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Effect of *Origanum majorana* L. Essential Oil on Extension Shelf Life of Strawberry Fruits

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ABSTRACT

Origanum majorana L.(oregano) essential oil was investigated for its efficacy in inhibiting grey mold decay and extending the shelf life of strawberries (*Fragaria x ananassa*) (). The severity of decay in strawberries subjected to spraying with 500ppm of oil was significantly (P<0.05) reduced by up to 60% after 8 days of storage at 7°C.Treatedfruits maintained better fruit quality with higher levels of vitamin C, phenolics, and titratable acid. However, the treatment did not have any effect on fruit weight, anthocyanins, total soluble solids,and total carbohydrates after 8 days of storage at 7°C. Results showed that oregano essential oil maybe considered a promising alternative to synthetic fungicidesin the application of new safe strategies to preserve the quality of strawberries during storage.

1. INTRODUCTION

Strawberries (*Fragaria x ananassa*) (*Rosaceae* family) are a soft fruit crop ranked first in production among the small berry crops, with a cultivation area of more than 400,000 hectares worldwide and a global production of approximately 9.118 million tons [1]. Strawberry fruits are rich in vitamins and antioxidants due to the presence of ascorbic acid (i.e., vitamin C) and other bioactive phytochemicals. Phytochemicals in strawberries are known to reduce the risk of cardiovascular disease and inhibit cyclooxygenase activity, allowing for modulation of the inflammatory process; they also possess anticarcinogenic properties and impede the aging process within the brain. Fresh strawberries have a short shelf life, and fruit losses can range up to 40% during storage [2]. Post-harvest decay of strawberries caused by *Botrytis cinerea* (gray mold rot) and *Rhizopus stolonifer* (soft rot) represent major loss during storage and shipment [3]. Control of these fungi during storage can be achieved by physical and chemical methods. Exposure tohigh CO_2 and low-temperature storage is effective in reducing fungal development. However, exposure to high CO_2 for prolonged periods could cause off-flavors of strawberries even though they can tolerate relatively high CO2 levels. The application of fungicides proves to be the most effective method in reducing post-harvest diseases in strawberries, however, the consumers are increasingly concerned with the chemical residues in the fresh produce. Furthermore, the development of resistance of post-harvest pathogens to chemical fungicides is also another problem [4]. Therefore, the use of natural plant extracts as an alternative to chemical fungicides has gained attention due to their safe and eco-friendly characteristics. Research studies have proven the antifungal property of some essential oils against post-harvest decay caused by fungi during fruit storage [5, 6,7]. Essential oils are volatile aromatic compounds extracted from plant materials by hydro-distillation or pressing processes [8]. The major components in the essential oils responsible for the antifungal properties are terpenes and terpenoids with different functional groups [9]. Therefore the objective of this study was to evaluate the possibility of using *Origanum majorana* L. **(**oregano) essential oil as an alternative to synthetic preservatives to control the common post-harvest decay of strawberries. Also, to determine if this treatment had any negative effects on the quality-related attributes of strawberry fruit.

2. MATERIALS AND METHODS

2.1 Plant materials

The aerial parts of the *Origanum majorana* L.*(*oregano) plant were collected at the flowering stages (June: 2021) from the Derna region which is located in the eastern part of Al-Jabel Al-Akhdar province–Libya. Samples were identified according to the Flora of Libya[10]. Samples were dried in a dark and well-ventilated area at room temperature (28ºC) for 15 days. The leaves used for the extraction of essential oil were separated from the rest of the plant, ground in a mortar, and kept in a clean container until use.

2.2 Preparation of essential oil

The essential oilwas extracted by hydro-distillation according to the method recommended by British Pharmacopoeia [11,12]with some modifications. For this, the dried and ground plant samples (100g) of *Origanum majorana* leaves were subjected to hydrodistillation for 4 hrs. then extracted with petroleum ether (40-60ºC; sigma) and dried over anhydrous sodium sulfate and filtered. The solvent was allowed to evaporate and the collected oil was stored in a dark bottle at 5ºC until use.

2.3 Strawberry fruits

Strawberry fruits (*Fragaria ananassa*) were harvested by hand at the mature red stage from a commercial greenhouse near Benghazi city and transferred to the laboratory within 1 h. The berries of uniform size, free of physical damage and fungal infection, and almost the same stage of maturity were selected. A quantity of 50 fruits was randomly distributed into five replicates of 10 fruits each for each treatment.

2.4 Preparation of oregano oil concentrations

The different concentrations of oregano oil were prepared by dissolving the wanted amount in 0.05% tween 20 solutions to obtain 250 ppm and 500 ppm of tested oil. The strawberry fruits were divided into three groups (50 fruits for each) and each group was treated with one of the oil concentrations using a hand-sprayerand leftto air dry at room temperature [13]. The treated fruits were aseptically transferred to clean plastic trays and wrapped with plastic film (Cling film,

falcon, U.A.E.) and stored at 7°C and 90% RH until 50% of the fruits of the control sample was spoiled. Strawberries were monitored daily for any fungal growth symptoms, and decayed fruits were removed from the lotto to avoid secondary infection. Different quality attributes were observed during 8 days of storage. Measurements were made every 4 days up to the end of the experiment.

