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Research Article
Impact of Aloe Vera and Grapefruit Seed Extracts on Flame Seedless Grape to Improve Quality by Control Gray Mold During the Storage

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Abstract

Background and Objective: Flame seedless is the earliest ripening red seedless cultivar of table grapes cultivated in the Egyptian vineyards for both exportation and local market. This cultivar has the ability of exporting and marketing, but appears to be very susceptible fungal infection during postharvest handling. Therefore, this study was conducted to evaluate the antifungal efficacy of Aloe vera and grapefruit seed extracts (GSE extracts to control decay formation caused by grey mould (*Botrytis cinerea*), *in vitro* and *in vivo* during cold storage to enhancement the storability of flame seedless grape. **Materials and Methods:** For this, bunches of flame seedless grape were sprayed with aloe vera and grapefruit seed extracts before harvest. In addition to control gray mold and improvement fruit quality after harvest and during cold storage for 40 days at 0°C±1 with 90-95% R.H. **Results:** The results indicated that all natural extracts have demonstrated good results for inhibiting the growth of pathogens over untreated. Similarly, all extracts reduced physiological loss in weight, decay, berry shatter and rachis browning. All the extracts were able to retain postharvest quality of berries without any adverse effect on quality parameters such as TSS, total acidity and total sugar percentage. Overall, the uses of aloe vera and GSE are 2 promising examples of treatments that are beginning to be adopted on a commercial scale. **Conclusion:** Thus, it is evident from this study that, combination of aloe vera at 250 mL L^{-1} and grapefruit seed extracts (GSE) at 0.1% have the potential to control gray mold, caused by *Botrytis cinerea* without causing any injury or harmful effects on bunches. Organic extracts of aloe vera and grapefruit seed can be recommended as a safe method for maintaining berry quality as anthocyanin, pectin methyl esterase (PME) and total phenol and extending storage life at the same time.

Key words: Flame seedless grape, berry decay, berry shatter, aloe vera and grapefruit seed extracts (GSE), cold storage

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Grapevine (*Vitis vinifera*) is the most widely cultivated fruit crop in the world and the most important in economic terms. Flame seedless is a popular red seedless table grape, with a firm, crispy texture and sweet, neutral taste. Due to its earliness, flame seedless is highly priced on the export market. Currently, it is the most widely planted red seedless table grape cultivar in the world. Rachis dehydration is a main decay factor (physical deterioration) of grapes during the pre or postharvest[1]. Skin browning of grapes is another main physiological problem associated with mature table grape cultivar[2]. Major cause for grape spoilage is fungal infection, which decreases the production of fruit[3].

Gray mold, caused by *Botrytis cinerea*, is the most destructive of the postharvest diseases of table grapes and limits their shelf-life[4]. It can develop in the vineyard and even more after harvest, at temperatures as low as 31°F (-0.5°C) and grows from berry to berry during long-distance transport, cold storage and shelf-life. Under commercial conditions, grapes may remain on the vines long after they are physiologically mature. Fungicides spray prevents decay of grapes while the health hazard effects with these applications have become restrictive. Harvested bunches are usually stored in the presence of sulfur dioxide. This compound is registered as an adjuvant in most countries, while it has been removed from the GRAS list and classified as a pesticide in USA. However, there are increasing regulatory restrictions on the use of synthetic fungicides and of sulfur dioxide which is not allowed on organic grapes[5]. The study of alternative means to control postharvest decay has progressed over the past several decades, along with the expansion of organic agriculture and the concern of consumers about the possible presence of fungicide residues on fruit.

The antifungal activity of aloe vera has observed against several pathogenic fungi including *Botrytis cinerea*, main causative agent to decay grapefruit[6]. The aloe gel is made up of water, vitamins, lipids, sterols, tannins and enzymes and contains phenol, saponin, anthraquinones components, have anti-bacterial, antiviral and antifungal properties. The two major liquid sources of aloe vera are a yellow latex (exudate) and clear gel (mucilage), which proceeds from the large leaf parenchymatic cells[7]. Aloe vera natural plant extracts can be applied as edible coatings for fruits as its biological activities prevent loss of moisture and firmness, control respiration rate and development and maturation, delay oxidative browning and reduce microorganism proliferation in fruits. The use of aloe vera prolong the shelf life and delay changes in parameters related to deterioration of quality in sweet cherry[8], table grapes[9] and nectarines[10]. Aloe vera gel coating reduced weight loss in coated fruit because of hygroscopic properties that enable the formation of a barrier to water diffusion between fruit and environment[8]. Clusters sprayed 24 h before harvest with the aloe vera extracts solution and then stored 35 day at 2°C had 1% decayed berries compared to 15% of the control[11].

Grapefruit seed extract (GSE) is a commercial product derived from the seeds and pulp of grapefruit (*Citrus paradisi* Macf. Rutaceae). Grapefruit seed extract (GSE) has been shown to possess safe antibacterial, antiviral, antifungal, antiseptic, cleaning properties and pre-and post-harvest plant protection agents. It contains large quantities of polyphenolic compounds, such as catechins, epicatechin, epicatechin-3-O-gallate, dimeric, trimeric and tetrameric procyanidins. Grapefruit seed extracts (GSE) have been applied on harvested 'Red Globe' grapes with the aim of controlling fungal rot and maintaining the keeping quality. Bunches immersed in grapefruit seed extracts and stored 4 weeks at 0°C had 6 infected berries/kg compared to 19 of the control. Bunches were artificially challenged with *B. cinerea* infected berries were 18 and 65/kg, when treated with grapefruit seed extracts or untreated, respectively. The time of appearance of initial gray mold symptoms was used to evaluate the efficacy of treatments on detached berries which were artificially wounded and inoculated with *B. cinerea*[12].

In the fact of increasing consumer demand for natural preservatives plant extracts and there are no recent report on the utilization of the use these extracts. Therefore, the objective of this study was conducted to evaluate the antifungal efficacy of aloe vera and GSE extracts to control decay formation caused by grey mould (*Botrytis cinerea*), *in vitro* and *in vivo* during cold storage to enhancement the storability of flame seedless grape.

MATERIALS AND METHODS

Isolation and identification of the pathogen: *Botrytis cinerea* was isolated from naturally infected flame seedless grape. These isolates were the most aggressive one in our collection and produced the largest lesions on fruits. These fungi were purified and maintained on potato dextrose agar (PDA) and stored at 4°C, with periodic transfers through citrus fruits to maintain its aggressiveness. Seedless grape were ready for examination under a stereoscopic binocular microscope (6-50 x) for the presence of fungi and to study their habit characters. When necessary the compound microscope was used for confirming the identification after having

examined the morphology of conidia and conidiophores. Fungi presented on infested seeds were identified by means of comparison with the description sheets of Commonwealth Mycological Institute, Kew, Surrey, England (CMI), Danish Government Institute of Seed Pathology (DGISP) publications as well as publication of Ellis[13].

Effect of aloe vera, grapefruit seed extracts and combinations on growth of fungi isolated from flame seedless grape

Linear growth: Aloe vera, grapefruit seed extracts and combinations were tested *in vitro* on the linear growth of the pathogenic fungus. Different concentrations were added to 10 mL of sterilized PDA before solidification and then poured in sterile petri-dishes. After solidification, the plates were inoculated with fungal disc (5 mm) in the center of the plate and incubated at 27±1°C. Three plates for each particular treatment for each fungus were used as replicates, 3 plates were prepared to serve as control for each fungus. Linear growth was observed daily and diameter of fungal colonies were recorded when plates of any treatment were filled with the fungal growth. Scanning electron microscopy micrographs were used for showing the microscopic structural changes of *Botrytis cinerea* hyphae in response to all treatments applied (Fig. 1).

Dry weight: About 100 mL of liquid PD medium in 250 mL Erlenmeyer flasks were amended with different concentrations of the tested compounds after autoclaving. Each flask was inoculated using 2 discs of 0.6 mm in diameter of fungal culture and then incubated at 20±2°C for 7 days. Control flasks contain no concentrations of these compounds. Three replicates were used for each concentration. At the end of incubation period, the mycelium was filtered off and washed several times with distilled water, then dried in an oven at 80°C for 48 h till constant weight[14].

