Khasab' fruit.

3.2. Fruit Weight Loss during Cold Storage

Weight loss is a significant factor that reduces postharvest fruit storage life and marketability [10,40]. Figure 1 presents the percentage of weight loss occurred during cold storage at 2 °C and 90–92% RH for 60 days in harvested fruit. The results revealed that 'Khasab' fruit weight was significantly ($p < 0.05$) influenced by the application of elicitors. In all fruit, weight loss occurred steadily at different levels for different treatments during storage. In comparison to the control, significant differences in weight loss among different treatments were apparent. After 60 days of storage, the lowest weight loss was observed in Ch+SA treated fruit followed by SA, Ch+Ca, Ca, Ch with 12.64, 14.74, 15.06, 15.76 and 16.57%, respectively (Figure 1). Weight loss during storage can be due to a rise in respiration rate and/or moisture loss from the fruit in general [10,26,41].

Table 2. Effect of different preharvest treatments on 'Khesab' fruit physical characteristics and phytochemical analysis at harvest.

	Fruit Weight (g)	Fruit Length (mm)	Fruit Width (mm)	TPC (mg/100 g)	TTC (mg/100 g)	TFC (mg/100 g)	IC50 (mg/mL)	ABTS (mg/g)
Control	10.2 ± 0.11 b	35.0 ± 0.38 b	23.5 ± 0.24 b	407.4 ± 12.2 c	73.9 ± 0.75 c	71.5 ± 0.34 bc	1.7 ± 0.03 b	1.9 ± 0.07 b
Ch	10.9 ± 0.22 b	35.2 ± 0.36 b	24.1 ± 0.27 ab	394.6 ± 8.89 c	42.0 ± 0.43 e	87.8 ± 0.42 b	3.3 ± 0.10 a	1.6 ± 0.11 c
Ca	10.0 ± 0.22 b	34.7 ± 0.38 b	23.9 ± 0.23 b	448.5 ± 11.3 b	61.9 ± 0.68 d	67.9 ± 0.22 c	1.7 ± 0.06 b	1.5 ± 0.09 c
SA	11.8 ± 0.25 a	36.7 ± 0.40 a	24.2 ± 0.25 ab	394.9 ± 10.7 c	47.8 ± 0.26 e	66.4 ± 0.31 c	2.1 ± 0.07 b	1.3 ± 0.17 d
Ch + SA	12.4 ± 0.46 a	37.2 ± 0.48 a	25.6 ± 0.32 a	407.2 ± 13.2 c	59.5 ± 0.45 d	86.6 ± 0.49 b	1.8 ± 0.05 ab	1.9 ± 0.18 b
Ch + Ca	10.8 ± 0.39 b	34.9 ± 0.51 b	24.2 ± 0.34 ab	451.1 ± 13.4 b	80.7 ± 0.64 b	82.9 ± 0.54 b	1.8 ± 0.04 ab	1.2 ± 0.12 d
Ch + Ca + SA	10.6 ± 0.16 b	35.2 ± 0.33 b	24.2 ± 0.21 ab	486.8 ± 12.7 a	116.2 ± 0.73 a	91.4 ± 0.41 a	1.5 ± 0.04 b	3.2 ± 0.13 a

Values are the mean (n = 25) ± SE for the physical characteristics, n = 3 for the phytochemical analysis. Means with different letters in the same column are significantly different at $p > 0.05$ using LSD test. TPC = Total Phenolic Content; TTC = Total Tannin Content; TFC = Total flavonoid Content. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

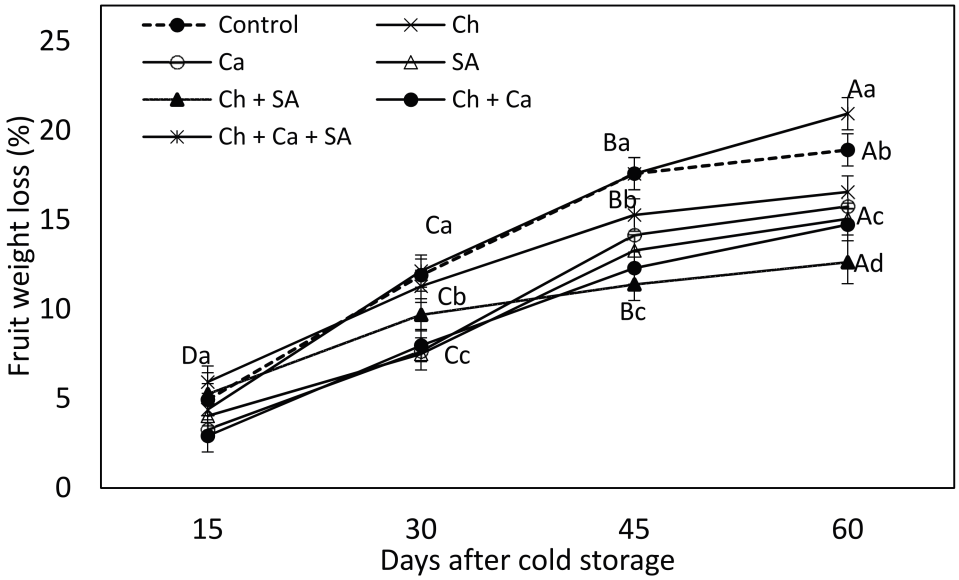

Figure 1. Effect of different preharvest treatments on weight loss of 'Khesab' fruit during cold storage, values are the mean (n = 25) ± SE. Means with different letters between treatments (small letter) at different time interval (capital letter) are significantly different at $p < 0.05$ using LSD test. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