2.5 Decay rate of strawberry fruits

The methodology used to determine decay percentage was a modification as previously described [14]. Strawberries were visually assessed at days 0, 4,and 8and then weighed. Strawberry fruits showing surface mycelia of fungi, visible lesions, brown spots, and softening are considered decayed. Results were expressed as the percentage of fruits decayed.

2.6 Weight loss percent

Weight loss was measured after 0, 4, and 8 days. The weight of individual fruits was recorded at the beginning of harvest as reference weight and at different sampling times and calculated by using the following formula:

$$\text{Weight loss (\%)} = \text{initial weight} - \text{recorded weight} / \text{initial weight} \times 100$$

2.7 Titratable Acidity (TA) and pH:

Titratable acidity was measured according to the method described in A.O.A.C [15]. For this 6 g of strawberry juice was weighed and 50 ml of distilled water and some drops of phenolphthalein indicator were added then, the mixture was titrated with 0.1N NaOH. The results were converted to percent citric acid and expressed grams of sample in terms of fresh weight.pH was determined according to the standard method [15].

2.8 Total soluble solids (TSS)

Total soluble solids were measured according to the standard method described by A.O.A.C.[15]. A random sample of berries (3 berries) was sampled per replicate, juiced, and filtered to get a clear sample. TSS content was determined usinga digital Abbe refractometer and results were expressed in °Brix.

2.9 Vitamin C content

The content of vitamin C was determined using the indophenol procedure as described by the standard method [15] using the 2, 6-dichloroindophenol titrimetric method. 10 ml of samples were filtrated and titrated against sodium 2, 6-dichlorophenol indophenol dye to a faint pink color which persisted for 5-10 seconds. It was expressed as mg vitamin C/ml fruit juice.

2.10 Anthocyanin determination

Determination of anthocyanin content was conducted by extracting 5 g of a fresh sample using 25 ml of acetone 80% containing 0.2% of formic acid. The content of anthocyanins was estimated by the pH difference method using Aquamate Plus UV/Vis Spectrophotometer (Thermo Scientific, England) at two wavelengths 496 nm and 700 nm. Results were expressed as milligrams of cyanidin-3-glucoside (C3G) equivalents per kg of fresh weight[16].

2.11 Determination of the total phenol content:

Total phenolic content was determined with Folin-Ciocalteu reagent by the method of Singleton and Rossi [17] using gallic acid as a standard and the results were expressed as mg of gallic acid equivalent/100g of fresh weight (mg GAE/100gm fw).

2.12 Estimation of total carbohydrate

Total sugars were estimated according to the standard methods [15] by following the anthrone method, which was carried out by using 100 mg of fruits. 5 ml of 2.5N HCL acid solution was added and heated in a water bath for 3 hrs, then cooled at room temperature and neutralized by sodium carbonate until the effervescence disappears. After the centrifugation process, 4 ml of anthrone reagent was added to 1 ml of the suspended liquid. The mixture was heated for 8 min and cooled rapidly. The absorbance was measured at a wavelength of 630 nm.Glucose was used as the standard material, and the concentration of carbohydrates was calculated by the standard curve. The results were expressed as mg/100gfw.

3. Statistical analysis

The data obtained in this study were analyzed using a completely randomized design (CRD) with 3 replications and the results were expressed as (mean ± standard deviation). Data were

subjected to two-way variance analysis (ANOVA) to evaluate differences between treatments at a significance level of $P \leq 0.05$ using SAS Software (Version 8.2; SAS Institute, Cary, NC, USA). Mean differences were separated by Duncan's Multiple Range Test (DMRT).

4. RESULTS AND DISCUSSION

4.1 The effect of oregano essential oil on strawberry decay

The effect of spraying fresh strawberry fruits with different concentrations of oregano essential oil on fruit decay is illustrated in Figure (1). It is clear that oregano treatment was highly effective in reducing gray mold and soft rot incidence in strawberry fruits caused usually by *B. cinerea* and *R. stolonifer*. The control of decay in fruits subjected to 500 ppm treatment was significantly ($P \leq 0.05$) high as it reduced gray mold incidences by more than 70% after 8 days of storage at 7°C. However, control samples and fruits exposure to 250 ppm showed significantly higher decay incidence reachingup to 50% compared to that of 500ppm treatment (20%).

