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Research Article
Evaluation of Natural Oils Impact on Flame Seedless Grape Quality at Harvest and Storage Marketing Process

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Abstract

Background and Objectives: Excessive use of chemical antifungal agents during the food production becomes a serious issue threatening our health especially in fresh fruits, which passes the applied chemicals into the human body quickly. The research evaluated the effect of different natural oils on flame seedless grapes (*Vitis vinifera* L.) as delicate fruits affected highly with fungal infection or temperature changes during different storage periods and marketing temperatures by testing the fruits quality and the antifungal properties of oils against post-harvest fungi. **Materials and Methods:** Thirteen years old flames seedless growing on sandy soil were sprayed with clove, black seed and garlic oil during two seasons 2016 and 2017. After harvest, the clusters were sorted in room or cold temperature for different storage periods. Clusters were analyzed for its physical, chemical and microbial prosperities. **Results:** Spraying natural oils (especially garlic oil) significantly improved the berries quality in terms of increasing berry weight, length, width, TSS and TSS/TA% compared to unsprayed ones. They also reduced the post-harvest fungal counts up to 75% decreasing in garlic oil treated samples. **Conclusion:** Using natural oils protected the fruits from post-harvest fungi and also generated protective coating layer decreased water loss during storage periods which increased the fruits quality and lifetime.

Key words: Antifungal agents, fungal infection, quality, flame seedless grapes, post-harvest fungi, black seed and garlic oil

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

INTRODUCTION

Fruit and vegetables consumption are widely accepted as health beneficial owing to our life style dramatic changes[1,2]. Fresh fruit or vegetables needs to presented in a state allows for immediate and direct use which put us in challenge with their shelf life time and any wounded tissue or any post-harvest infection[3]. Grape (*Vitis vinifera* L.) is one of the most economic and important fruit in the world and the second important fruit after citrus in Egypt, represented important source of antioxidant[4,5]. Fungal disease causes more than 12% post-harvest losses of world crops[6]. Scientists estimate losses due to fungal decay as 10-40% of total grape production throughout the world[7], this loss made fruits post-harvest diseases the most severe reasons of fruits production loss[8,9]. These molds have a great ability to produce mycotoxins in the infected fruits which have serous effects on human health[10].

Various synthetic chemicals (antifungal agents) have been used to inhibit the pathogenic fungi attacks of plants but they developed fast resistance against these chemicals, also these chemicals have bad effect in our health and cause environmental pollution by accumulation in the environment with slow biodegradation abilites[11]. Antifungal edible coating can effectively protect fresh fruit against fungal contamination by its antimicrobial activities and reducing water loss[12-14], e.g., organic acids, fatty acid esters, polypeptides, plant essential oils and many others. Essential oils generally recognized as safe materials for environment and human health[15]. They defined as the products obtained by hydro-distillation, dry distillation, steam distillation or by mechanical process without heating from different plant parts[16]. They characterized by oily texture, volatile, rarely colored, strong odor, have lower density[17] and several biological properties (anti-cancer, antimicrobial, anti-inflammatory, antioxidant and insect repellent)[18-20].

The antifungal mechanisms of essential oils basically owing to their hydrophobicity which able them to disrupt the fungal cell membrane lipid structure, cell structures and made them highly permeable[21,22]. Other activity acts on the proteins of cytoplasmic membrane and inhibit some enzymes activity involved in metabolic pathways[23].

Natural essential oils effects on the physical and chemical properities fruits, Asghari Marjanlo *et al.*[24] indicated that essential oil-treated fruits with thymol, eugenol and menthol vapours had higher TSS, TA, lycopene and β-carotene contents than control fruits. Essential oil concentration effects on the fruits rate of respiration which related to the fungus infection degree[25]. Other researchers showed that fruit decay and weight loss percentage were less in fruits treated with essential oils after harvesting in several plants[26]. Therefore, the aim of this research was to evaluate the effect of three natural oils (clove, black seed and garlic) as antifungal and protective agents on grapes (as a delicate fresh eaten fruit affected highly with microbial infection or temperature) at harvest and during different storage temperatures and periods.