The low weight reduction observed in fruit sprayed with chitosan alone might be due to the film forming properties which significantly decreased evaporation rate of water from the fruit, as noted in case of other fruit treated with chitosan [19,42,43]. Also, Ca and SA were found to have significantly lower weight loss compared to control in constant to Atia et al. [44] reported that pre-storage treatment with Ca and SA reduced weight loss in 'Barhi' dates at Khalal stage. Also, Kassem et al. [40] and Mohamed et al. [10] found that the preharvest application of SA reduced weight loss during storage in treated 'Khesab' fruit compared to the control. SA is well known to reduce chilling injuries, inhibit ripening, and act against various abiotic and biotic factors [45]. This suggests the positive physiological condition of fruit treated with SA presumably owing to decreased respiration rate that can also be associated with the improved fruit turgidity. In dates, typically, the weight of the fruit increases as the maturation advance and at its peak at the Khalal stage, then promptly decreases as ripening progresses [46]. Based on the above results, the weight loss reduction observed in fruit treated with Ch alone or in combinations with SA and Ca, indicates the good physiological conditions of the treated fruit, most likely as a result of the reduced respiration and transpiration rates, and the regulating effects of these elicitors on the ripening process.

3.3. Total Soluble Solids (TSS)

The TSS concentrations exhibited significant variation ($p < 0.05$) among different treatments (Figure 2). Overall, in all fruit, the concentration of TSS increased gradually with advance of ripening during cold storage. At harvest and after 60 days of cold storage the control fruit had the highest TSS concentration (from 32 to 42%) followed by Ch+SA treated fruit [35]. The Ca treated fruit had the lowest TSS concentration (from 28% to 38%). Similarly, Ch+Ca, and SA, treated fruit had significantly ($p < 0.05$) lower levels of TSS compared to the control fruit after 15, 30, 45 and 60 days of cold storage (Figure 2). It has been reported that the effectiveness of elicitors is determined by the reactivity of the fruit tissues, which decrease with ripening progress [19]. In this study, the observed TSS concentrations were similar to those described by Kassem et al. [40], in SA treated date fruit. However, Mohamed et al. [10] reported no significant difference between SA treated and the control date fruit, in relation to TSS content. The results from Ca treatment might be attributed to the high concentration of the Ca (3%) in the treatment. Normally, TSS increase during cold storage could be attributed to the degradation of large polysaccharide molecules into smaller sugars via enzymatic activities and water loss [10,47]. These results are consistent with weight loss results shown in Figure 1. Based on our findings, the degradation of polysaccharides was slowed down by Ca alone or in combination with Ch and SA, resulting in reduced TSS concentrations in treated fruit, and thus a lower rate of ripening.

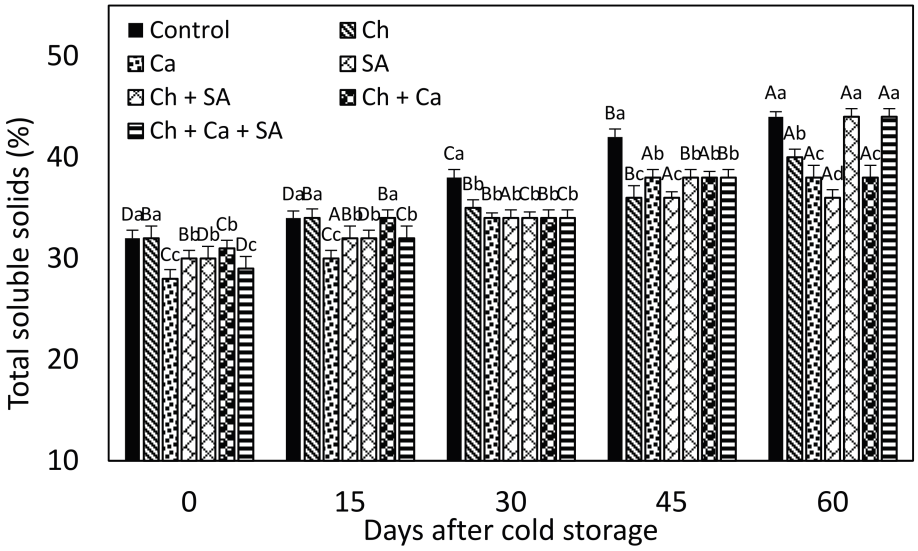

Figure 2. Effect of different preharvest treatments total soluble solids content of 'Khesab' fruit during cold storage, values are the mean ($n = 25$) ± SE. Means with different letters between treatments (small letter) at different time interval (capital letter) are significantly different at $p < 0.05$ using LSD test. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