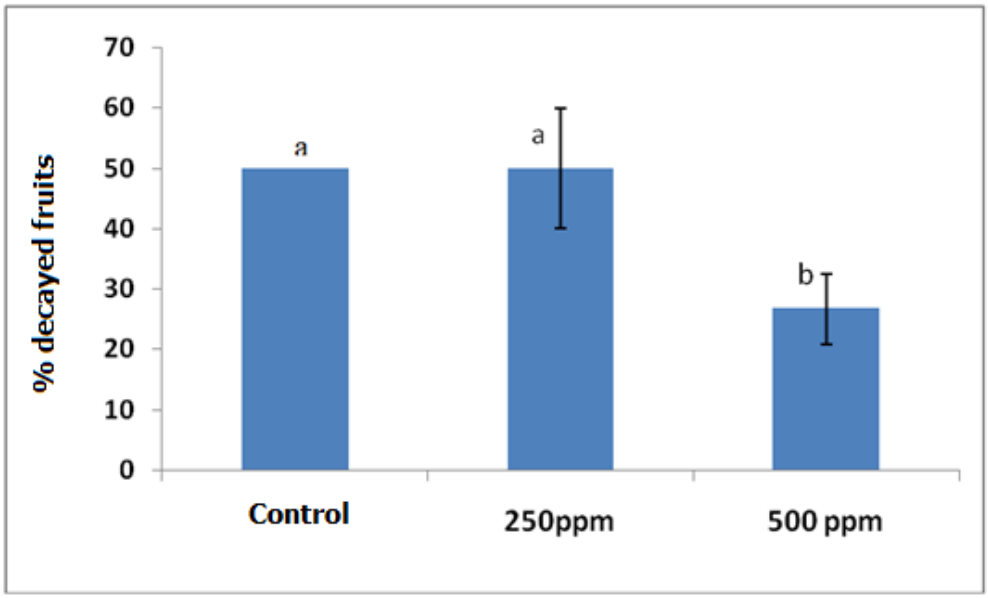

Figure (1): The effect of the essential oil of oregano on the % decay of strawberry fruits stored at 7°C for 8 days.

Averages bearing the same letters have no significant differences at $P \leq 0.05$.

These results are similar to those of Guang et al.[18] who used different amounts (0 to 1.656 microcapsules/gm) of microcapsules of oregano essential oil wrapped in small polypropylene non-woven package to increase the shelf life of strawberries during storage in a room temperature (16~18℃) for 4 days. The results showed that this treatment effectively inhibited the decay of strawberries and fruits presented the best quality when the number of microcapsules reached 0.828g/500 g of strawberries. Wang et al.[19] showed that treatments with thymol, eugenol, and menthol reduced decay in strawberries, with thymol being the most effective at slowing berry decay compared to the other two compounds. Similarly, p-cymene, linalool, carvacrol, anethole, and perillaldehyde effectively retarded blueberry mold formation [20]. Also, Vitoratoset al. [21] confirmed the effect of essential oils of thyme, oregano, and lemon with different concentrations, on strawberry, tomato, and cucumber, to evaluate the reduction of propagation of different fungi. The vapor of thyme oil (extracted from Thymus plants of the *Lamiaceae* (mint family) was used to fumigate sweet cherries and showed effective control of grey mold rot (*Botrytis cinerea*) [22].Wang et al.,[19] found that treatment with thymol and eugenol extended strawberry shelf life and increased fruit-free radical scavenging capacity, thereby enhancing resistance to spoilage and deterioration. It was evident that essential oils increased membrane permeability and their compounds dissolved in the membranes caused swelling and reduced membrane function[23]. A lipophilic property of essential oils affects their antifungal activity via their ability to penetrate the cell wall and affects the enzymes responsible for wall synthesis reactions, therefore they altered the morphological characters of fungi [24].

4.2 % Weight Loss

Changes in weight loss of strawberries are shown in Figure.(2). Weight losses of strawberries treated with oregano essential were significantly ($p< 0.05$) lower than those of control strawberries. The highest weight loss (7.5%) of strawberries was observed in control strawberries after 8 days of storage, whereas the lowest weight loss (5.0%) was reported in strawberries that were treated with either 250 or 500 ppm of oregano oil.

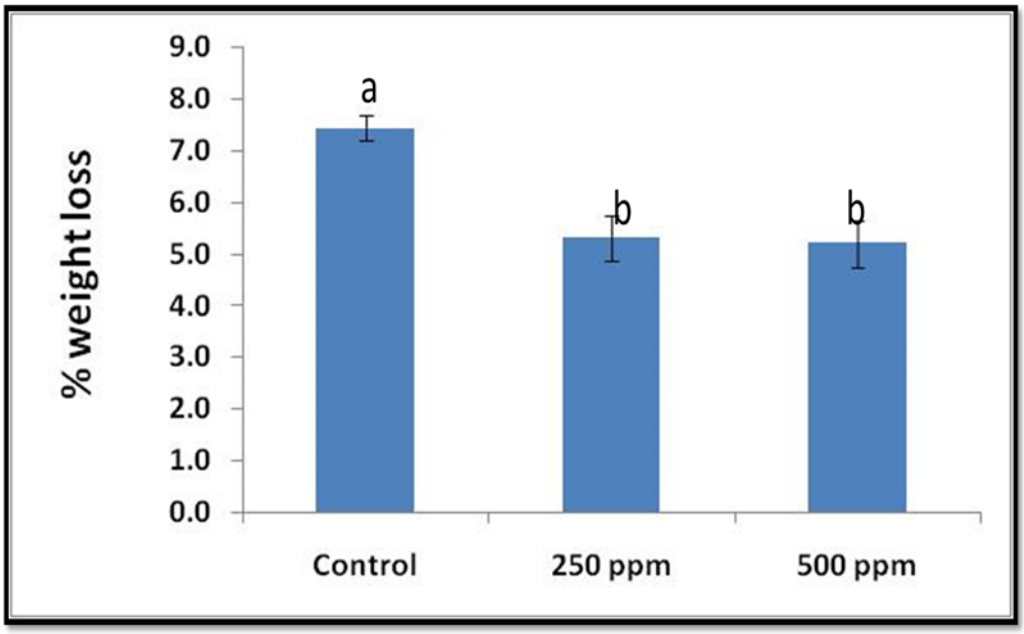

Figure (2): Effect of oregano treatment on weight loss changes of strawberries stored at 7°C for 8 days

Averages bearing the same letters have no significant differences at $P \leq 0.05$.