Disease infection: It was determined according to the following equation:

$$\text{Disease infection (\%)} = \frac{\text{Number of natural infected fruits}}{\text{Number of total fruits}} \times 100$$

Field study: The present investigation was carried out during the 2 successive seasons of 2016 and 2017 on 7 years old flame seedless grapevines. Table grape vines were cultivated on sandy clay soil under drip irrigation system, planted at 2×3 m grown in private vineyard orchard at EL-Nubaria region, (60 km Alexandria Cairo desert road) Behera Governorate. All vines received there commended regular fertilizer and other horticulture practices. Fifty four vines of nearly similar vigour and bud load were chosen for the spraying applications according to completely randomized block design with 3 replicates each one was represented by 3 vines. The 24 h before harvest, clusters of the selected vines were sprayed as follows:

Treatments:

- Spray clusters with aloe vera (150 mL L^{-1})
- Spray clusters with aloe vera (250 mL L^{-1})
- Spray clusters with grapefruit seed extracts (GSE) 0.1%
- Spray clusters with aloe vera (150 mL L^{-1})+grapefruit seed extracts (GSE) (0.1%)
- Spray clusters with aloe vera (250 mL L^{-1})+grapefruit seed extracts (GSE) (0.1%)
- Control (spray cluster with water)

Preparation of extracts solutions

Aloe vera: The fully extended mature leaves of aloe vera were harvested. The leaves were then stored in plastic papers and transported to the laboratory with in same day.

Leaves of aloe (var. *Barbadenisis miller*), were selected for the study. The leaves were washed under running water, to get rid of dirt, insects and plankton. They were dried overnight in the laboratory with an electric oven at 40°C. About 100 g of the material (leaves) were pulverized by an electric mixer and preserved in labeled glass bottles that were sealed until use.

Plant extracts were prepared by macerating fresh plant parts with an equal volume of sterile distilled water (1:1, w/v) in a Waring blender following the standard procedure. This solution was treated as standard plant extract of 100% concentration.

Grapefruit seed extracts (GSE): (Citricidal™), 60% grapefruit extract and 40% vegetable glycerine) was purchased from Bio/chem. Research (CA, USA). The GSE was dissolved in distilled water with 0.05% (v/v) Tween-80 as surfactant to make 1% (v/v) stock solution[12].

The surfactant super film as a wetting agent was added at the rate of 40 cm/100 L water to all spraying solutions in order to obtain best penetration results.

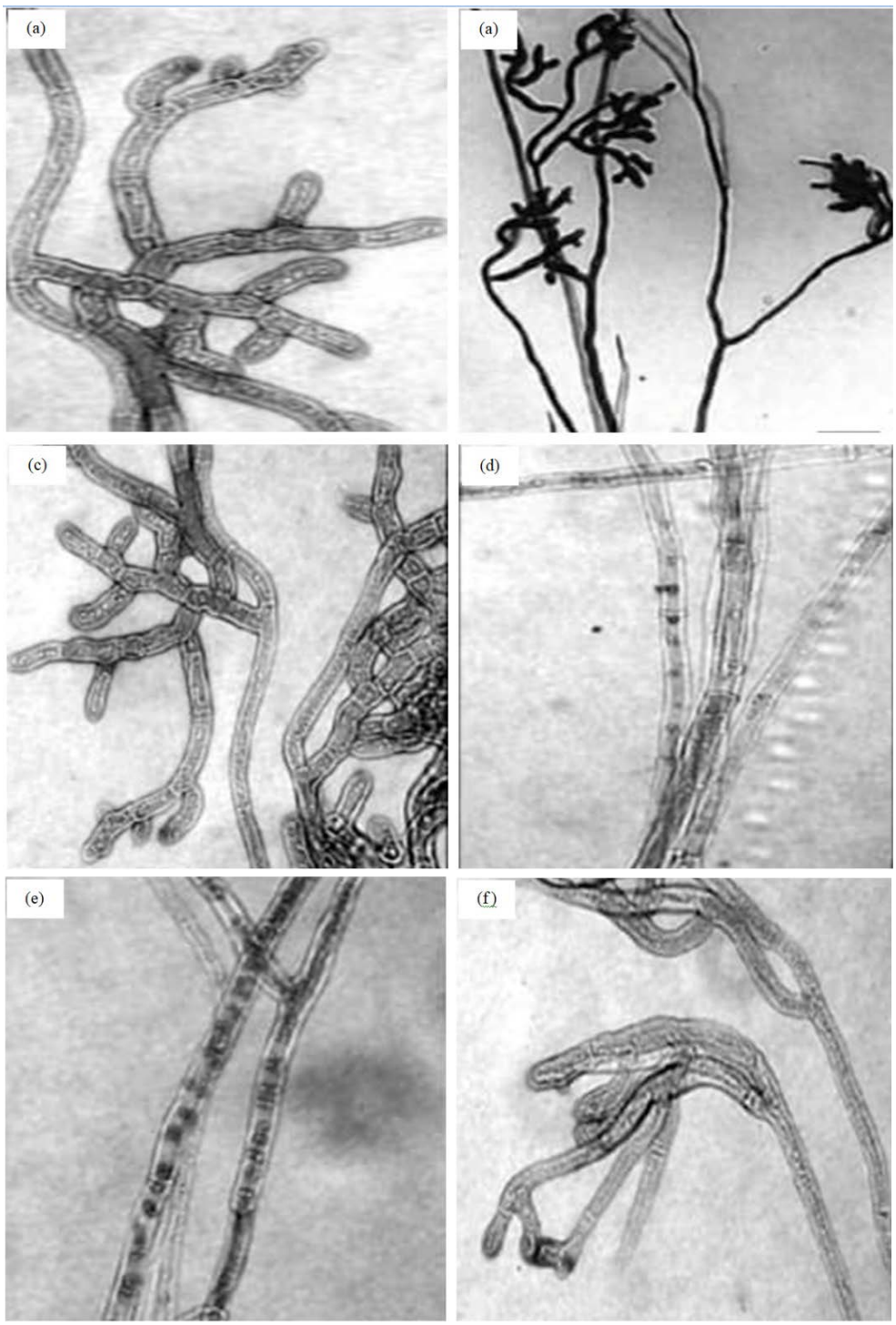

Fig. 1(a-f): Scanning electron microscopy micrographs of *Botrytis cinerea* hyphae. The mycelium hyphae in (a) Control (untreated clusters) contained normal cytoplasm and sporangia are stained dark, (b) Treated with aloe vera 150 mL L^{-1} contained little cytoplasm and sporangia are stained (c) Treated with aloe vera 250 mL L^{-1} contained little cytoplasm and sporangia are stained, (d) Treated with grapefruit seed extracts (GSE) 0.1% contained little cytoplasm and sporangia are stained, (e) Treated with aloe vera (150 mL L^{-1})+GSE (0.1%) contained little cytoplasm and sporangia are stained lightly and (f) Treated with aloe vera (250 mL L^{-1})+GSE (0.1%) contained little cytoplasm and sporangia are stained lightly

Harvest time was adjusted when the berry skin was full red color and SSC (%) in berry juice of the control treatment reached 17-18% in the 2 seasons. Clusters were picked at morning and immediately transported to the laboratory. Samples from each replicate were taken to study the effect of each treatment on clusters and berries quality at harvest time to determine the initial properties. For storage study, clusters of all treatments were sorted to remove any infected and berry damaged. All clusters were weighted put in ventilated carton box (50×35×15 cm). The total numbers of carton boxes were 36 for all treatments. Every treatment was represented by 6 carton boxes, each box containing 6 clusters. All boxes were stored 40 days at 0°C±1 with 90-95% R.H., then 3 carton boxes for each treatment were taken 20 days intervals to evaluate the storability of flame seedless grape as affected by the pre-harvest treatments.

Berry physical parameters

Cluster weight loss (%): It was calculated according to the following equation:

$$\text{Cluster weight loss}\,(\%) = \frac{\text{Initial cluster weight} - \text{Weight at sampling date}}{\text{Initial cluster weight}} \times 100$$

Berry decay (%): Decayed berries were estimated according to the following equation:

$$\text{Berry decay}\,(\%) = \frac{\text{Weight of decayed berries}}{\text{Initial cluster weight}} \times 100$$

Berry shatter (%): Shattering berries were determined according to the following equation:

$$\text{Berry shatter}\,(\%) = \frac{\text{Weight of berry shatter}}{\text{Initial cluster weight}} \times 100$$

Rachis browning: The rachis browning of the clusters was rated according to Crisosto *et al.*[4] as described, 1 healthy (entire rachis including the pedicels are fresh and green), 2 slight (rachis in good condition, but noticeable browning of pedicels), 3 moderate (browning of pedicels and secondary rachis) and 4 severe (pedicels, secondary and primary rachis completely brown).

