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Inhibitory effect of *Origanum vulgare* L. essential oil and *Bacillus subtilis* antagonist on blue mold (*Penicillium expensum*) and post-harvest quality parameters of sweet cherry (*Prunus avium* L.)

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ABSTRACT

Fruit rot caused by the pathogenic fungus Penicillium is one of the most critical factors in reducing the post-harvest life of fruit crops worldwide. Inthe present study, the effects of marjoram essential oil and Bacillus bacteria on the control of rot caused by a blue mold of cherry (*Prunus avium* L.) in the horticultural laboratory of Urmia University was evaluated.Marjoram essential oil was extracted at the flowering stage by Clevenger apparatus and hydro-distillation. First, cherry fruits were infected with mushroom suspension at a concentration of 10^5 ha/ml. Then, experimental treatments, including suspension with a concentration of 10^8ha/ml selected strains of antagonist bacteria and marjoram essential oil, were applied at five levels of concentration (0, 250, 500, 750, and 1000 µl/l). The fruits were stored in the refrigerator at a temperature of 0 °C and relative humidity of 90-95% for 30 days.Finally, different qualitative traits including titratable acidity, pH, soluble solids, tissue firmness, weight loss, antioxidant activity (DPPH) and fungal rot of the fungus were evaluated at three times (0, 15 and 30 days) and in three replications. The marjoram essential oil at a concentration of 750 µl/l and Bacillus bacteria was the most effective treatment in maintaining titratable acidity, lowering pH and soluble solids on the 15th day. Also, antioxidant activity was enhanced by increasing the concentration ofmarjoram essential oil. The lowest rate of fruit weight loss was observed at a concentration of 500 µl/l of essential oil during the 15th day. According to our findings, marjoram essential oil compounds, especially carvacrol, as well as Bacillus bacteria, as natural compounds and guaranteeing human health, can be suggested as alternatives to chemical compounds in the control of pathogenic fungi in fruit products.

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1. Introduction

Molds caused by pathogenic fungi will cause serious health risks to consumers by producing mycotoxins in food. These toxins include secondary metabolites called mycotoxins, which cause harmful effects including carcinogenesis, abnormalities, growth retardation, reduction of the immune system, and mutagenesis in organisms. Citrinin is one of these examples[1] and patulin[2] and penicillin acid[3] Produced by a fungal species*The pencil was spent*He pointed out that they cause abnormalities in fetal growth and carcinogenesis [1]. Fruits and vegetables are one of the most important human food sources and in fact provide vitamins and antioxidants needed by the body and are essential for maintaining health. Cherries are nutritious fruits, rich in polyphenols and have high antioxidant potential. However, due to high humidity, high acidity and the type of food and wounds caused by harvesting and transportation, it is prone to attack by pathogenic fungi. Due to the existence of limitations in the control of fungal diseases, such as the shortage and high cost of antifungal drugs, their side effects, as well as the resistance of drugs or the reduction of the sensitivity of fungi to these types of drugs, researchers have paid attention to the search for biological metabolites in order to control biological spoilage. and increasing the shelf life of perishable products should be more focused [2].

Marjoram medicinal plant*Oregano vulgaris*L.) from the mint family is one of the most important and best-selling medicinal plants in the world. Marjoram essential oil has a wide range of antimicrobial activities, and carvacrol and thymol compounds are responsible for these properties [3]. Many studies have proven the protective effect of marjoram essential oil in various fruits such as cherries, bananas, kiwi, grapes, pears, avocados and strawberries [4]. In addition to the shelf life of fruits, reports show that marjoram essential oil with 71% carvacrol and 4.5% The percentage of thymol has the ability to improve the shelf life of food [5].

On the other hand, biological control alone or in combination with other methods of controlling pathogens is one of the most effective alternative methods in reducing the use of chemical poisons [6]. Antagonist bacteria of the Bacillus genus are effective in inhibiting fungal pathogens by producing antibiotics, extracellular secretions, and volatile compounds. Hadian et al. [7] in their study, the effect of four essential medicinal plants, Shirazi thyme, aloe vera, garlic, and cumin, as well as antagonistic activity Three bacterial isolates included*Bacillus sp*□*Pseudomonas sp*،*Streptomycess sp*investigated against Penicillium fungus on citrus fruits and reported that the highest inhibition of fungal growth was related to thyme essential oil and isolate*Bacillus cereus*In a similar study on citrus fruits, Yang et al. [8] emphasized the antifungal effects of carvacrol against Penicillium. Marjoram plant essential oil has antifungal and antioxidant compounds, which a number of researchers have the ability to inhibit mycelial growth and spore production of pathogenic fungi. They have reported the harvest in apple fruit [9] and grape fruit [10]. Considering the use of natural compounds subject to preventing the decline of quantitative and qualitative indicators of fruit products in response to consumer pressure to reduce or stop the use of synthetic chemicals, it is possible to use them in the food industry and post-harvest processing to a large extent. Agriculture became operational. Based on this, the present study aims to obtain a compound with high antifungal and antioxidant activity from marjoram essential oil and Bacillus subtilis bacteria against fungus.*P. spent*In order to maintain the appearance quality of cherry fruit during storage, it was examined.