MATERIALS AND METHODS

Experiment site and natural oil application: The present study was performed during two seasons 2016 and 2017 on 13 years old Flame Seedless cv. (*Vitis vinifera* L.), grown on sandy soil at Assiut Governorate, Egypt. Seven treatments were applied with three replicates for each treatment (1 replicate = 2 vine) were 1-Spraying with 0.5% clove (*Syzygium aromaticum*) oil, 2-Spraying with 1% clove oil, 3-Spraying with 0.5% black seed (*Nigella sativa*) oil, 4-Spraying with 1% black seed oil, 5-Spraying with 0.5% garlic (*Allium sativum*) oil, 6-Spraying with 1% garlic oil and 7-Spraying with water (control). All vines were sprayed once at the 2nd week of May (4 weeks before harvest date). Trees under study were sprayed in early morning by the above listed materials using a back gun sprayer 20 L.

Storage conditions and sample size: Harvest date was determined when berries reached full color and the soluble solids content in berry juice reached about 15-18%. In this respect clusters were harvested in the 2nd week of June and transported to the Laboratory of in Agriculture Research Station in Assiut, Egypt for analysis. At the beginning of the experiment, samples of 3 clusters from each treatment were taken to determine the initial characteristics. Each treatment containing 18 clusters and each cluster was packed using perforated bag and weighted. Clusters were stored in two different temperatures, room temperature (28$\pm$2°C) and cold storage in refrigerator (5$\pm$2°C). Clusters kept at room temperature were examined every 2 days but under cold storage conditions, the samples were examined every 4 days to study the change in clusters and berry characteristics through marketing and under cold storage conditions.

Physical and chemical properties of grapes

Cluster weight loss: Cluster weight loss was calculated according to the Eq. 1:

$$\text{Cluster weight loss (\%)} = \frac{\text{Initial cluster weight} - \text{Final cluster weight}}{\text{Initial cluster weight}} \times 100 \quad (1)$$

Decay rate (life storage): Decay rate was calculated according to the Eq. 2:

$$\text{Berry decay (\%)} = \frac{\text{Weight of decayed berries}}{\text{Initial cluster weight}} \times 100 \qquad (2)$$

Total soluble solids: Total soluble solids (TSS%) were determined using hand refractometer.

Titratable acidity: Titratable acidity (TA%) was determined by titration of 10 mL of berry juice against 0.1 N sodium hydroxide solution using phenolphthalein as indicator. Titratable acidity was expressed as gram tartaric acid per/100 mL juice according to AOAC[27].

Microbiological assay

Assay medium: Potato dextrose agar medium (PDA) used for fungal isolation containing (g L^{-1}): Potato (scrubbed and diced), 200, dextrose, 15.0 and agar, 20.0, distilled water, 1000 mL and initial pH 5.6. The medium was supplemented with rose-bengal (1/30000) and chloramphenicol (250 mg mL^{-1}) added to the medium as bacteriostatic and bactericidal agents, respectively[28]. For potato preparation, potato discs were boiled in water for 1 h and passed the mixture through a fine sieve (cloth chess), add dextrose, stir, add agar and boiled until dissolving then autoclaved[29] at 121°C, 1.5 P for 20 min.

Isolation of fungi: Fungi associated with grapes isolated using dilution plate method as described by Pitt and Hocking[30], randomized selected berries were immersed in flask containing sterilized distilled water and shaken for 15 min. After shaken, serial dilutions were prepared until reach to the suitable dilution. About 1 mL of the suspension was transferred to each sterilized Petri dish and covered with sterilized melted and cooled medium. Plates in three replicates were incubated at 28±1°C for 7 days and the developing fungi were isolated and purified. The counts were calculated as colony forming units (CFU) per gram of fresh grapes.