Skin hue color (h°): Peel color of berries were measured using a hand-held colorimeter (CR-10; Minolta Co., Ltd., Osaka, Japan) and Spectra-Match software, set to L*, a*, b* mode. The values of L*, a*, b* were recorded and hue angle (h°) was calculated using the methods described by McGuire[15] as the following equation:

$$h^\circ = \tan^{-1}\left(\frac{b}{a}\right)$$

Where:

a = Interval of color between green (-) and red (+)
b = Interval of color between blue (+) and yellow (-)
h° = Skin hue color

Berry firmness: It was measured as (lb inch^{-2}) using Push-Pull (Hand Dynamometer Model DT101) with a thump (1 mm), 3/16 inch plunger.

Berry chemical parameters

Total soluble solids (TSS): It was measured in berries juice by using a Carl-Zeiss hand refractometer apparatus and expressed as a percent according to AOAC[16].

Total titratable acidity: Titratable acidity of berry juice was determined in terms of anhydrous tartaric acid percentage after titration 10 mL of berries juice with (0.1 N) NaOH solution using phenolphthalein as an indicator according to AOAC[16].

Anthocyanin content: It was measured in berry skin with the extractions solvent ethanolic HCl and absorbance was noted at 535 nm wave length by spectrophotometer. The content of total anthocyanin in berry skin was calculated using the following equations:

$$\text{Total anthocyanin content mg}/100\text{ g} = \frac{\text{Total absorbance}/100\text{ g skin}}{98.2\ (\text{E})}$$

The (E) value for 1% solution at 535 nm is equal to 98.2. Therefore, the absorbance of a solution containing 1 mg is equal to 98.2 as described by Ranganna[17].

Total sugar contents (%): The extract was prepared by taking 0.5 g of fresh berries and extracting the same with 80% ethanol by centrifuging 3 times. The supernatant was collected and measured quantity of distilled water was added to it and heated until all ethanol got evaporated. Then volume of sample was made up to 150 mL by adding distilled water. The total sugar was estimated using anthrone's reagent[17].

Pectin methyl esterase activity determination (PME): Frozen tissue samples of berries (50 g) were assayed for PME enzyme activity via a titration technique developed by Anthon and Barrett[18]. The volume of 0.02 N NaOH consumed to adjust the pH to 7.5 was recorded and the results were expressed as g g^{-1} FW according to AOAC[16].

Total phenols: Phenol extraction was carried out with 80% ethanol and the absorbance was measured at 765 nm by spectrophotometer against a blank as described by Slinkard and Singleton[19]. Total phenols was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid and the results expressed as mg g^{-1} FW gallic acid equivalent.

Statistical analysis: Data of both seasons of the study were designed by using analysis of variance (ANOVA), with 2 factors, time and temperature. Differences between the conducted treatments means were compared using Duncan's multiple tests at $p<0.05$ and means separation using the CoStat program.

RESULTS

This study was estimated the effect of aloe vera and grapefruit seed extracts as organic agents on storability of Flame seedless grape during cold storage by controlling gray mold.

Linear growth and dry weight of fungi isolated from flame seedless grape: Data in Table 1 show the effect of aloe vera, grapefruit seed extracts and combinations on linear growth and dry weight of *Botrytis cinerea* isolated from flame seedless grape. It was noticed that the reduction in linear growth and dry weight were correlated to the increase in aloe vera and grapefruit seed extract concentrations. Aloe vera (150 mL L^{-1})+GSE (0.1%) and aloe vera (250 mL L^{-1})+GSE (0.1%) treatments completely inhibited the linear growth and dry weight of *Botrytis cinerea*.

Effect on disease infection percentage of flame seedless grape: Data in Table 2 showed the effect of aloe vera, grapefruit seed extracts and combinations on disease infection of flame seedless grape during 40 days in cold storage (0±1°C with 90-95% RH). In both seasons, all treatments resulted decrease in disease infection. On the other hand, the most effective treatment aloe vera (250 mL L^{-1})+GSE (0.1%) gave reduction in disease infection caused by *Botrytis cinerea*.

Table 1: Effect of aloe vera and grapefruit seed extracts on linear growth and dry weight (g) of *Botrytis cinerea* isolated from flame seedless grape

	Linear growth (cm)			Dry weight (g)		
Treatments	3 days	6 days	9 days	3 days	6 days	9 days
Aloe vera (150 mL L^{-1})	2.23^{b}	3.46^{b}	4.92^{b}	0.30^{b}	0.47^{b}	0.66^{b}
Aloe vera (250 mL L^{-1})	0.92^{d}	1.85^{d}	2.08^{d}	0.12^{d}	0.25^{d}	0.28^{d}
Grapefruit seed extracts (GSE) (0.1%)	1.31^{c}	2.77^{c}	3.23^{c}	0.21^{c}	0.37^{c}	0.44^{c}
Aloe vera (150 mL L^{-1})+GSE (0.1%)	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}
Aloe vera (250 mL L^{-1})+GSE (0.1%)	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}
Control (spray cluster with water)	4.00^{a}	6.10^{a}	9.00^{a}	0.54^{a}	0.82^{a}	1.22^{a}

Means followed by the same letters are not significantly different by Duncan's multiple range test at 0.05 levels

Table 2: Effect of aloe vera and grapefruit seed extracts on disease infection percentage of flame seedless grape during cold storage 2016 and 2017 seasons

		Disease infection (%)	
Treatments	Storage period (days)	Season 2016	Season 2017
Aloe vera (150 mL L^{-1})	Initial	0.00^{j}	0.00^{k}
	20	17.51^{ef}	18.19^{ef}
	40	23.67^{c}	24.02^{c}
Aloe vera (250 mL L^{-1})	Initial	0.00^{j}	0.00^{k}
	20	16.29^{f}	17.34^{f}
	40	21.78^{d}	21.08^{d}
Grapefruit seed extracts (GSE) 0.1%	Initial	0.00^{j}	0.00^{k}
	20	18.19^{e}	18.86^{e}
	40	21.74^{d}	22.67^{c}
Aloe vera (150 mL L^{-1})+GSE (0.1%)	Initial	0.00^{j}	0.00^{k}
	20	13.61^{g}	13.05^{h}
	40	14.48^{g}	15.71^{g}
Aloe vera (250 mL L^{-1})+GSE (0.1%)	Initial	0.00^{j}	0.00^{k}
	20	6.17^{i}	6.69^{j}
	40	7.54^{h}	8.46^{i}
Control (spray cluster with water)	Initial	0.00^{j}	0.00^{k}
	20	28.43^{b}	29.72^{b}
	40	59.43^{a}	62.28^{a}

Means followed by the same letters are not significantly different by Duncan's multiple range test at 0.05 levels

Table 3: Effect of aloe vera and grapefruit seed extracts on weight loss, decay and shatter (%) of flame seedless grape during cold storage 2016 and 2017 seasons

Treatments	Storage period (days)	Weight loss (%)	Decay (%)	Shatter (%)
Season 2016				
Aloe vera (150 mL L^{-1})	Initial	0.00^{k}	0.00^{k}	0.00^{m}
	20	3.00^{j}	4.92^{i}	5.40^{l}
	40	4.40^{g}	6.65^{c}	11.10^{b}
Aloe vera (250 mL L^{-1})	Initial	0.00^{k}	0.00^{k}	0.00^{m}
	20	4.60^{f}	5.00^{h}	6.14^{k}
	40	5.20^{c}	6.12^{d}	10.50^{c}
Grapefruit seed extracts (GSE) (0.1%)	Initial	0.00^{k}	0.00^{k}	0.00^{m}
	20	4.50^{fg}	5.11^{g}	7.11^{h}
	40	5.00^{d}	6.11^{d}	10.00^{e}
Aloe vera (150 mL L^{-1})+GSE (0.1%)	Initial	0.00^{k}	0.00^{k}	0.00^{m}
	20	4.77^{e}	5.32^{f}	6.90^{j}
	40	5.00^{d}	5.66^{e}	9.88^{f}
Aloe vera (250 mL L^{-1})+GSE (0.1%)	Initial	0.00^{k}	0.00^{k}	0.00^{m}
	20	3.70^{i}	4.23^{j}	6.50^{j}
	40	4.20^{h}	5.17^{g}	9.11^{g}
Control (spray cluster with water)	Initial	0.00^{k}	0.00^{k}	0.00^{m}
	20	5.56^{b}	7.99^{b}	10.15^{d}
	40	9.71^{a}	16.70^{a}	17.11^{a}
Season 2017				
Aloe vera (150 mL L^{-1})	Initial	0.00^{i}	0.00^{k}	0.00^{l}
	20	3.33^{h}	5.11^{i}	4.30^{k}
	40	5.10^{d}	6.75^{c}	9.90^{b}
Aloe vera (250 mL L^{-1})	Initial	0.00^{i}	0.00^{k}	0.00^{l}
	20	4.05^{f}	5.32^{h}	6.84^{h}
	40	5.71^{b}	6.47^{d}	9.20^{d}
Grapefruit seed extracts (GSE) (0.1%)	Initial	0.00^{i}	0.00^{k}	0.00^{l}
	20	4.00^{f}	5.30^{h}	6.90^{h}
	40	5.33^{c}	6.37^{e}	9.00^{e}
Aloe vera (150 mL L^{-1})+GSE (0.1%)	Initial	0.00^{i}	0.00^{k}	0.00^{l}
	20	3.75^{g}	5.10^{i}	6.14^{j}
	40	5.26^{c}	6.14^{f}	8.50^{f}
Aloe vera (250 mL L^{-1})+GSE (0.1%)	Initial	0.00^{i}	0.00^{k}	0.00^{l}
	20	4.13^{f}	4.29^{j}	5.71^{j}
	40	4.31^{e}	5.43^{g}	7.70^{g}
Control (spray cluster with water)	Initial	0.00^{i}	0.00^{k}	0.00^{l}
	20	5.11^{d}	8.35^{b}	9.40^{c}
	40	9.00^{a}	17.50^{a}	18.31^{a}