3.4. Fruit Ripening

The results of fruit ripening during cold storage at 2 °C for 60 days are presented in Figure 3. Different treatments significantly ($p < 0.05$) delayed fruit ripening during the storage time. Generally, Rutab percentage gradually increased throughout the cold storage period, in all treatments with varying degrees. At the end of storage time, the highest Rutab percentage was observed in the control fruit (90.3%) compared to the treatments; SA, Ch+SA, Ch+Ca, Ch, Ch+SA+Ca, and Ca treated fruit with a range from 79–83%, respectively. The Ch+SA+Ca alone treatment significantly delayed the normal ripening of the Bisr fruit compared to the other treatments that combined with Ca. These results are consistent with TSS results shown in Figure 2, where Ca, Ch+Ca, Ch+SA+Ca, and SA treated fruit had lower TSS content in comparison to the control and other treatments.

Fruit ripening (Rutab %) was positivity correlated with the increase in TSS and weight loss (Table 4). These observations may also be viewed as evidence of elicitors delaying 'Khesab' fruit ripening during cold storage. Calcium application is well known for slowing the rate of respiration and delaying fruit ripening [26–28]. Similarly, the preharvest application of SA delayed date fruit ripening by three weeks compared to the control fruit [40]. In addition, pre- and postharvest treatments with SA have been reported to improve quality, shelf life, and prevent deterioration in many fruit [20,26,48]. Also, fruit ripening can be slowed by the film forming ability of Ch, creating a barrier for gas exchanges and decreased respiration. It should be noted that a less ripe fruit is less susceptible to postharvest rot [19].

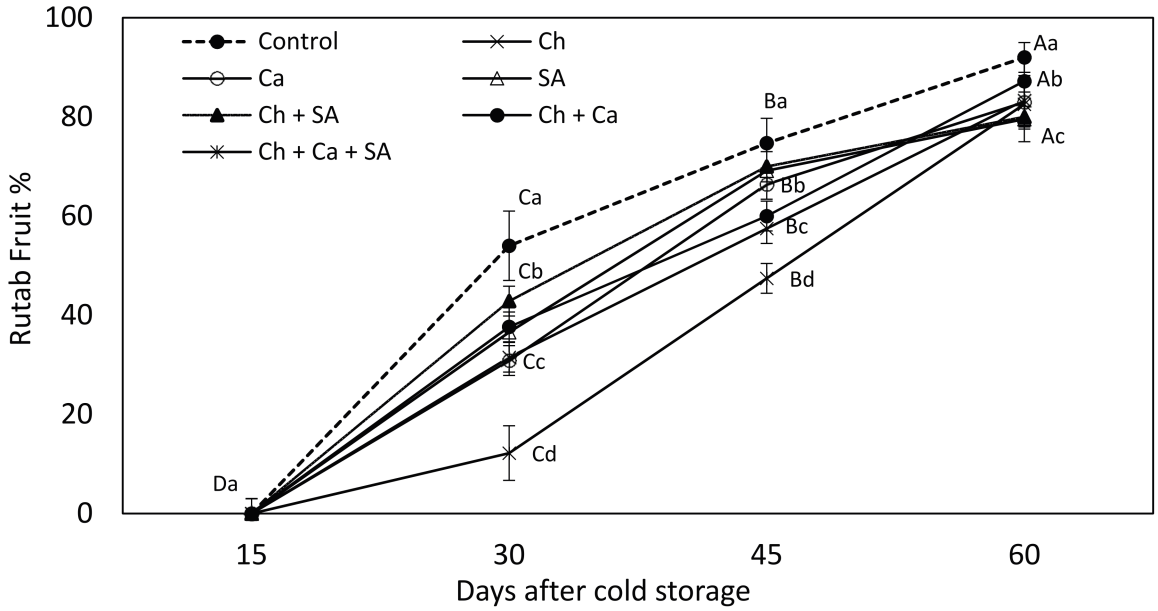

Figure 3. Effect of different preharvest treatments on 'Khesab' fruit ripening (Rutab %) during cold storage. Values are the mean (n = 25) ± SE. Means with different letters between treatments (small letter) at different time interval (capital letter) are significantly different at $p < 0.05$ using LSD test. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

Fruit maturation is a natural phenomenon that leads to membrane impermeability and gradual senescence of the fruit tissues due to oxidation of essential membrane components [8]. Ethylene is the main responsible hormone with the processes of ripening and senescence [40]. Accordingly, it is possible that sprayed elicitors may control the production or action of ethylene, and as a result decelerated fruit ripening (retard of Rutab occurrence). Our results showed the delaying effect of Ch, Ca, and SA in different combinations on fruit ripening compared to the control. Nevertheless, date maturation is not an asynchronous process in all date fruit in the same bunch, as there can be fruit in various stages of maturation in the same bunch at any given time. In the same bunch, Bisr fruit that received the same treatment, for example, could enter the Rutab stage later than the control one. As a result, various major biological variations among individual fruit within the same bunch can be accountable for certain contradictory findings [10].