The higher reduction of weight loss by oregano oil would indicate the role of this oil in lowering the dehydration process. The same results were obtained when strawberries were stored in a box with 0.828 g oregano oil microcapsules as treated fruits had the lowest weight loss (16%) after 4 days of storage at room temperature (16~18°C) whereas the highest weight loss (26%) was observed in control strawberries [18,25,26].

4.3 Total Soluble Solids (TSS)

The TSS evaluation showed that untreated fruits had the highest value of %TSS, whereas the TSS content of oregano treated fruits at 250 ppm dropped significantly ($p<0.05$) to the lowest value of 6.45% as compared with control treatment after 8 days of storage at 7°C (Figure 3). It is interesting to point out that %TSS of strawberries treated with higher oil concentration (500 ppm) had no significant differences in comparison with control or 250 ppm treated fruits. Same observations were reported by Yan et al.[1] who revealed that no significant differences in TSS content were found between strawberry fruits exposed to the vapors of *Origanum heracleoticum* essential oil and control samples after 36 days of storage at 25°C. However, our results disagreed

with the results of others [27] who found that treating table grapes with eugenol as an essential oil active component delayed the change in maturity index with depends on TSS but differently depending on the added compound of oil active materials. In the present study, at the end of the storage period,the %TSS of control was higher than that of treated fruits and this may be related to high weight loss in control fruits compared to treated samples.

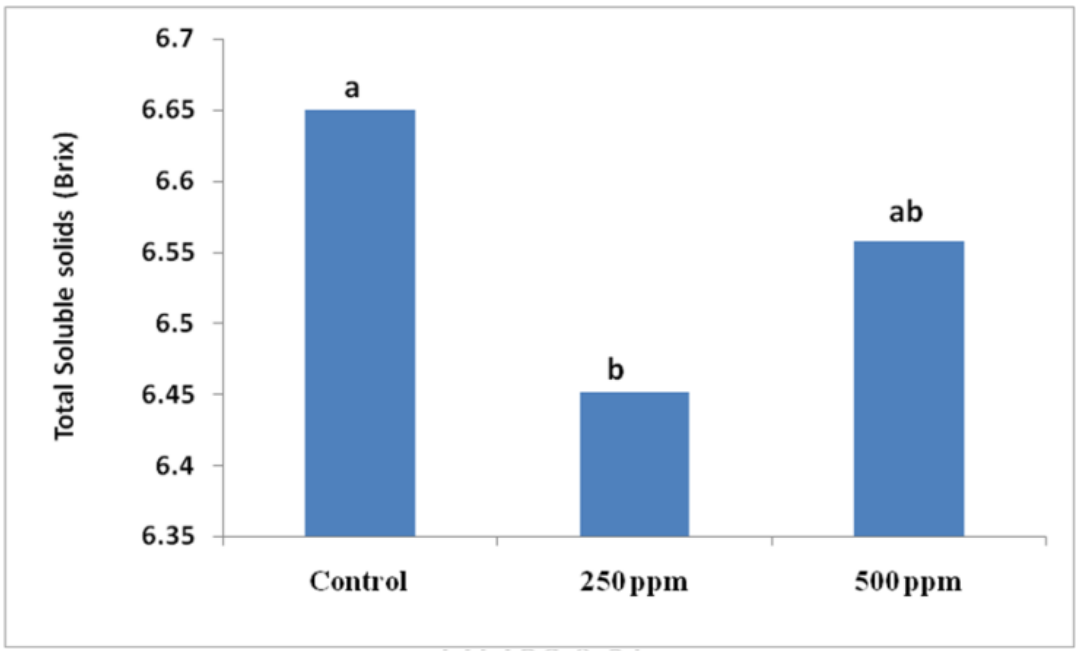

Figure (3) Effect of oregano essential oil on %TSS of strawberry fruits after 8 days of storage at 7°C.

Averages bearing the same letters have no significant differences at $P \leq 0.05$.