Statistical analysis: All the treatments of the field experiment were arranged in complete randomized block with three replicates and the storage treatments were arranged in a split-plot design with three replicate and statistically analyzed according to Snedecor and Cochran[31] using new L.S.D. at the level of 0.05.

RESULTS

Spraying oil treatments significantly gave the best cluster and berries quality in terms of increasing berry weight, length, width, total soluble solids (TSS%), TSS %/titratable acidity (TA%), anthocyanin pigment contents in berry skin and decreased only TA % compared to unsprayed ones at harvest time as cleared in Table 1. The heaviest berry weight was 2.08 g (1% garlic oil) in the first season, whereas the highest total soluble solids observed was 17.5% (1% black seed oil) in the second season. Anthocyanin pigment recorded its highest value in the first season with 2.15 mg g^{-1} fresh skin berries (1% garlic oil). Data in Fig. 1 revealed that, cluster weight loss gradually increased with the advanced of storage period at both temperatures and reached its maximum values at the end time of the storage. However storage in room temperature (6 days) had high loss than cold temperature (12 day), also garlic oil recorded the less cluster weight loss comparing to other treatments. Cold storage gave the lowest fruits decayed percentage and until 8 days almost no decayed fruits recorded in all oil sprayed fruits during the both studied seasons. Garlic treatment significantly reduced the percent of decayed berries during all the storage periods as revealed in Fig. 2. Applying the natural oils decreased TSS% significantly compared to untreated berries and increased with the storage period (Fig. 3). The minimum TSS% obtained by garlic after 6 days under room temperature while, black seed oil gave the highest values after 12 days under cold temperature. Titratable acidity percentage was markedly decreased with advanced of storage periods, however

Table 1: Effect of different natural oils on berry weight, berry length, berry width, Total soluble solids (TSS), Titratable acidity (TA) and anthocyanin content of flame seedless grapes at harvest time during 2016 and 2017 seasons

	Season 2016						Season 2017					
Treatments	Berry weight (g)	Berry length (cm)	Berry width (cm)	TSS (%)	TA (%)	TSS /TA	Berry weight (g)	Berry length (cm)	Berry width (cm)	TSS (%)	TA (%)	TSS /TA
Clove oil (0.5%)	2.01	1.46	1.51	17.00	0.595	28.57	1.91	1.37	1.39	16.50	0.579	28.50
Clove oil (1%)	2.02	1.48	1.52	16.75	0.590	28.39	1.93	1.39	1.40	17.25	0.585	29.49
Black seed oil (0.5%)	2.06	1.51	1.55	16.50	0.590	27.97	1.98	1.41	1.41	17.00	0.587	28.96
Black seed oil (1%)	2.03	1.48	1.52	16.50	0.588	28.06	2.03	1.49	1.51	17.50	0.590	29.66
Garlic oil (0.5%)	2.07	1.50	1.55	16.00	0.599	26.71	2.01	1.46	1.47	16.00	0.587	27.26
Garlic oil (1%)	2.08	1.49	1.51	16.50	0.591	27.92	2.01	1.47	1.51	16.50	0.586	28.16
Control (no oil)	1.91	1.40	1.41	15.00	0.608	24.67	1.85	1.37	1.35	15.00	0.606	24.75