3.5. Fruit Decay during Storage

The results of fruit decay percentage during cold storage at 2 °C for 60 days are presented in Figure 4. It was observed that different preharvest spray treatments significantly ($p < 0.05$) impacted fruit decay incidence during cold storage period. Fruit decayed progressively during storage, with the exception of Ch and Ch+ SA treated fruit with no decay observed until 45 days (Figure 4). Among all the treatments, after 60 days of storage, control samples had the highest fruit decay percentage with about 55.6% while Ch+SA+Ca, Ch, and Ch+SA had the lowest (11.6, 25.9–25%) respectively compared to other treatments (Figure 5). Likewise, SA at concentrations of 1 and 2 mmol L-1 reported

to be effective in reducing berry drop and decay in grapes [22]. SA and Ca, alone or in combination, increased fruit hardness and delayed softening, resulting in less decay in strawberry fruit [49]. Similarly, use of elicitors such as SA, Ca, and oxalic acid have been reported to induced defense response and decreased the decay in other fruit [20,50,51]. The findings of the present study demonstrated that using Ch in combination with Ca and SA could substantially protect 'Khesab' fruit from rotting during cold storage, likely by triggering defensive mechanisms in the fruit and maintained solidity of the exocarp.

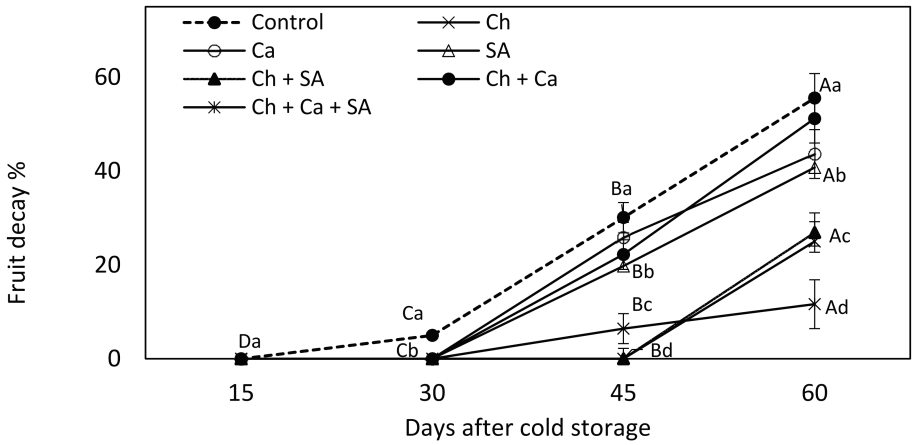

Figure 4. Effect of different preharvest treatments on 'Khesab' fruit decay during cold storage, values are the mean (n = 25) ± SE. Means with different letters between treatments (small letter) at different time interval (capital letter) are significantly different at $p < 0.05$ using LSD test. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