4.4 Total carbohydrates

The effect of oregano oil on total carbohydrates in treated strawberry fruits is illustrated in Figure (4). The treatments showed no significant change in the carbohydrate content during the first 4 days of the storage period. On the contrary, a significant dropping in carbohydrate content occurred on day 8 for all treatments compared to day 0 and day4 although no significant differences were reported between treatments on day 8 indicating no efficacy of oil on carbohydrate content (Figure 4).These results are consistent with that previously reported by Sonker et al [28] where two studies on *Vitis vinifera* L. fruits treated with essential oil vapors indicated a decrease in sugar content in fruits due to treatment. In *Vitis vinifera* L. fruits

fumigated with mugwort essential oils, the total sugar content in treated berries was lower than in non-treated control samples [28]. A similar situation was observed when active packaging was developed by adding eugenol or thymol to table grapes stored for 56days under a modified atmosphere; the highest contents of glucose and fructose were recorded in the control samples in both types of storage—cold and normal (shelf-life storage) [27]. Similarly, Meng et al. [29] reported that using methyl jasmonate as a fumigant for the *Agaricus bisporus* fruiting body delayed the decrease in sugar content.

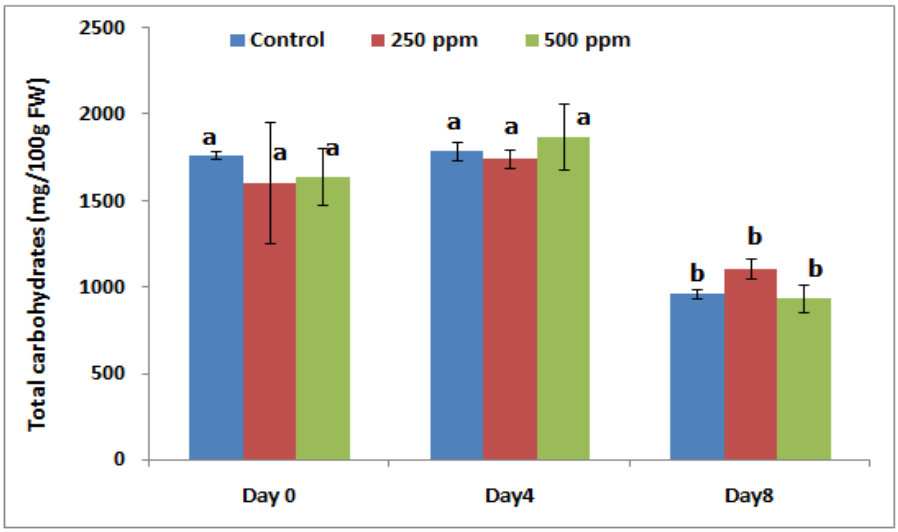

Figure (4): Effect of oregano essential oil on total carbohydrates in strawberry fruits after 8 days of storage at 7°C.

Values followed by a common letter are not significantly different at ($P \leq 0.05$).

4.5 Titratable Acidity (TA):

The amount of titratable acidity (TA) in strawberries is directly related to the content of organic acids present in the fruit. Concerning organic acids, the major one in strawberry fruits is citric acid with levels of 0.83 ±0.023 g/100 g at 0 times in this experiment. Figure (5) shows that at the end of the storage period a significant reduction in TA was reported for control strawberries while acidity retention was shown for treated fruits. This could be due to the delay in physiological aging and alteration in metabolism, which ultimately resulted in higher retention of acidity in treated fruits. Meanwhile, decrease TA in control fruits had high changes of acidity probably due to high respiratory rate and therefore acids consumption quickly and related to

increases in metabolic activity. These results are in line with those obtained by Mahajan et al. [30] suggesting that organic acids were used in the respiratory process. Similar results were observed in the study of Shehata et al. [31] and Gómez-Contreras et al. [32] who found that fruits coated with essential oils reduce water loss, respiration rate, and microbial growth, and thus reduced the consumption of organic acids in respiratory metabolic activities of strawberry fruits, and this significantly checks the loss of titrated acid, which led to extending the shelf life of strawberries during storage.

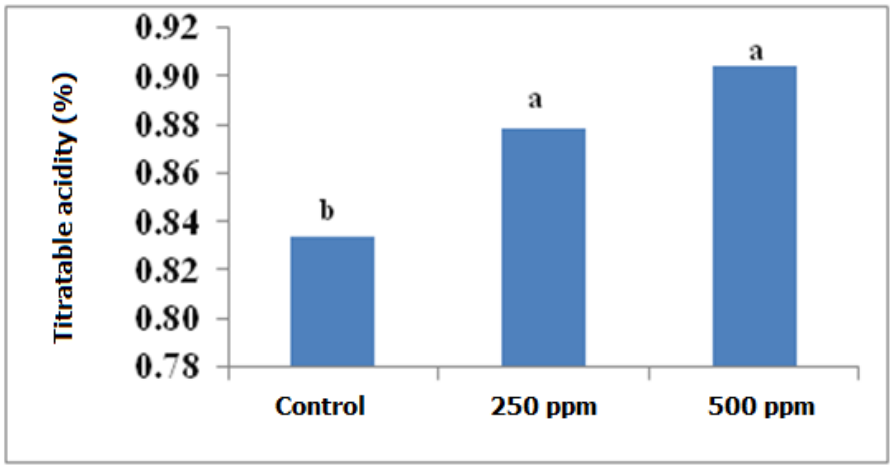

Figure (5): Effect of oregano essential oil on the titratable acidity of strawberry fruits after 8 days at 7^{O}C.