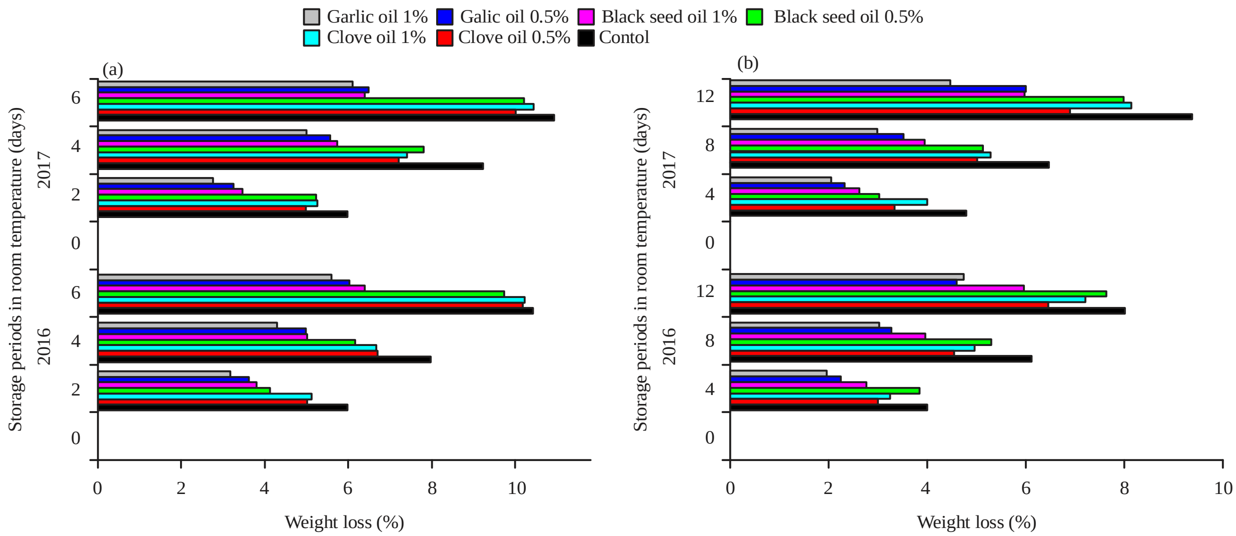

Fig. 1(a-b): Effect of different natural oils on weight lose (%) under (a) Room and (b) Cold storage temperatures of flame seedless grapes during 2016 and 2017 seasons

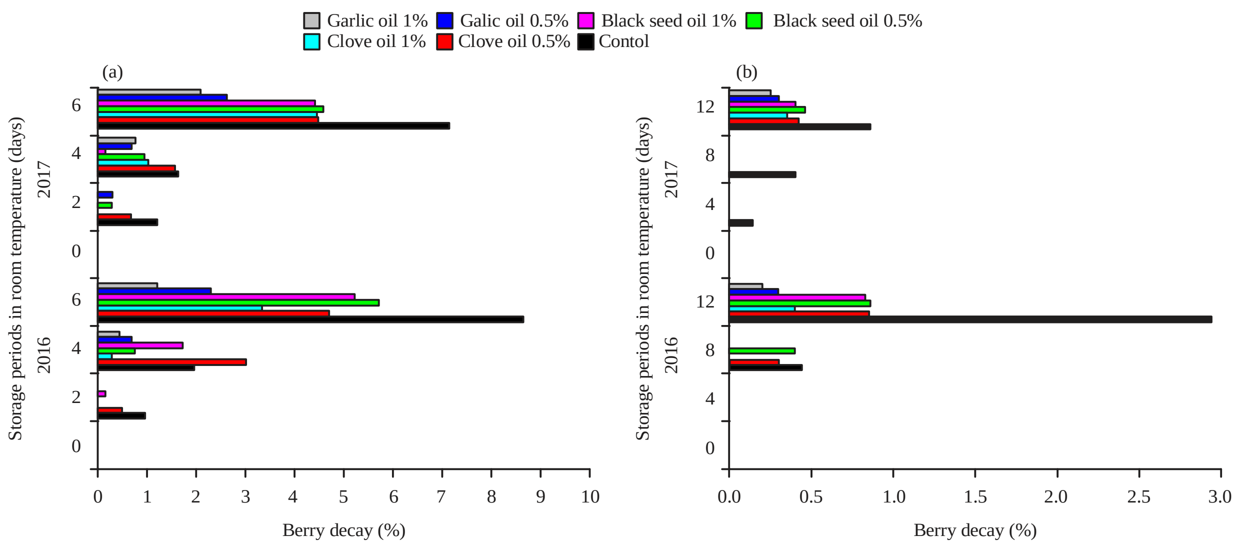

Fig. 2(a-b): Effect of different natural oils on berry decay (%) under (a) Room and (b) Cold storage temperatures of flame seedless grapes during 2016 and 2017 seasons