Means followed by the same letters are not significantly different by Duncan's multiple range test at 0.05 levels

Effect on berry physical parameters of flame seedless grape

Cluster weight loss (%): Weight loss is one of the most critical quality attributes of postharvest life and quality of grape during storage. Weight loss increased during storage, although it was significantly higher in the control than in treated grape clusters. Effects of aloe vera and grapefruit seed extracts (GSE) treatments on grapes are shown in Table 3. Data disclosed that the physiological loss in weight of all grape clusters were significant increased gradually during 40 days in cold storage (0°C, 90-95% RH). The data also disclose that all applied treatments significantly reduced the percent of loss in cluster weight than the control under cold storage. Since, the highest weight loss was observed in control clusters (9.71 and 9.00%) after 40 days of cold storage in both seasons, respectively. The lowest significant weight loss was determined in clusters treated with aloe vera (250 mL L^{-1}) in combination with grapefruit seed extracts (GSE) at 0.1% (4.20 and 4.31%) after 40 days of cold storage in the 2 seasons, respectively. Since, these treatments were more effective for reducing the percentage of decayed and shattering berries.

Berry decay (%): Data in Table 3 revealed that the combination treatment of aloe vera at 250 mL L^{-1} with grapefruit seed extracts (GSE) at 0.1% decreased significantly decay rate in clusters (5.17 and 5.43%) during cold storage. At the end of the storage, the percent of decayed berries in control reached 16.70 and 17.50% after 40 days of cold storage in the 2 seasons, respectively. Whereas, all treatments markedly delayed the decay incidence of clusters after 40 days of cold storage in the 2 seasons, respectively. The results revealed that, Aloe vera treatments combined with grapefruit seed extracts treatment were more effective in inhibiting fungal decay (5.17 and 5.43) during storage than each treatment alone.

In this respect, micrographs in (Fig. 1a cleared that, through the scanning electron microscopy the mycelium hyphae of *Botrytis cinerea* in control (untreated clusters) were contained normal cytoplasm and sporangia are stained dark (Fig. 1a). Moreover, the mycelium hyphae treated with aloe vera (150 mL L^{-1}) contained little cytoplasm and sporangia are stained (Fig. 1a, b). The mycelium hyphae treated with aloe vera (250 mL L^{-1}) contained little cytoplasm and sporangia are stained (Fig. 1a-c). Since, the mycelium hyphae treated with grapefruit seed extracts (GSE) at 0.1% alone contained little cytoplasm and sporangia are stained (Fig. 1a-d). While, the mycelium hyphae treated with aloe vera (150 mL L^{-1})+GSE (0.1%) contained little cytoplasm and sporangia are stained lightly (Fig. 1a-f). Also, the mycelium hyphae treated with Aloe vera (250 mL L^{-1})+GSE (0.1%) contained little cytoplasm and sporangia are stained lightly (Fig. 1a-f).

Berry shatter (%): Correlation analysis (Table 3) showed that, there is a strong positive correlation between physiological loss weight and berry shatter indicating that water loss causes not only berry softening but also berry shattering. Berry shatter is mainly triggered by mechanical damage occurring during harvesting, packaging and transportation. As shown in Table 1 the berry shatter increased significantly with the advanced in storage period. In all the cases, excessive water loss leads to berry shatter. All treated clusters exhibited a significantly lower shatter percentage compared to the control.

Similarly to weight loss, the grape clusters sprayed with combination of aloe vera (250 mL L^{-1}) and grapefruit seed extracts GSE (0.1%) maintained a significantly lower berry shatter percentage (9.11 and 7.70%) after 40 days of cold storage during both seasons, respectively. Whereas, berry shatter was very high in control clusters presented 17.11 and 18.31% during both seasons, respectively.

Rachis browning index: In the study, there were significant differences ($p<0.05$) between treatments in terms of their effects on rachis browning during storage (Table 4). During the storage period, all treatments maintained better stem color than control. Also, clusters showing severe rachis browning symptoms lost more weight loss than clusters with moderate and slight stem browning symptoms. Control clusters reached to the highest rachis browning values ranged 3.4 and 3.6 (score 4) at the end of 40 days of cold storage. It is clear that the rachis of clusters sprayed with (GSE) 0.1% were slight browning (score 2) ranged 1.40 and 1.70 after 40 days of cold storage during both seasons, respectively.

Berry firmness (lb $inch^{-2}$): In the present study, reduced firmness with prolonged storage was prominent in control fruit compared to fruit treated. Data in Table 4 showed that berry firmness declined during storage both in treated and control fruit. However, the loss of firmness was significantly reduced by all treatments applied compared to untreated clusters. Since, the values of berry firmness of the control clusters were 3.74 and 3.00 lb $inch^{-2}$ after 40 days under cold storage through both seasons, respectively. Meanwhile, grape clusters applied with aloe vera (250 mL L^{-1})+GSE (0.1%) presented higher berry firmness than all treatments used or the control after 40 days of cold storage (6.13 and 5.93 lb $inch^{-2}$) in both seasons, respectively.

Skin hue color (h°): It is obvious that during 40 days of cold storage, hue angle (h°) declined progressively. In this respect, the grape clusters sprayed with different doses of aloe vera either alone or in combination with grapefruit seed extracts (GSE) showed a decrease in berry hue angle color (increased red skin color) and berries appears darker and redder in contrast to control group.

As shown in Table 4 the lowest value of hue angle (the highest red skin color) (17.50 and 18.13 h°) was recorded by the application of aloe vera (250 mL L^{-1})+GSE (0.1%) after 40 days of cold storage in the 2 seasons, respectively. On the contrary, control grapes resulted in the highest values of hue angle (the lowest red skin color) (39.00 and 38.70 h°) at the last sampling date after 40 days of cold storage, respectively during both seasons. However, for clusters treated with grapefruit seed extracts (GSE) 0.1% alone reduced hue more than the grape clusters sprayed with different doses of aloe vera alone after 40 days of cold storage during both seasons, respectively.

Effect on berry chemical analysis of flame seedless grape

Total soluble solids (TSS%): Regarding to the effect on TSS percentage, Table 5 revealed that during 40 days of cold storage, clusters treated with aloe vera either alone or in combination with grapefruit seed extracts (GSE) effectively retarded the degradation of TSS on the contrary to control clusters which reduction of TSS (%) were detected (16.90 and 16.70%) after 40 days of cold storage in both seasons, respectively.

Meanwhile, clusters treated with grapefruit seed extracts (GSE) 0.1% alone presented higher percent of TSS (18%) than all treatments used or the control after 40 days of cold storage in both seasons, respectively.