Values followed by a common letter are notsignificantly different at ($P \leq 0.05$).

4.6 Ascorbic acid

Vitamin C, including ascorbic acid and dehydroascorbic acid, is one of the most important nutritional quality factors in many crops and is one of the most important antioxidants that eliminate free radicals. The amount of ascorbic acid is dependent on the intensity of the oxidation process by ascorbate oxidase due to enzymatic activity, and its high value may influence the spoilage and ripening of crops [33]. Due to this fact, its concentration in plants is dependent on the storage time, the temperature during storage, and the amount of oxygen in the atmosphere. Hence, methods of reducing oxygen availability, such as coatings or modified atmosphere packages, are believed to be appropriate for maintaining high vitamin C levels [33].In the current study, the levels of ascorbic acid at 0 times for all treatments were 0.43±0.01

mg/ml, which increased throughout cold storage at 7°C after oregano treatment (Figure. 6). On the contrary, no increase was observed in control berries except a slight increase (0.81±0.23 mg /ml) was reported on day 4 of the storage period although it was not significant. The addition of oregano oil significantly increased the ascorbic acid contents in treated strawberries after 4 days of cold storage to reach a value of 1.26 mg/ml (500 ppm) then declined thereafter to around 0.75± 0.17 mg/ml for fruits treated with either 250 or 500 ppm of oregano oil (Figure. 6).

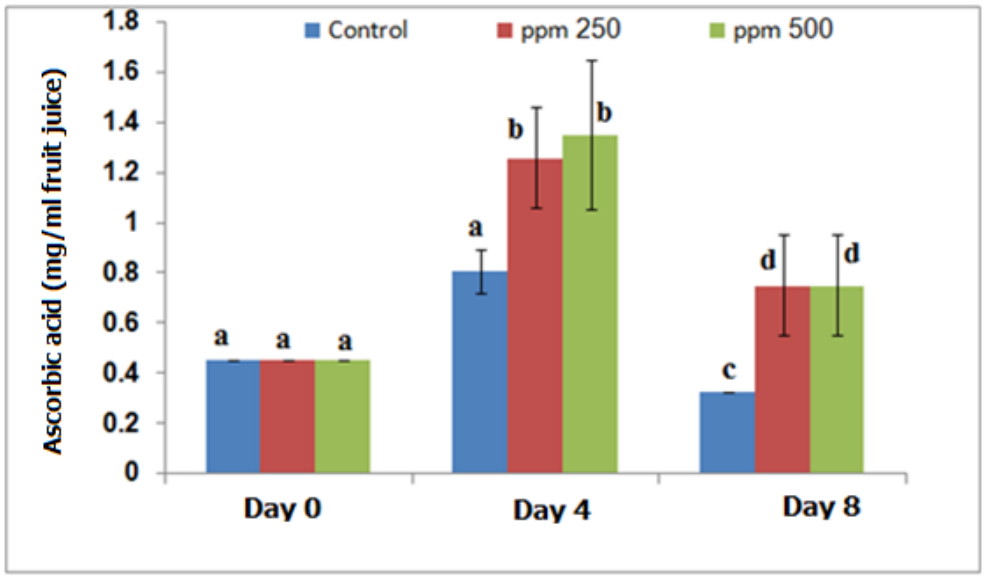

Figure (6): Effect of oregano essential oil on ascorbic acid (Vitamin C) content in strawberry during 8 days of cold storageat 7°C.

Values followed by a common letter are notsignificantly different at ($P \leq 0.05$).

Similar findings were observed by Sahar and Abd El waha [34] who stated that vitamin C decreased by increasing the storage time of Florda 7/2' nectarine treated with several Eos including thyme and rosemary and stored for 4 weeks at 0°C and the highest vitamin C content was obtained from nectarine fruits treated with essential oils compared to control fruits. In the current study, the results agreed with those of Tzortzakis,et al. [6] who found that the amount of vitamin C in tomato fruits enriched by oregano essential oil vapor and increased to reach twice the initial value after the second week of storage. Therefore, it was observed that in normal conditions, the content of vitamin C decreases due to oxidation processes; however, essential oil treatments are capable of maintaining or even increasing the level of vitamin C, probably in