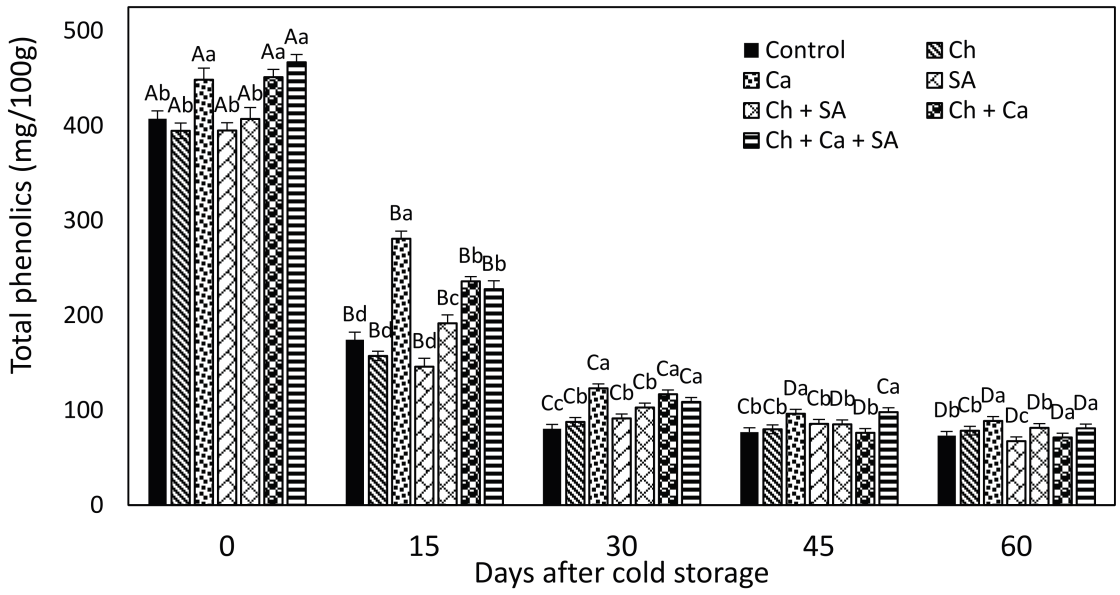

Figure 5. Effect of different preharvest treatments on total phenol content of 'Khesab' fruit during cold storage, values are the mean (n = 3) ± SE. Means with different letters between treatments (small letter) at different time interval (capital letter) are significantly different at $p < 0.05$ using LSD test. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

3.6. Color Attributes

Color is an essential visual attribute of food, especially for fresh fruit, that has a direct impact on the product attractiveness and acceptability by the consumers [52]. Thus, the effect of treatments on color differences was considered for evaluating the quality of fresh 'Khesab' fruit. Important color parameters, i.e., ΔE, C^*, L^*, and h^o were determined to follow

up the color changes in the fruit during the cold storage after the elicitor treatments and are presented in Table 3. Generally, color parameters were significantly ($p < 0.05$) impacted by the application of elicitors. Also, the color values gradually decreased in all fruit with the progress of storage time. L^* values of all fruit decreased with storage progress, with significant reduction in fruit lightness from 37 to 21 after 60 days. After 30 days significant differences were noticed between treatments, as Ch and Ch+SA had the lowest decrease in the L^*, C^* and h^o.

After 60 days, the lowest reduction in L^* value was observed in fruit treated with Ca followed by SA, Ch and Ch+SA (Table 3). The same treatments also had lower ΔE values compared to other treatments including the control. Similarly, by the end of the storage time, C^* was highest in Ch+Ca followed by Ca, Ch, control, Ch+Ca+SA, Ch+SA, and SA treated fruit. L^*, C^*, and h^o values were positively correlated as presented in Table 4, with the increasing TSS and the ripening, whereas ΔE was opposite. The noticed delay in the color change of the fruit treated with Ca, SA and Ch could be attributed to the disturbance on the ethylene action, unmasking chlorophyll pigment, decreasing the respiration rate of the fruit that led to slowdown the color change [27]. Similarly, Ch and SA treated pistachio fruit maintained the color quality as compared to the control [53].

Deteriorative color reactions (date fruit browning), in particular the enzymatic ones, are a naturally occurring phenomenon triggered mainly by action of peroxidase and polyphenol oxidase, during the ripening and storage of fruit, and are associated with fruit market value loss [54,55]. These reactions implicated phenolic compounds oxidation and the development of dark brown pigments in date fruit [54]. In the present study, the steady decrease in TPC during storage (Figure 4) was positively correlated with the reduction in color parameters i.e., h^o, C^*, L^* and negatively with ΔE (Table 3), in all fruit by different levels. Thus, based on the present results, it is possible that the application of Ca alone or in combination with Ch and SA delayed the reactions of color change in different degrees and accordingly preserved fruit color.

3.7. Total Phenolic Content of Fruit at Harvest and during Storage

The TPC content of 'Khesab' fruit at harvest and during cold storage are presented in Figure 5. The type of elicitor applied significantly affected the TPC content at harvest and during cold storage. Generally, TPC gradually decreased in all fruit with the progress of storage time. Likewise, in different dates, the content of phenols declined from young stage of development to maturation and ripening [54]. In the present study, Ch+Ca+SA treated fruit had the highest TPC concentration which progressively decreased from 486.8 mg 100 g^{-1} GAE at day 0 to 66.9 mg 100 g^{-1} GAE at day 60, followed by Ca (from 448.5 to 82.8) and Ch (394.6 to 68.5 mg 100 g^{-1} GAE) as compared to control and other treatments. However, variations in the concentration of TPC among different date cultivars have been reported by several studies (50–400 mg 100 g^{-1}) [7,10,54], which may be attributed to cultivar variations and/or environmental factors. In other fruits, SA has previously been shown to decrease peach chilling injury for the duration of cold storage [56], owing to its ability to induce antioxidant systems as well as heat shock proteins [56]. The application of elicitors such as chitosan, SA, and Ca has been reported to improve the levels of polyphenols in fruit and therefore enhances their quality [29]. In the present study, the Ch+SA+Ca treatment had low decay incidence, during the period of cold storage (Figure 5) and had the lowest Rutab percentage after Ca treatment (Figure 3). Also, TPC showed negative correlation with weight loss, Rutab and decay percentage (Table 4), signifying an important role in date fruit ripening/senescence. Based on these findings, preharvest application of Ch, Ch+Ca and Ch+SA+Ca improved the levels of TPC, which enhanced the quality and shelf life of 'Khasab' fruit.

Table 3. Effect of different preharvest treatments on 'Khesab' fruit color attributes during cold storage.