Table 4: Effect of aloe vera and grapefruit seed extracts on rachis browning, berry firmness and skin hue color (h°) of flame seedless grape during cold storage 2016 and 2017 seasons

Treatments	Storage period (days)	Rachis browning index	Berry firmness (lb inch^{-2})	Skin hue color (h°)
Season 2016				
Aloe vera (150 mL L^{-1})	Initial	0.00^{j}	7.20^{a}	53.00^{a}
	20	1.20^{hi}	6.23^{d}	50.50^{b}
	40	1.70^{ef}	5.94^{f}	32.70^{fg}
Aloe vera (250 mL L^{-1})	Initial	0.00^{j}	7.20^{a}	53.00^{a}
	20	1.30^{gh}	5.93f	47.00^{c}
	40	1.90^{cd}	5.14^{h}	29.60^{h}
Grapefruit seed extracts (GSE) (0.1%)	Initial	0.00^{j}	7.20^{a}	53.00^{a}
	20	1.10i	6.02^{ef}	32.50^{g}
	40	1.40^{g}	5.39^{g}	23.40^{j}
Aloe vera (150 mL L^{-1})+GSE (0.1%)	Initial	0.00^{j}	7.20^{a}	53.00^{a}
	20	1.60^{f}	6.63^{c}	36.00^{e}
	40	1.90^{cd}	6.03^{ef}	22.10^{j}
Aloe vera (250 mL L^{-1})+GSE (0.1%)	Initial	0.00^{j}	7.20^{a}	53.00^{a}
	20	1.80^{de}	6.83^{b}	33.70^{f}
	40	2.15^{b}	6.13^{de}	17.50^{k}
Control (spray cluster with water)	Initial	0.00^{j}	7.20^{a}	53.00^{a}
	20	2.00^{c}	4. 66^{i}	50.50^{b}
	40	3.40^{a}	3.74^{j}	39.00^{d}
Season 2017				
Aloe vera (150 mL L^{-1})	Initial	0.00^{g}	6.80^{a}	52.20^{a}
	20	1.30^{f}	6.03^{d}	48.20^{c}
	40	1.70^{d}	5.00^{h}	32.10^{h}
Aloe vera (250 mL L^{-1})	Initial	0.00^{g}	6.80^{a}	52.20^{a}
	20	1.20^{f}	5.67^{f}	45.13^{d}
	40	1.70^{d}	5.00^{h}	28.20^{j}
Grapefruit seed extracts (GSE) (0.1%)	Initial	0.00^{g}	6.80^{a}	52.20^{a}
	20	1.30^{f}	5.93^{de}	36.90^{f}
	40	1.70^{d}	5.20^{g}	23.20^{k}
Aloe vera (150 mL L^{-1})+GSE (0.1%)	Initial	0.00^{g}	6.80^{a}	52.20^{a}
	20	1.50^{e}	6.53^{b}	31.30^{i}
	40	2.00^{c}	5.80^{ef}	22.70^{l}
Aloe vera (250 mL L^{-1})+GSE (0.1%)	Initial	0.00^{g}	6.80^{a}	52.20^{a}
	20	1.70^{d}	6.33^{c}	35.55^{g}
	40	2.00^{c}	5.93^{de}	18.13^{m}
Control (spray cluster with water)	Initial	0.00^{g}	6.80^{a}	52.20^{a}
	20	2.30^{b}	4.53^{i}	50.10^{b}
	40	3.60^{a}	3.00^{j}	38.70^{e}

Means followed by the same letters are not significantly different by Duncan's multiple range test at 0.05 levels

Titratable acidity (TA%): As described in Table 5 with progress of storage, total acidity in berry juice decreased significantly ($p<0.05$) when compared with the initial values after 40 days of cold storage. In the study, reduction of TA content was followed by decline in visual quality. After the end of 40 days of storage during both seasons TA contents of all the treatments were significantly higher than non-treated control clusters which realized 0.50 and 0.49, respectively.

On the 40th day, the highest TA content was determined in aloe vera (250 mL L^{-1})+GSE (0.1%) treated clusters (0.59 and 0.58%) during both seasons, respectively.

Total sugar (%): Considering to the effect on total sugar, data in Table 5 reveal that the values of total sugar were progressively increased by the storage period advanced from harvest till 40 days at cold storage. With regard to the effect of these treatments on total sugar the data reveal that, clusters treated with aloe vera (250 mL L^{-1})+GSE (0.1%) produced a higher value of total sugar after 40 days of cold storage since the values averaged about 16.80 and 16.20% under the 2 seasons, respectively.

Anthocyanins (mg 100 g^{-1} FW): In the study, statistical analysis showed that total anthocyanin content of Flame seedless grape berries were significantly ($p = 0.05$) increasing during 40 days of cold storage (Table 6). Also, all treatments applied increased the content of anthocyanin in berry skin than the control under cold storage.

Table 5: Effect of aloe vera and grapefruit seed extracts on TSS (%), titratable acidity and total sugar (%) at berries juice of flame seedless grape during cold storage 2016 and 2017 seasons

Treatments	Storage period (days)	TSS (%)	Acidity (%)	Total sugar (%)
Season 2016				
Aloe vera (150 mL L^{-1})	Initial	17.00^{b}	0.68^{a}	13.60^{f}
	20	18.00^{a}	0.59^{c}	15.90^{bc}
	40	17.80^{a}	0.56^{cd}	16.10^{bc}
Aloe vera (250 mL L^{-1})	Initial	17.00^{b}	0.68^{a}	13.60^{f}
	20	18.20^{a}	0.65^{ab}	15.10^{de}
	40	17.30^{b}	0.53^{de}	15.49^{cde}
Grapefruit seed extracts (GSE) (0.1%)	Initial	17.00^{b}	0.68^{a}	13.60^{f}
	20	18.20^{a}	0.58^{c}	15.60^{bcd}
	40	18.00^{a}	0.53^{de}	16.19^{ab}
Aloe vera (150 mL L^{-1})+GSE (0.1%)	Initial	17.00^{b}	0.68^{a}	13.60^{f}
	20	17.80^{a}	0.65^{ab}	15.06^{de}
	40	17.90^{a}	0.54^{d}	16.25^{ab}
Aloe vera (250 mL L^{-1})+GSE (0.1%)	Initial	17.00^{b}	0.68^{a}	13.60^{f}
	20	18.20^{a}	0.63^{b}	16.00^{bc}
	40	17.90^{a}	0.59^{c}	16.80^{a}
Control (spray cluster with water)	Initial	17.00^{b}	0.68^{a}	13.60^{f}
	20	17.80^{a}	0.58^{c}	13.90^{f}
	40	16.90^{b}	0.50^{e}	14.90^{e}
Season 2017				
Aloe vera (150 mL L^{-1})	Initial	17.50^{c}	0.73^{a}	14.00^{e}
	20	18.30^{ab}	0.68^{b}	15.40^{bcd}
	40	17.80^{abc}	0.57^{c}	15.90^{ab}
Aloe vera (250 mL L^{-1})	Initial	17.50^{c}	0.73^{a}	14.00^{e}
	20	18.00^{abc}	0.53^{def}	15.40^{bcd}
	40	17.70^{bc}	0.51^{efg}	15.80^{ab}
Grapefruit seed extracts (GSE) (0.1%)	Initial	17.50^{c}	0.73^{a}	14.00^{e}
	20	18.00^{abc}	0.55^{cd}	15.70^{abc}
	40	18.00^{abc}	0.50^{fg}	16.00^{ab}
Aloe vera (150 mL L^{-1})+GSE (0.1%)	Initial	17.50^{c}	0.73^{a}	14.00^{e}
	20	18.40^{a}	0.54^{de}	15.70^{ab}
	40	17.90^{abc}	0.52^{defg}	16.00^{ab}
Aloe vera (250 mL L^{-1})+GSE (0.1%)	Initial	17.50^{c}	0.73^{a}	14.00^{e}
	20	18.00^{abc}	0.70^{ab}	15.80^{abc}
	40	17.90^{abc}	0.58^{c}	16.20^{ab}
Control (spray cluster with water)	Initial	17.50^{c}	0.73^{a}	14.00^{e}
	20	17.50^{c}	0.55^{cd}	14.90^{d}
	40	16.70^{d}	0.49^{g}	15.00^{cd}

Means followed by the same letters are not significantly different by Duncan's multiple range test at 0.05 levels

The results showed that, the combination treatment of aloe vera (250 mL L^{-1}) and GSE (0.1%) maintained anthocyanin in berry skin (51.66 and 56.66 mg 100 g^{-1} FW) after 40 days under cold storage comparison with other treatments used or the control during both seasons. However, control clusters presented lower values of anthocyanin in berry skin (43.66 and 45.33 mg 100 g^{-1} FW) after 40 days of cold storage through the both seasons under the study.

Enzymatic activity of pectin methyl esterase (PME mg g^{-1} FW): Irrespective of treated or control, pectin methyl esterase (PME) activity of grape berries increased slowly up to 20 days and then exhibited a sharp rise coming to a peak on 40 days of storage (Table 6). However, lower PME activity was shown by the clusters treated with combination of aloe vera and GSE in contrast to all applied clusters or the control. In this respect aloe vera (250 mL L^{-1})+GSE (0.1%) produced the lowest activity values (10.0 and 12.0 mg g^{-1} FW) of pectin methyl esterase (PME) during both seasons, respectively.