connection with the changes in the level of ascorbate-glutathione pathway enzymes [33]. Additionally, a study on the influence of thyme and savory (*Saturejamontana* L.) essential oil vapors on vitamin C content showed that the treatment slows down the ascorbic acid loss in *Prunus persica*var. nucipersica (nectarines) fruits [35]. Also, Valero et al.[27] studied the effect of active packaging by adding eugenol or thymol on table grapes' quality stored for 56 days under a modified atmosphere (MAP). They noticed that levels of ascorbic acid decreased throughout cold storage; however, control berries suffered the greatest losses of ascorbic acid, which were significant during 28 days storage period. Treatment with methyl jasmonate was proven to help maintain ascorbic acid content by maintaining higher levels of ascorbate and dehydroascorbate in raspberries than in the control samples[36]. In the present study, the maximum retention of Vitamin C was observed with essential oil treatment suggesting that the oil treatment reduced the oxidation in the fruits as the main compounds of oils had antioxidant properties and inhibited damage that causedoxidation of ascorbic acid. This result agrees with Lin et al. [37] who found that the decrease in vitamin C levels was associated with the reduced capability of preventing oxidative damage and with the incidence of physiological disorders during storage. In this study, degradation of vitamin C was highest in control fruits probably due to physiological disorders, decay, and weight loss. Also, the high respiration rate of control fruits rapid the deteriorative oxidation reaction of vitamin C and so, vitamin C declined to the lowest level in control fruits. A similar effect was reported by Cordenunsi et al [38] who studied the effects of temperature (6,15, and 25^{o}C) on the chemical composition and antioxidant activity of three strawberry cultivars.Their results showed an increase in vitamin C contents, indicating that a new biosynthesis had taken place during storage especially when the fruits of the three strawberry cultivars were stored at 16^{o}C, as an increase of at least 10% was detected at the end of storage. However,the same positive effect on vitamin C level was not achieved by lowering the temperature further to 6^{o}C which disagreed with the results of our study. This may be attributed to oil treatment and/or the type of strawberry cultivar as cultivar type can be defined as another important factor affecting vitamin C content which influences the adaptation of the fruit to low temperatures [38]. The increase in ascorbic acid content in fruits is thought to be an indication that the fruits are still in the ripening stage, while a decrease indicates senescent fruit[39]. These results are also similar to that reported on some essential oils extracted from thyme, clove, and orange which were effective in maintaining ascorbic acid [40].

4.7 Total phenols

The effect of oregano oil on the content of total phenols of treated strawberry fruits is illustrated in Figure (7). Fruits treated with oregano oil showed a significant increase in total phenols content as the storage period was prolonged. At the end of the storage period (day 8), the oregano-treated fruits exhibited a significant (p<0.05) higher accumulation in total phenolics compared to control samples (Figure7). It was assumed that the effect of oil treatments on maintaining total phenol content can be attributed to a delay in the senescence process. Also, coating fruits with essential oils contributed to the enhancement of phenolic content as these oils contain high concentrations of phenolic compounds, which are characterized by their antioxidant properties that protect cells from deterioration, as mentioned in several studies that matched these results [27,34].

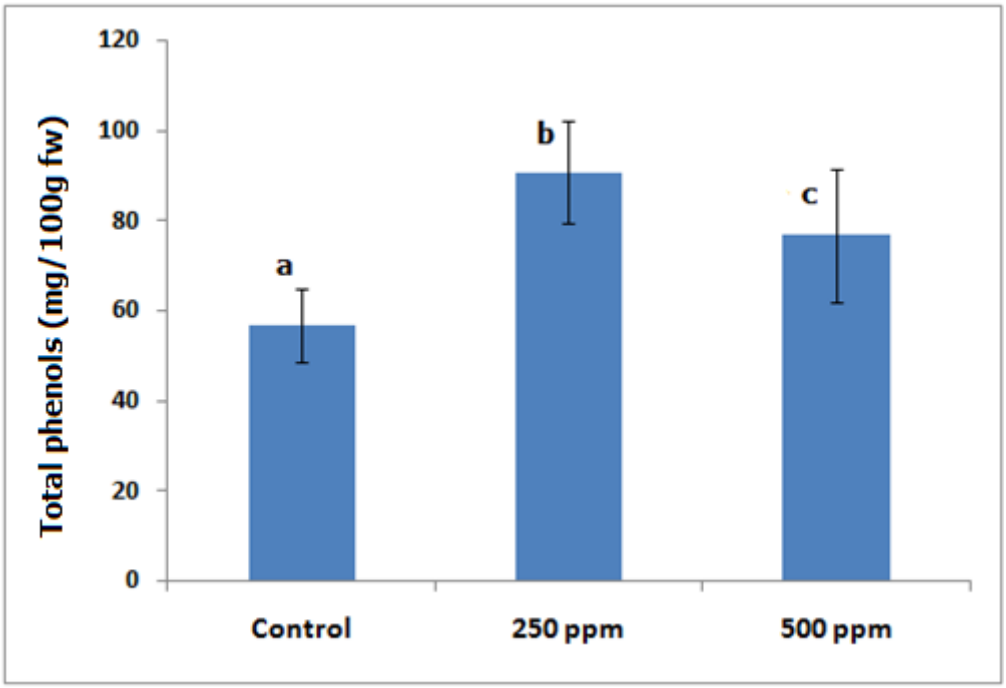

Figure (7): Effect of oregano essential oil on the content of total phenols in strawberry during 8 days of cold storageat 7^{O}C.

Values followed by a common letter are notsignificantly different at ($P\leq$ 0.05).