the combinations with oils was significantly increased the total acidity compared as showed in Fig. 4. Total counts of isolated fungi indicated large variations between fungal counts associated with grapes in room temperature and cold temperature as revealed in Fig. 5 and 6. In both seasons (2016 and 2017) the total counts of fungi was highest in room storage than cold storage during seasons. Garlic oil (1%) showed the highest fungal count decreasing in all storage periods and temperature followed by the other concentration 0.5%. The total counts ($x10^3$) during 2016 and 2017 for room storage of control samples were 34, 42, 64, 80 (0, 2, 4, 6 days) and 58, 81, 92, 112 (0, 2, 4, 6 days), respectively, while garlic oil 1% total counts were 9, 17, 16, 18 (0, 2, 4, 6 days season 2016) and 6, 14, 23, 30 (0, 2, 4, 6 days season 2017). The total counts ($x10^3$) for cold storage of the control samples were 34, 29, 35, 49 (0, 4, 8, 12 days season 2016) and 58, 39, 54, 64 (0, 4, 8, 12 days season 2017), while garlic oil 1% total counts were 9, 12, 15, 17 (0, 4, 8, 12 days season 2016) and 6, 5, 9, 13 (0, 4, 8, 12 days season 2017).

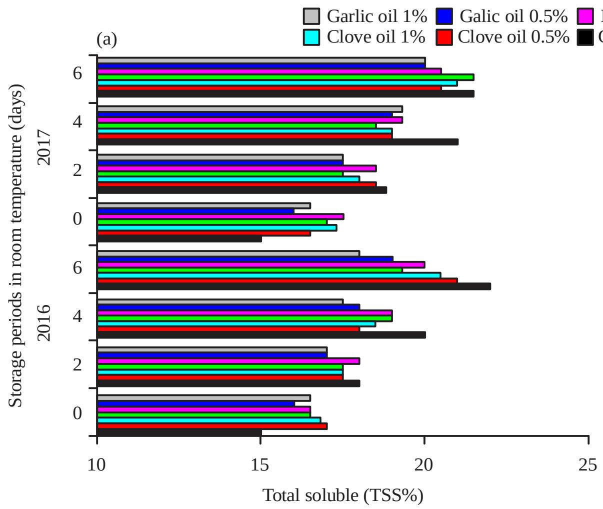

Fig. 3(a-b): Effect of different natural oils on total soluble solids (TSS%) under (a) Room and (b) Cold storage temperatures of flame seedless grapes during 2016 and 2017 seasons

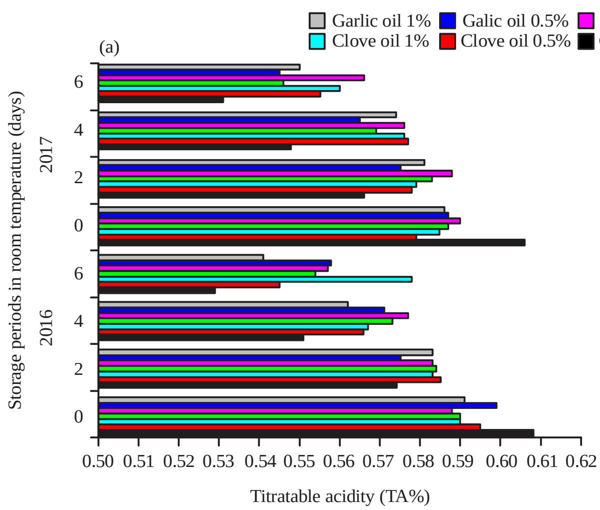

Fig. 4(a-b): Effect of different natural oils on Titratable acidity (TA%) under (a) Room and (b) Cold storage temperatures of flame seedless grapes during 2016 and 2017 seasons