Total phenols content (TPC mg 100 g^{-1} FW): Total phenolic content phenolic compounds are an important group of secondary metabolites in grape and strongly influence the berry quality such as color, flavour, bitterness and astringency. In the study, the levels of total phenolic in grapes at harvest were found to be 52.0 and 52.30 mg 100 g^{-1} FW during both seasons. Total phenolic content of clusters showed decreasing towards end of cold storage period (Table 6). Total phenol content (TPC) of all clusters samples decreased slowly

Table 6: Effect of aloe vera and grapefruit seed extracts on anthocyanin, pectin methyl esterase (PME) and total phenol content (TPC) of flame seedless grape during cold storage 2016 and 2017 seasons

Treatments	Storage period (days)	Anthocyanin mg 100 g^{-1} FW	PME mg g^{-1} FW	Total phenol mg 100 g^{-1} FW
Season 2016				
Aloe vera (150 mL L^{-1})	Initial	37.46^{j}	0.08^{ef}	52.00^{a}
	20	42.36^{h}	0.09^{de}	40.60^{e}
	40	45.56^{e}	0.16^{a}	33.00^{h}
Aloe vera (250 mL L^{-1})	Initial	37.46^{j}	0.08^{ef}	52.00^{a}
	20	43.20^{g}	0.08^{ef}	45.00^{d}
	40	46.20^{d}	0.14^{b}	39.00^{f}
Grapefruit seed extracts (GSE) (0.1%)	Initial	37.46^{j}	0.08^{ef}	52.00^{a}
	20	44.43^{f}	0.09^{de}	46.10^{c}
	40	47.43^{c}	0.16^{a}	39.00^{f}
Aloe vera (150 mL L^{-1})+GSE (0.1%)	Initial	37.46^{j}	0.08^{ef}	52.00^{a}
	20	46.00^{d}	0.08^{ef}	45.60^{cd}
	40	50.00^{b}	0.12^{c}	38.00^{g}
Aloe vera (250 mL L^{-1})+GSE (0.1%)	Initial	37.46^{j}	0.08^{ef}	52.00^{a}
	20	47.86^{c}	0.07^{f}	48.60^{b}
	40	51.66^{a}	0.10^{d}	40.00^{e}
Control (spray cluster with water)	Initial	37.46^{j}	0.08^{ef}	52.00^{a}
	20	40.40^{i}	0.09^{de}	38.60^{fg}
	40	43.66^{g}	0.17^{a}	29.00i
Season 2017				
Aloe vera (150 mL L^{-1})	Initial	40.60^{j}	0.09^{e}	52.30^{a}
	20	45.66^{h}	0.10^{de}	38.60^{f}
	40	48.33^{f}	0.15^{a}	31.20^{j}
Aloe vera (250 mL L^{-1})	Initial	40.60^{j}	0.09^{e}	52.30^{a}
	20	47.00^{g}	0.12b^{c}	40.70^{d}
	40	50.33^{d}	0.15^{a}	34.20^{h}
Grapefruit seed extracts (GSE) (0.1%)	Initial	40.60^{j}	0.09^{e}	52.30^{a}
	20	49.33^{e}	0.11^{cd}	42.10^{c}
	40	50.66^{d}	0.13^{b}	36.30^{g}
Aloe vera (150 mL L^{-1})+GSE (0.1%)	Initial	40.60^{j}	0.09^{e}	52.30^{a}
	20	50.00^{de}	0.11^{cd}	42.10^{c}
	40	54.00^{b}	0.13^{b}	34.00^{h}
Aloe vera (250 mL L^{-1})+GSE (0.1%)	Initial	40.60^{j}	0.09^{e}	52.60^{a}
	20	51.66^{c}	0.10^{de}	44.60^{b}
	40	56.66^{a}	0.12^{bc}	39.40^{e}
Control (spray cluster with water)	Initial	40.60^{j}	0.09^{e}	52.30^{a}
	20	42.33^{i}	0.13^{b}	30.60^{j}
	40	45.33^{h}	0.16^{a}	22.00^{j}

Means followed by the same letters are not significantly different by Duncan's multiple range test at 0.05 levels

compared to control clusters which produced the lowest concentration of total phenols (29.0 and 22.0 mg 100 g^{-1} FW) after 40 days of cold storage during both seasons (Table 6). Preharvest application of aloe vera (250 mL L^{-1})+GSE (0.1%) significantly affected total phenol content (TPC) of berries which gave the highest concentration (40.0 and 39.40 mg 100 g^{-1} FW) maintained the highest TPC after 40 days of cold storage during both seasons.

DISCUSSION

From this study it can be reported that all natural extracts have demonstrated good results for inhibiting the growth of pathogens of flame seedless grape over untreated. The combination extracts of aloe vera at 250 mL L^{-1} and grapefruit seed extracts (GSE) at 0.1% have the potential to control gray mold, caused by *Botrytis cinerea* without causing any injury or harmful effects on bunches. Also, organic extracts of aloe vera and grapefruit seed significantly maintaining berry quality as anthocyanin, pectin methyl esterase (PME) and total phenol and extending storage life at the same time. Overall, the uses of aloe vera and GSE are 2 promising examples of treatments that are beginning to be adopted on a commercial scale as a safe method.

Grapes are non-climacteric fruits with a relatively low physiological activity and are subject to serious water loss and softening after harvest, which can result in stem browning, berry shatter, wilting, shriveling of berries[4]. Water loss is strictly a physical factor related to the evaporative potential of the surrounding air. The higher the evaporative potential of the air

surrounding the fruit, the more water is lost from the fruit. This relationship may be expressed directly as the vapour pressure deficit a term which indicates the combined influence of temperature and relative humidity[20]. The highest weight loss suppression was achieved with the interaction of Aloe vera and grapefruit seed extracts (GSE). Aloe vera gel coating reduced weight loss in coated fruit because of hygroscopic properties that enable the formation of a barrier to water diffusion between fruit and environment[8].

Most grapes are usually stored after harvest. During cold storage, losses of economic importance are produced by several decays due to fungal rot. In this respect, *Penicillium expansum* and *Botrytis cinerea* are well-known postharvest pathogens. They produce blue and gray rots, respectively[21]. Aloe vera and grapefruit seed extracts (GSE) completely inhibited the linear growth and dry weight of *Botrytis cinerea*. This result is in agreement with the finding of Latifa *et al.*[22] on citrus who reported complete inhibition of mycelia growth of *Penicillium itelicum* which was generally associated with complete inhibition of sporulation by organic acids and salts.

Moreover, aloe vera treatments combined with grapefruit seed extracts treatment were more effective in inhibiting fungal decay during storage than each treatment alone. This might be a result of direct inhibition of microbial growth or activating defense responses in clusters and thus contributed to alleviate and reduce tissue colonization by pathogen. Among the work reported in the literature, most of them focused on the use of these organic extracts to ensure microbial safety of produce. The primary cause of postharvest loss in table grapes is grey mold disease caused by *B. cinerea*[23]. Also, grapes and sweet cherries coated with aloe vera showed retardation of the ripening process by delayed evolution of the parameters related to organoleptic quality, as well as by a reduction of fruit decay, with high acceptance from the sensory panel[8].

In addition, the application of aloe vera as an edible coating in table grapes imparted beneficial effects in terms of maintenance of total antioxidant activity (TAA), for both skin and pulp, either during cold storage or after the periods of shelf life. Moreover, the higher TAA observed in aloe vera-treated grapes as well as its maintenance during prolonged storage could be attributed to the presence of a large number of chemical compounds in the composition of aloe vera gel[7], but it is thought that aloe emodin, an anthraquinone derivative, is one of the main components that contribute to antioxidant activity these compounds.

There appear to be three types of berry shatter, physiological, pathological and mechanical. The first is associated with the thickening and hardening of the pedicel and production of an abscission layer[24]. Some researchers reported that ethylene stimulates berry shatter. The suppression of formation of ethylene which is the primary trigger of abscission process might cause reduction in berry shatter in treated clusters in contrast tocontrol[25]. The increase of berry shatter has been recommended the pedicle and stalk of cluster behave in a climacteric process showing respiration and ethylene peaks[26].