Storage Time	L^*							h^0						
	Control	**Ch**	**Ca**	**SA**	**Ch+SA**	**Ch+Ca**	**Ch+Ca+SA**	**Control**	**Ch**	**Ca**	**SA**	**Ch+SA**	**Ch+Ca**	**Ch+Ca+SA**
Day 0	33.1 ± 0.44b	33.1 ± 0.36b	32.6 ± 0.58b	31.2 ± 0.52b	33.4 ± 0.83b	37.6 ± 0.67a	37.7 ± 0.86a	27.7 ± 0.34a	26.0 ± 0.36b	24.9 ± 0.38c	23.8 ± 0.37c	27.3 ± 0.47a	24.2 ± 0.32c	27.4 ± 0.41a
Day 15	27.4 ± 0.54b	29.5 ± 0.38a	29.8 ± 0.64a	27.9 ± 0.51b	29.6 ± 0.54a	29.6 ± 0.70a	30.8 ± 0.91a	28.9 ± 0.46b	30.3 ± 0.45a	26.8 ± 0.31d	25.4 ± 0.33d	27.8 ± 0.39c	26.1 ± 0.46d	29.9 ± 0.49a
Day 30	25.2 ± 0.59b	26.7 ± 0.64b	28.4 ± 0.85a	26.0 ± 0.91b	28.5 ± 0.78a	27.1 ± 0.94a	27.4 ± 0.89a	38.1 ± 0.51a	35.9 ± 0.53b	29.9 ± 0.45d	28.8 ± 0.44d	34.2 ± 0.84c	34.5 ± 0.91c	38.7 ± 0.81a
Day 45	22.0 ± 0.32c	23.4 ± 0.43b	25.2 ± 0.69a	22.9 ± 0.47bc	23.2 ± 0.60b	24.6 ± 0.48a	24.1 ± 0.44a	38.0 ± 0.53b	38.3 ± 0.67b	35.8 ± 0.74c	34.4 ± 0.68c	37.5 ± 0.73b	38.3 ± 0.94b	40.6 ± 0.90a
Day 60	21.2 ± 0.52b	21.7 ± 0.43b	23.7 ± 0.88a	21.2 ± 0.44b	21.7 ± 0.36b	23.3 ± 0.67a	22.2 ± 0.41b	41.9 ± 0.46a	41.2 ± 0.66a	37.1 ± 0.62c	36.8 ± 0.41c	39.4 ± 0.45b	39.4 ± 0.82b	42.2 ± 0.44a
	C^*							ΔE						
	Control	**Ch**	**Ca**	**SA**	**Ch+SA**	**Ch+Ca**	**Ch+Ca+SA**	**Control**	**Ch**	**Ca**	**SA**	**Ch+SA**	**Ch+Ca**	**Ch+Ca+SA**
Day 0	30.7 ± 0.47bc	31.8 ± 0.82b	29.9 ± 0.39c	28.7 ± 0.38c	32.8 ± 0.76b	32.6 ± 0.56b	34.3 ± 0.714a	0e	1.4 ± 0.06d	1.7 ± 0.04d	3.4 ± 0.13b	2.3 ± 0.07c	5.3 ± 0.10a	5.9 ± 0.16a
Day 15	27.4 ± 0.56c	29.8 ± 0.46b	29.4 ± 0.44b	28.1 ± 0.41bc	32.1 ± 0.54a	27.6 ± 0.42c	28.6 ± 0.43b	6.7 ± 0.14a	3.9 ± 0.08bc	3.5 ± 0.08c	5.8 ± 0.15b	3.7 ± 0.09c	4.7 ± 0.08b	3.3 ± 0.05c
Day 30	10.4 ± 0.23d	12.8 ± 0.21c	15.6 ± 0.24b	13.1 ± 0.25bc	18.4 ± 0.23a	11.8 ± 0.18c	14.4 ± 0.22b	21.9 ± 0.36a	19.1 ± 0.33b	15.8 ± 0.15c	18.9 ± 0.31b	13.4 ± 0.23d	19.9 ± 0.34b	17.7 ± 0.24b
Day 45	6.6 ± 0.12c	7.5 ± 0.11b	8.7 ± 0.13a	6.1 ± 0.11c	7.3 ± 0.12b	5.8 ± 0.14c	8.9 ± 0.12a	26.5 ± 034a	25.3 ± 0.46a	23.5 ± 0.37b	26.6 ± 0.41a	25.5 ± 0.53a	26.4 ± 0.43a	23.8 ± 0.35b
Day 60	5.2 ± 0.14a	5.7 ± 0.9a	5.8 ± 0.11a	3.7 ± 0.08b	4.6 ± 0.10a	4.9 ± 0.05a	4.9 ± 0.09a	28.2 ± 0.57a	27.5 ± 0.72b	26.6 ± 0.51b	29.5 ± 0.64a	28.5 ± 0.69a	28.8 ± 0.47a	27.7 ± 0.42ab

Values are the mean (n = 25) ± SE. Means with different letters in the same row for each color index are significantly different at $p > 0.05$ using LSD test. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

Table 4. Pearson's correlation coefficients between some biochemical and physical characteristics of 'Khesab' fruit.