DISCUSSION

Spraying flame seedless grapevines with different natural oils e.g., clove oil, black seed oil and garlic oil evaluated best cluster traits and berry quality in terms of increasing berry weight, length, width, TSS, TSS/TA% and anthocyanin contents compared to unsprayed ones. Best berries quality obtained with vines sprayed with 1% garlic oil. Many researchers[32-34] attributed the antimicrobial effects of garlic oil to its higher content with various antioxidants e.g., organosulfur compounds (allinin, s-allylcysteine, diallyl disulfide and allicin). Cluster stored for 6 days at room temperature showed higher weight loss than cluster stored for 12 days under cold storage, this behavior in fruits may attribute to the water loss either by transpiration through the surface or respiration[35]. Moreover, oil treated cluster have the lowest weight loss which may attribute to the oil ability to form a thin coating film around the fruit surface inducing protective layer from water loss as cleared by Mohammadi and Aminifard[36]. Garlic oil showed great results in decreasing berries decayed specially in cold storage, this decrease probably due to the increased in berries surface defense by garlic oils which eventually delayed the microbial infection, also low storage temperature could extend fruits shelf life

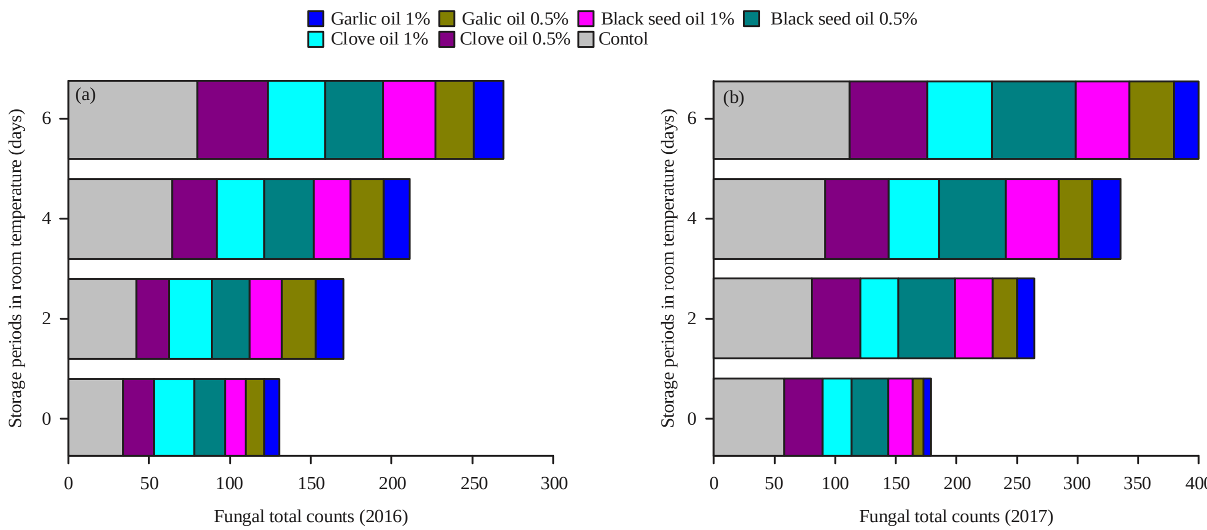

Fig. 5(a-b): Effect of natural oils on total counts of fungi associated with grapes (CFU per g of fresh grapes $\times 10^3$) during storage periods 0, 2, 4 and 6 days in room temperature (seasons (a) 2016, (b) 2017)