Phenol compounds are responsible for the flavor and color of fruits [41] and act as antioxidants [39]. Polyphenol oxidization (PPO) activity is responsible for the browning of tissue fruits

through the oxidation of phenolic compounds [42, 43]. In this study, it is evident that oregano oil treatment gave the lowest decrease in total phenols with the advancing storage period compared with the control fruits; this may be that oxidized phenols are more active with decayed fruits by increasing the enzyme activity of Polyphenol oxidize (PPO) responsible for the oxidation of phenolic compounds, and the deficiency of phenols in untreated fruits as a result of the respiration process and cell breakdown [32, 44]. Also,Abdolahi et al.[24] stated that postharvest essential oils treatments kept on the total phenolic content as essential oils containing more phenolic compounds. In addition, Miller and Evans[45] reported that phenolic compounds have been found to play a protective effect on plant tissue.In the current study, the presence of phenolic in the fruit cells may help to maintain the ascorbic acid content value. Tzortzakiset al. [6] found that ascorbic acid content in volatile-treated tomato fruits increased up to 62% comparedto the control following storage in ambient air for 2 weeks. Previous studies suggested that the increase of the phenolic compounds resulted in the increase of the antioxidant activity in sweet basil by Methyl Jasmonate treatment, which in consequence stimulated plant defense mechanisms. In this regard, it is worth noting the marked stimulation in ascorbic acid for oil-treated fruits that might increase the phenolic content [6].

4.8 Anthocyanin content

As shown in Figure (8), anthocyanin content was maintained within the cold storage period. At the end of the cold storage, no significant difference in anthocyanin content was observed between control and treated fruits. However, fruits treated with 500ppm of oil had lower content of anthocyanin (308.6 ±46.0 mg/kg fw) than that of control (344±88.3 mg/kg fw) although no significant difference was observed ($p > 0.05$). Generally, the concentrations of total phenols and total anthocyanins in the fruits depend on varieties and the maturity stage of strawberries at harvest in the open field or during storage [31]. In this study, the increase, especially in control, could be explained as a natural process during fruit ripening, in addition to the effect of the high weight loss that might have contributed to the concentration of pigments [31].

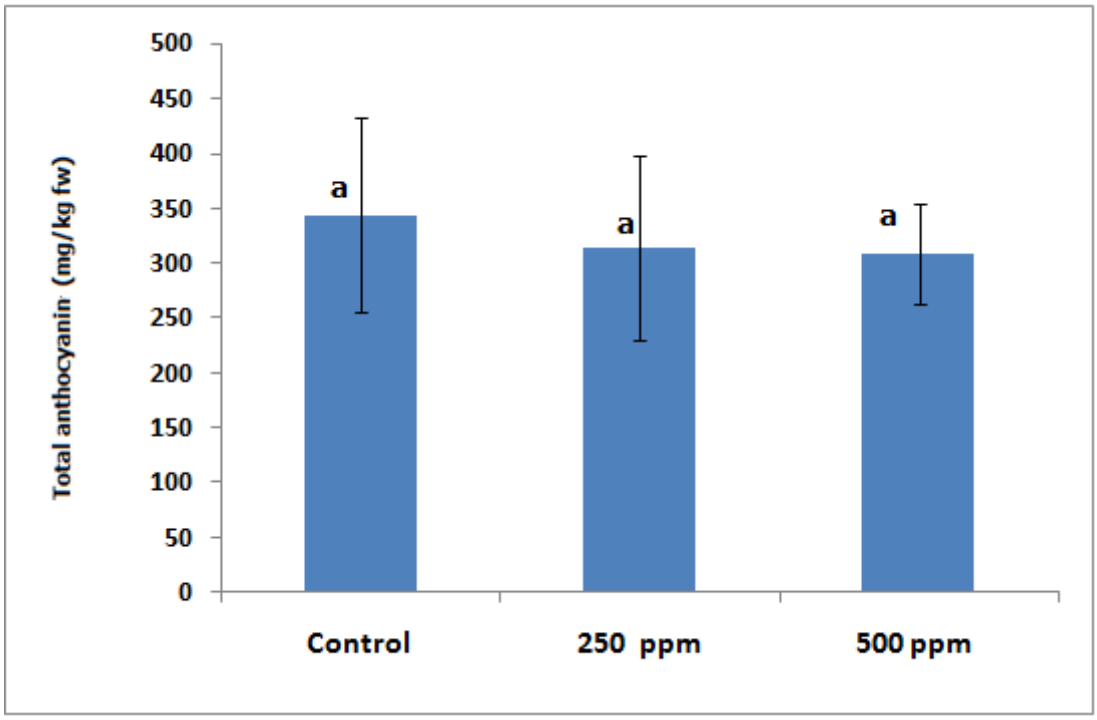

Figure (8): Effect of oregano essential oil on the content of anthocyanin dye in strawberry during 8 days of cold storageat 7^{O}C.

Values followed by a common letter in each period of storage are not significantly different at ($P \leq 0.05$).(Total anthocyanin expressed as mg Equiv. cyanidin-3-glucoside /kg fw).

5. CONCLUSION

This study demonstrates the noticeable antifungal activity of essential oil from oregano leaves against spoilage fungi of strawberry fruits. It can be concluded that oregano essential oil may be a promising natural agent in postharvest disease control of strawberry fruits as it delayed the fruit decay up to 7 days during cold storage without negative changes in the important chemical compositions such as total carbohydrates or vitamin C contents. The treatment maintained better fruit quality and fruit weight, anthocyanins, and total soluble solids.

Acknowledgments:

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