Traits	TSS	TPC	Rutab %	Decay %	Weight Loss %	L^*	ΔE	C^*
TPC	−0.73 ***							
Rutab %	0.74 ***	−0.92 ***						
Decay %	0.61 ***	−0.42 ***	0.56 ***					
Weight loss %	0.52 ***	−0.77 ***	0.65 ***	0.43 ***				
L^*	−0.67 ***	0.85 ***	−0.91 ***	−0.39 ***	−0.60 ***			
ΔE	0.68 ***	−0.81 ***	0.88 ***	0.47 ***	0.55 ***	−0.96 ***		
C^*	−0.63 ***	0.72 ***	−0.85 ***	−0.49 ***	−0.52 ***	0.93 ***	−0.94 ***	
h^o	−0.67 ***	0.81 ***	−0.89 ***	−0.55 ***	−0.63 ***	0.92 ***	−0.90 ***	0.91 ***

(***) significant at level $p = 0.001$.

3.8. Total Tannin, Flavonoids, and Antioxidants Concentrations at Harvest

The effect of different elicitors on total tannin (TTC), flavonoids, and antioxidant activity was determined at harvest (Table 2). TTC, TFC, and antioxidant activity as impacted by the applied elicitors showed significant variation among different treatments. In comparison to all treatments, the Ch+Ca+SA treated fruit had the highest TTC and TFC with 116.5 mg 100 g^{-1} CE and 91.0 mg 100 g^{-1} CE, respectively, followed by Ch+Ca (80.0, and 82.7 mg 100 g^{-1} CE), compared to control and other treatments as the lowest treatment for theses phytochemicals contents was SA (23.8 and 56.3 mg 100 g^{-1} CE), and Ch (21, and 73.6 mg 100 g^{-1} CE). These antioxidant compounds content showed activity as well as the antioxidants activity was higher in CH+SA+Ca treated fruit as measured by ABTS (323.7 mg 100 g^{-1} TE) and DPPH with lowest (IC50 = 1.5 mg mL^{-1}) radical scavenging activities (Table 2). The lower IC50 values in the DPPH radical scavenging activities determination showed a reduction in the extract concentration needed to scavenge the DPPH radical by 50%, indicating an improvement in antioxidant activity of the phenolic compounds that found in the fruit. This result is consistent with low ripening (low Rutab percentage) and decay observed with the same treatment (Figures 3 and 5). Generally, in dates, moving from early stage (Bisr) to the ripening stages, the concentration of antioxidants declined [54]. These results are consistent with those found for TPC at harvest (Table 2), highlighting the key role of phenolic compounds in the antioxidant capacity of 'Khesab' fruit. Also, a positive correlation was reported between the antioxidant compounds content and the antioxidant capacity in five date cultivars [54].

In strawberry fruit, preharvest spray with chitosan significantly increased flavonoids and phenolic compounds up to 2.6-fold relative to untreated control [17]. The application of SA was found to increase the antioxidant compounds in table grape [22]. Comparable findings were also reported by Mohamed et al. [10], who found that preharvest spray of SA significantly increased DPPH scavenging activity in Bisr stage of date fruit compared to the control fruit. Date as a climacteric fruit, during the maturation and ripening, the oxidative stress is considered to be responsible for the reduction in the antioxidant compounds (Figure 4) at Bisr and/or Rutab stage as a consequence of the decrease in free radical scavenging capacity (Mohamed et al. 2014). Hence, more reactive oxygen species (ROS) including H_2O_2 and superoxide may develop and be involved in the ripening/senescence of the date fruit and other fruit, through the development of free radicals [33]. According to the above findings, the preharvest application of Ch+SA+Ca increased the levels of antioxidant compounds and the antioxidant activity that enhanced quality and shelf life of 'Khesab' fruit possibly through the reduction of harmful radicals.

3.9. Microbiological Quality of Fruit

The microbial load (Log_{10} CFU g^{-1}) on 'Khesab' samples at harvest and at the end of cold storage are presented in Figure 6. Generally, total bacteria count (TBC) and fungal/mold count (FMC) showed significant variation at harvest and by the end of the storage time as affected by elicitors application. At day 0, Ch, SA, Ca, Ch+Ca, Ch+SA, and Ch+Ca+SA, treated date fruit had lower TBC counts compared to control., while FMC counts were significantly lower in Ch, Ca, and Ch+Ca+SA treated fruit compared to other

treatments and control. After 60 days, SA and Ch treated fruit had a lower TBC followed by Ch+SA and Ch+Ca+SA compared to control and other treatments, while same treatments had significantly lower FMC counts compared to control and other treatments (Figure 6). Consistently, various elicitors such as SA showed the best results in inducing defense reactions and minimizing microbial attack and decay in pear fruit [50]. Chitosan has also been shown to stimulate plant defenses against a variety of pathogens, including fungi and bacteria [19]. Moreover, the Ch and SA treatments, either separately or in combination, dramatically reduced bacterial and fungal growth in pistachio fruit [53]. Based on the present results, combination treatment with different elicitors exhibited better antimicrobial effect compared to other treatments. The same treatment showed the highest antioxidant activity and the least decay percentage which explained theses microbial analysis results [27]. Thus, the increase in antioxidant activity and phenols by the treatment of different elicitors alone or in combination may induce defense mechanisms leading to pathogen resistance and an increase in the fruit's shelf life as also reported in other fruit [19,51,57].