Rachises browning due to weight loss and *Botrytis* infection are the 2 main factors which reduce table grape postharvest quality. Rachis quality of bunches has been investigating extensively among producers and exporters because of its high impact on the cluster freshness that determines consumers[27]. Stem browning in cold storage has been commonly associated with water loss and oxidation processes[28]. Moreover, the stem is a physiologically active part with greater respiration intensity than the berry and it is a key issue in grape storage[4].

Grape berries develop as clusters with each berry attached to the bunch stem (rachis and branches) via a pedicel which contains vascular bundles. Thus, penetration and absorption take place not only through the thin exocarp of the berry but also via bunch stems, which enhances infiltration of applied chemicals. The beneficial effect of applied grapefruit seed extracts (GSE) was clear in delaying rachis dehydration and browning, which is associated with decayed berries. These symptoms first appeared on pedicels followed by lateral branches and finally on the central axis, as has been reported for flame seedless grapes due to increase polyphenol oxidase activity[29]. Moreover, the variation parameters of weight loss, decay, shattering and browning show a significant interaction during 40 days of cold storage.

Firmness is one of the main indicators in judging the quality of table grapes for fresh consumption. Grapes lose their firmness by loss of water and by changes in their structure composition during postharvest storage[30]. The effect of softening is an important part of the ripening process in most fruit and it is widely recognized that changes in cell walls accompany fruit softening. During the ripening process, the progressive loss of firmness is the result of a gradual transformation of protopectin into pectin which is degraded by the enzyme, polygalacturonase, in the cell wall[31]. This could be explained that aloe vera delays fruit softening by affecting major cell wall degrading enzymes activity such as cellulase, polygalacturonase and xylanase through the reduction of ethylene production. It may be concluded that there is a close relation between water loss and berry firmness during storage. The aloe vera works as a barrier to O_2 and CO_2 and acts as moisture barrier and thus reduces weight loss, browning, softening and growth of yeast and molds. The material contains antimicrobial compounds and thus prevents decay[32].

As flame seedless berries mature, peel color changes from a relatively pure green to yellow and eventually to red. At the optimum harvest stage, 'flame seedless' berries are characterized by a reddish purple skin color. From this study it clear that, the highly colored fruits have lower anthocyanin concentrations. Thus, anthocyanin concentrations had strongly negatively correlated effect on hue color of the berries. A decrease in hue angle could indicate the senescence process. Decreases in berry color at the end of cold storage may be attributed to the degradation of anthocyanins by glycosidases and peroxidases[33].

Khan *et al.*[34] confirmed that the increment in TSS may be related with enzymes which are presented when amino acids enhanced the synthesis of different proteins, acids and sugars. This explanation is in the line with using foliar application of green algae extract on Superior grapes which significantly improved TSS synthesis[35].

The lower total soluble solids in aloe vera gel coated might be due to delayed fruit ripening. Similarly a delayed and a smaller increase in TSS have been reported in aloe vera gel coated sweet cherry and table grapes[8].

In the present study, TSS increased over 20 days of storage and then showed a slight declining trend. Increase in TSS (%) from harvest to 20 days of storage is mainly due to reduction in water transport through the xylem to the berry once detached from the vine and also subsequent back flow of water from the berry to the rachis. This occurs due to differential rates of evaporation[36].

Moreover, total soluble solids may augment during fruit ripening because of the action of sucrose-phosphate synthase (SPS), a key enzyme in sucrose biosynthesis. The enzyme activity is under the influence of ethylene and the ripening process itself during storage. Thus, the use of an edible coating based on aloe vera gel as a postharvest treatment exogenously reduce the rates of respiration and ethylene production thus inhibiting ripening related changes within the berries resulting lower degradation of TSS in treated samples and maintain quality and safety[10].

The acidity level is a very important quality factor in table grapes and those used for wine production. Consumer acceptance of table grapes and grape juice is strongly influenced by the sweetness to acid balance. Aloe vera either alone or in combination with grapefruit seed extracts (GSE) treatment would induce a lower physiological maturation in table grapes, since both sugars and organic acids are substrates of the fruit respiration. The organic acids play an important role in flavor perception for consumers and fruit acidity usually tends to decrease due to using of organic acids as substrates for respiratory metabolism during storage period[37].

It was reported that the accumulation of sugars in grapes preceding the rise in anthocyanin content, thus a strong correlation is found between the accumulation of certain amount of sugars in the berries and formation of anthocyanin[38]. Furthermore, it has been found that the percentage of sugar has a triggering effect on anthocyanin accumulation.

Anthocyanin concentration of grapes is an important fruit quality parameter for marketing. Anthocyanins contribute to grape quality by effecting both color intensity and color quality. Anthocyanin considers one of the important flavonoids classes. Red and black grapes owe their attractive color to their anthocyanin pigments. Moreover, anthocyanin levels and the other flavonoid classes in grape skin are parameters available for evaluating grape quality[30].

It is well known that changes in the pectin matrix affect cell wall structure during fruit ripening and senescence. Pectic substances, cellulose and hemicellulose are the major cell wall polysaccharides, some of which are depolymerized during ripening leading to fruit softening. In many fruits, including grapes, pectin degradation is occurred initially by the action of PME. In this context, pectin methyl esterase (PME) activity constitutes a key control point for both the assembly and disassembly of pectin networks. It catalyses the hydrolysis of methyl-ester groups from galacturonosyl residues and plays important roles in determining the extent to which demethylated polygalacturonans are accessible to degradation by polygalacturonases[39]. Therefore, the positive effect of aloe vera and (GSE) on suppression of fruit softening enzymes might account for firmer berries in the treated group as compared to control.

Generally, phenolic compounds are an important group of grapes secondary metabolites and strongly influence the berry quality such as color, flavor, bitterness, astringency and berry functional properties[40]. They are also involved in enhancing antimicrobial properties of grape berries. In our experiment, correlation analysis showed that there is significant negative correlation between decay incidence and TPC (Table 3, 6).

Anthocyanins and phenolic compounds are important constituents in human health and there is rising awareness among consumers about their functional properties. Table grapes are a good source of health promoting compounds and being a colored variety, the flame seedless is also rich in these important antioxidants. As observed in the study, the levels of total anthocyanins and phenols remained

significantly higher in the combination treatment of aloe vera (250 mL L^{-1}) and GSE (0.1%) treated clusters in contrast to the control in which a significant loss of these compounds was detected. Aloe vera treatments delayed the loss of phenolic compounds in both skin and pulp of flame seedless grape during cold storage.

CONCLUSION

Based on the results of this study, it can be concluded that, the foliar applications of aloe vera at 250 mL L^{-1} and grapefruit seed extracts (GSE) at 0.1% had more pronounced effect on clusters quality of flame seedless grape than the control clusters during cold storage (at 0±1°C with 90-95% R.H.). Application of aloe vera and grapefruit seed extracts have the potential to control gray mold, caused by *Botrytis cinerea* without causing any injury or harmful effects on bunches.

SIGNIFICANCE STATEMENT

This study discovered the possible effect of natural preservatives plant extracts such as aloe vera and grapefruit seed extracts (GSE) that can be beneficial for enhancing the storability of flame seedless grape. This study will help the researchers to uncover the critical areas of inhibiting the growth of pathogens of grey mould (*Botrytis cinerea*) with natural preservatives that many researchers were not able to explore. Thus a new theory of aloe vera and grapefruit seed extracts is two promising examples that are beginning to be adopted on a commercial scale which may be arrived at retain postharvest quality of grape clusters without any adverse effect on quality parameters.