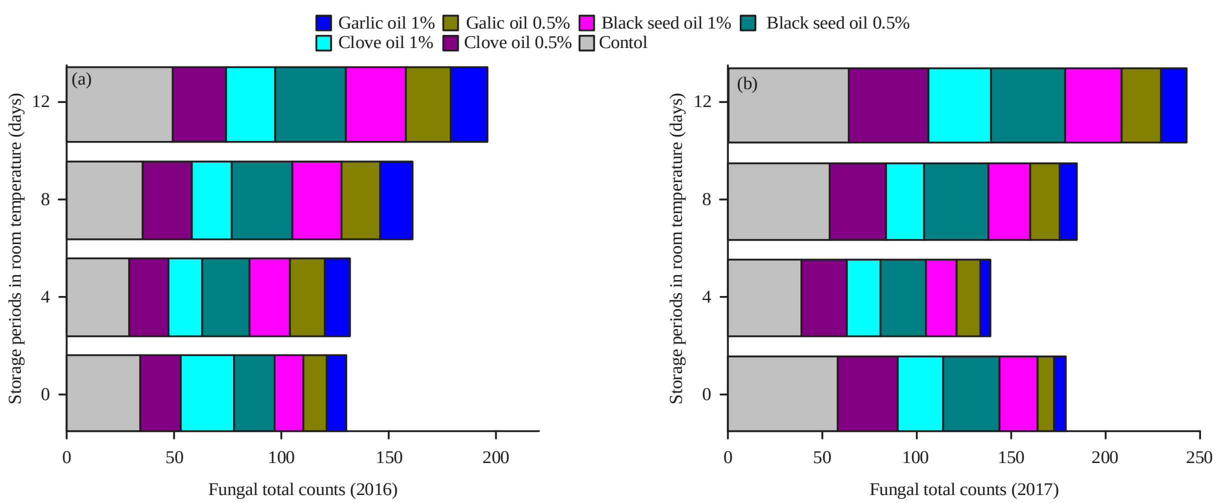

Fig. 6(a-b): Effect of natural oils on total counts of fungi associated with grapes (CFU per g of fresh grapes $\times 10^3$) during storage periods 0, 4, 8 and 12 days in cold temperature (season (a) 2016, (b) 2017)

by reducing the respiration rate, browning and surface damage[37]. Oils treated samples have high TSS%, TSS/TA% and anthocyanin content, these results were in agreement with Ju *et al.*[38], who reported that natural oil reserved the TSS% by retarding the fruit ripening process or due to the delaying of physiological ageing, metabolism alternation and respiration slowing[39]. Asghari Marjanlo *et al.*[24] indicated that essential oil-treated fruits had higher TSS, TA, lycopene and β-carotene contents than control fruits. Total counts of isolated fungi indicated high decreased in natural oil treated samples, this attributes to the antifungal prosperities of natural oils[40,41]. The lowest fungal total counts in both temperatures recorded by samples treated with garlic oil especially with high concentration 1%. Garlic consists of various active biologically metabolites[42] and garlic oil offers greater therapeutic potential as reported by Ross *et al.*[43]. Arora and Kaur[44] tested anti-microbial activity of different spices, only garlic and clove exhibit this property and could be a solution to decrease the fruit infection which alter the fruits flavor and color[45,46].

CONCLUSION

Using natural oils protected the fruits from post-harvest fungi and also generated protective coating layer decreased water loss during storage periods which increased the fruits quality and longtivity. Garlic oil has a great efficiency in protection of grapes and also on maintains its quality during different storage temperatures and periods.

SIGNIFICANCE STATEMENT

This study revealed the large benefits of using natural oils to increase the fruits quality of flame seedless grapes (*Vitis vinifera* L.) and protect them against the highly spread post harvest fungi. The research tested the grapes quality during conditions and periods similar to the actual storage process e.g., storage in room temperature and in refrigerators, which give this research huge applicability on the actual marketing process. This research will also help the researchers and farmers to protect their fruits from less quality or fungal pathogenicity during harvest and marketing times. Thus, a new way cleared in this research to protect our fresh eaten fruits from chemical agents and protect our health.

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