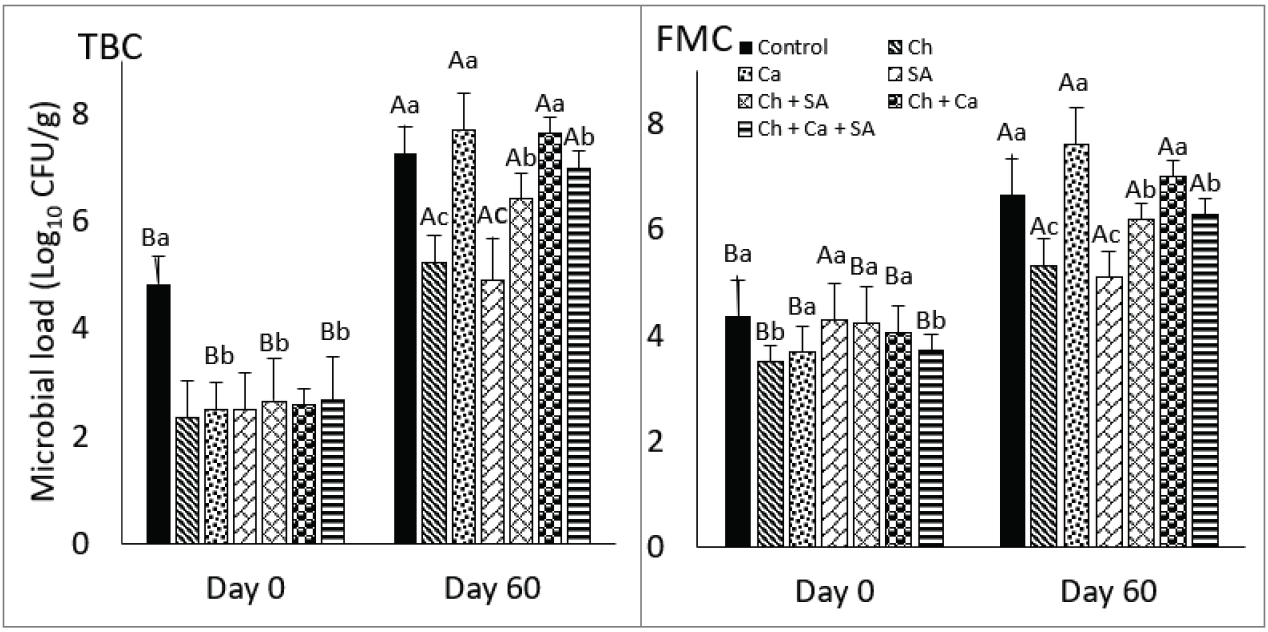

Figure 6. The effect of preharvest treatment on viable microbial load (Log_{10} CFU/g) of 'Khesab' date fruit at day 0 and day 60 of cold storage. Values are the mean (n = 3) ± SE. Means with different letters between treatments (small letter) at different time interval (capital letter) are significantly different at $p < 0.05$ using LSD test. TBC = Total Bacterial Count, FMC = Yeast and Mold Count. Ch: chitosan; Ca: calcium chloride; SA: salicylic acid.

4. Conclusions

The findings of this study revealed that applications of different elicitor combinations improved biochemical characteristics of 'Khesab' fresh fruit throughout the maturation and storage period. The synergistic effect of the elicitor was noticed when combining different treatments; Ch+Ca and Ch+Ca+SA and Ch+SA treated fruit in harvested Bisr date fruit, had lower decay and less microbial load. By the end of cold storage, Ca followed by Ch+Ca+SA had the highest TPC concentration as they delayed the natural ripening. Antioxidant activity was found to be high in Ch+SA, followed by Ch+SA+Ca and Ch compared to other treatments. Based on these findings, we can conclude that the delay in ripening and the reduction in fruit decay might be owing to the regulatory effect of the sprayed elicitors on the ripening progress and thus retarding of 'Khesab' fresh fruit senescence. As a result, out of the combinations of elicitors, the (Ch+SA and Ch+SA+Ca) can be suggested for a wide scale use and recommended be tested on different date cultivars and other fruit. The application of natural materials can be a more efficient, cost-effective,

and natural way to improve date fruit quality/marketability and protection against a wide range of decaying microbes.

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