REFERENCES

1. Chauhan, S., K.C. Gupta and M. Agrawal, 2014. Application of biodegradable *Aloe vera* gel to control post-harvest decay and longer the shelf life of grapes. Int. J. Curr. Microbiol. Applied Sci., 3: 632-642.
2. Vial, P., C. Crisosto and G. Crisosto, 2005. Early harvest delays berry skin browning of 'Princess' table grapes. California Agric., 59: 103-108.
3. Thanaboripat, D., 2011. Control of aflatoxins in agricultural products using plant extracts. KMITL Sci. Technol. J., 11: 35-42.
4. Crisosto, C.H., D. Garner and G. Crisosto, 2002. Carbon dioxide-enriched atmospheres during cold storage limit losses from Botrytis but accelerate rachis browning of 'Redglobe' table grapes. Postharvest Biol. Technol., 26: 181-189.
5. Gabler, F.M., J.L. Smilanick, M.F. Mansour and H. Karaca, 2010. Influence of fumigation with high concentrations of ozone gas on postharvest gray mold and fungicide residues on table grapes. Postharvest Biol. Technol., 55: 85-90.
6. De Rodriguez, D.J., D. Hernandez-Castillo, R. Rodriguez-Garcia and J.L. Angulo-Sanchez, 2005. Antifungal activity *in vitro* of *Aloe vera* pulp and liquid fraction against plant pathogenic fungi. Ind. Crops Prod., 21: 81-87.
7. Ni, Y., D. Turner, K.M. Yates and I. Tizard, 2004. Isolation and characterization of structural components of *Aloe vera* L. leaf pulp. Int. Immunopharmacol., 4: 1745-1755.
8. Martinez-Romero, D., N. Alburquerque, J.M. Valverde, F. Guillen and S. Castillo, D. Valero and M. Serrano, 2006. Postharvest sweet cherry quality and safety maintenance by *Aloe vera* treatment: A new edible coating. Postharvest Biol. Technol., 39: 93-100.
9. Serrano, M., J.M. Valverde, F. Guillen, S. Castillo, D. Martinez-Romero and D. Valero, 2006. Use of *Aloe vera* gel coating preserves the functional properties of table grapes. J. Agric. Food Chem., 54: 3882-3886.
10. Ahmed, M.J., Z. Singh and A.S. Khan, 2009. Postharvest *Aloe vera* gel-coating modulates fruit ripening and quality of 'Arctic Snow' nectarine kept in ambient and cold storage. Int. J. Food Sci. Technol., 44: 1024-1033.
11. Castillo, S., D. Navarro, P.J. Zapata, F. Guillen, D. Valero, M. Serrano and D. Martinez-Romero, 2010. Antifungal efficacy of *Aloe vera in vitro* and its use as a preharvest treatment to maintain postharvest table grape quality. Postharvest Biol. Technol., 57: 183-188.
12. Xu, W.T., K.L. Huang, F. Guo, W. Qu, J.J. Yang, Z.H. Liang and Y.B. Luo, 2007. Postharvest grapefruit seed extract and chitosan treatments of table grapes to control *Botrytis cinerea*. Postharvest Biol. Technol., 46: 86-94.
13. Ellis, M.B., 1971. Dematiaceous Hyphomycetes. 1st Edn., Commonwealth Mycological Institute, Kew, Surrey, UK., ISBN-13: 978-0851986180, Pages: 608.
14. El-Morsy, T.H.A., 1993. Ecological and physiological studies on fungi present in water and its relation to pollutants in Dakahlia province. M.Sc. Thesis, Botany Department, Faculty of Science, Mansoura University, Egypt.
15. McGuire, R.G., 1992. Reporting of objective color measurements. HortScience, 27: 1254-1255.
16. AOAC., 2005. Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists, Washington, DC., USA.
17. Ranganna, S., 1979. Manual of Analysis of Fruit and Vegetable Products. Tata McGraw Hill Publishing Company Limited, New Delhi, India, Pages: 634.
18. Anthon, G.E. and D.M. Barrett, 2006. Characterization of the temperature activation of pectin methylesterase in green beans and tomatoes. J. Agric. Food Chem., 54: 204-211.
19. Slinkard, K. and V.L. Singleton, 1977. Total phenol analysis: Automation and comparison with manual methods. Am. J. Enol. Vitic, 28: 49-55.

20. Ngcobo, M.E.K., U.L. Opara and G.D. Thiart, 2012. Effects of packaging liners on cooling rate and quality attributes of table grape (cv. Regal seedless). Packaging Technol. Sci., 25: 73-84.
21. Calvo, J., V. Calvente, M.E. de Orellano, D. Benuzzi and M.I.S. de Tosetti, 2007. Biological control of postharvest spoilage caused by *Penicillium expansum* and *Botrytis cinerea* in apple by using the bacterium *Rahnella aquatilis*. Int. J. Food Microbiol., 113: 251-257.
22. Latifa, A., T. Idriss, B. Hassan, S.M. Amine, B. El Hassane and A.A.B. Aoumar, 2011. Effects of organic acids and salts on the development of *Penicillium italicum*: The causal agent of citrus blue mold. Plant Pathol. J., 10: 99-107.
23. Snowdon, A.L., 1990. A Colour Atlas of Post-Harvest Diseases and Disorders of Fruits and Vegetables: General Introduction of Fruits. Vol. 1, Wolfe Scientific, London, UK., ISBN-13: 9780723416364, Pages: 302.
24. Ling, X., S.I. Kusakari, H. Toyoda and S. Ouchi, 1999. Role of fungi in the shattering of grape berries during storage. Bulletin of the Institute for Comprehensive Agricultural Sciences No. 7, Kinki University, Japan, pp: 97-106.
25. Lydakis, D. and J. Aked, 2003. Vapour heat treatment of Sultanina table grapes. I: Control of *Botrytis cinerea*. Postharvest Biol. Technol., 27: 109-116.
26. Fayed, T.A., 2010. Effect of some antioxidants on growth, yield and bunch characteristics of Thompson seedless grapevine. Am.-Eurasian J. Agric. Environ. Sci., 8: 322-328.
27. Balic, I., A. Moreno, D. Sanhueza, C. Huerta, A. Orellana, B.G. Defilippi and R. Campos-Vargas, 2012. Molecular and physiological study of postharvest rachis browning of table grape cv Red Globe. Postharvest Biol. Technol., 72: 47-56.
28. Lichter, A., T. Kaplunov, Y. Zutahy, A. Daus, V. Alchanatis, V. Ostrovsky and S. Lurie, 2011. Physical and visual properties of grape rachis as affected by water vapor pressure deficit. Postharvest Biol. Technol., 59: 25-33.
29. Carvajal-Millan, E., T. Carvallo, J.A. Orozco, M.A. Martinez and I. Tapia *et al*., 2001. Polyphenol oxidase activity, color changes and dehydration in table grape rachis during development and storage as affected by *N*-(2-chloro-4-pyridyl)-*N*-phenylurea. J. Agric. Food Chem., 49: 946-951.
30. Mori, K., S. Sugaya and H. Gemma, 2005. Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. Sci. Hortic., 105: 319-330.
31. Asghari, M., L. Ahadi and S. Riaie, 2013. Effect of salicylic acid and edible coating based *Aloe vera* gel treatment on storage life and postharvest quality of grape (*Vitis vinifera* L. cv. Gizel Uzum). Int. J. Agric. Crop Sci., 5: 2890-2898.
32. Valverde, J.M., D. Valero, D. Martinez-Romero, F. Guillen, S. Castillo and M. Serrano, 2005. Novel edible coating based on *Aloe vera* gel to maintain table grape quality and safety. J. Agric. Food Chem., 53: 7807-7813.
33. Gagne, S., K. Esteve, C. Deytieux, C. Saucier and L. Geny, 2006. Influence of abscisic acid in triggering "véraison" in grape berry skins of *Vitis vinifera* L. cv. Cabernet-Sauvignon. J. Int. Sci. Vigne Vin, 40: 7-14.
34. Khan, A.S., B. Ahmad, M.J. Jaskani, R. Ahmad and A.U. Malik, 2012. Foliar application of mixture of amino acids and seaweed (*Ascophylum nodosum*) extract improve growth and physico-chemical properties of grapes. Int. J. Agric. Biol., 14: 383-388.
35. Abd El Moniem, E.A. and A.S.E. Abd-Allah, 2008. Effect of green alga cells extract as foliar spray on vegetative growth, yield and berries quality of superior grapevines. J. Agric. Environ. Sci., 4: 427-433.
36. Chervin, C., A. Aked and C.H. Crisosto, 2012. Grapes. In: Crop Post-Harvest: Science and Technology, Volume 3: Perishables, Rees, D., G. Farrell and J. Orchard (Eds.). Chapter 9, Blackwell Publishing Ltd., West Sussex, UK., ISBN-13: 9781444354638, pp: 187-211.
37. Valero, D. and M. Serrano, 2010. Postharvest Biology and Technology for Preserving Fruit Quality. 1st Edn., CRC Press, New York, USA., ISBN-13: 9780429093333, Pages: 287.
38. Pirie, A. and M.G. Mullins, 1980. Concentration of phenolics in the skin of grape berries during fruit development and ripening. Am. J. Enol. Vitic., 31: 34-36.
39. Deytieux-Belleau, C., A. Vallet, B. Doneche and L. Geny, 2008. Pectin methylesterase and polygalacturonase in the developing grape skin. Plant Physiol. Biochem., 46: 638-646.
40. Chamkha, M., B. Cathala, V. Cheynier and R. Douillard, 2003. Phenolic composition of champagnes from Chardonnay and Pinot Noir vintages. J. Agric. Food Chem., 51: 3179-3184.