2- Materials and methods

2-1- Preparation of fruit

1. lemon
2. speed up
3. penicillic acid

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Mashhad single seed cherry fruits were harvested in 2017 from a commercial orchard located in Ashnoye city at the ripening stage (TSS=17%) following technical points. Fruits with healthy appearance, uniform shape and level of ripening and maturity were selected and immediately transferred to the laboratory and cold storage of Horticultural Sciences Department of Urmia University for treatment.

2-2- Preparation of essential oil of the medicinal plant marjoram

Marjoram medicinal plant was obtained from the research farms of the Faculty of Agriculture of Urmia University. 300 grams of plant material were crushed, then their essential oil was prepared by Cloninger machine and distilled with water for 4 hours. After dehydrating, the obtained essential oil was stored in dark glasses in the refrigerator at a temperature of 4 degrees Celsius until the test and analysis of its compositions [11].

2-3- Preparation and cultivation of pathogenic mushrooms

A pathogenic fungal strain*P. spent* It was prepared from the microbial collection of the Department of Plant Medicine of Urmia University and cultured on potato dextrose agar (PDA) culture medium and kept at 27 degrees Celsius for seven days. After the completion of the mushroom growth, they were placed at a temperature lower than 4 degrees Celsius [12].

2-4- Preparation and cultivation of antagonistic bacteria

Bacterial strains*B.subtilis* They were obtained from the microbial collection of the department of herbal medicine of Urmia University. After growing the strains on Nutrient Agar (NA) medium for short-term storage, they were placed at 30 °C for 2 days. After the growth of bacterial colonies was completed, it was transferred to a lower temperature (4 degrees Celsius). Bacteria suspension in 25% glycerol at -20 degrees Celsius was used for long-term storage of bacteria [13].

5-2- Preparation of the suspension of the fungus causing the disease

To prepare the spore suspension of the disease-causing fungus, a 7-day mushroom culture on PDA culture medium was used. In this way, some of the spore of the disease-causing fungus was removed and immersed in ten milliliters of sterile distilled water containing 0.05% Tween 80. Using a hemocytometer slide, count the mushroom spore suspension with the number of $10^5 1 \times$ spore per ml was prepared [14].

6-2- Preparation of antagonistic bacteria suspension

In order to prepare Bacillus bacteria suspension, a loop was removed from the plate containing the bacterial colony and mixed with 50 milliliters of NA Nutrient Broth liquid medium. Then, Erlenmeyer was kept on a shaker at a speed of 2000 rpm for 48 hours at a temperature of 30 degrees. Then, the bacterial cells were separated from the liquid medium by centrifugation at 3000×g for ten minutes and washed twice with sterile distilled water. Finally, the bacterial suspension at a concentration of 108 cells per milliliter was prepared by a spectrophotometer [15].

2-7- Treating fruits with pathogenic fungi and antagonistic bacteria

Contamination of cherry fruits with pathogenic fungus suspension at a concentration of 10^5 Spores in milliliters of pathogenic fungi were carried out by immersion method. Then the fruits infected with the fungus with the antagonist bacterial cell suspension at a concentration of 10^8 were sprinkled After drying, the treated fruits were placed in polyethylene containers and kept for 30 days at a temperature of 0±1 degree Celsius and a relative humidity of 90-95%. We used sterile distilled water to treat control fruits.

8-2- Treating fruits with pathogenic fungi and marjoram medicinal plant essence

Contamination of cherry fruits with pathogenic fungus suspension at a concentration of 10^5 Spores in milliliters of pathogenic fungi were carried out by immersion method. Essential oil emulsion was prepared in Tween 80 0.05 solution, then five levels (zero (control), 250, 500, 750 and 1000 microliters/liter) of marjoram essential oil were sprayed on the infected fruits. After drying, the treated fruits were placed in polyethylene containers and kept for 30 days at a temperature of 0±1 degree Celsius and a relative humidity of 90-95%. We used sterile distilled water to treat control fruits.

9-2- Measured attributes

2-9-1- pH of fruit extract

To measure the pH of fruit juice, a digital pH meter (pH-Meter CG 82) was used according to the Iranian National Standard No. 4124 (Iranian National Standard, 1375).

2-9-2- Soluble solids (TSS)

In order to measure the dissolved solids, an ATAGO hand-held refractometer was used, and the percentage of dissolved solids was read in Brix degrees.

2-9-3-Tetratable acidity (TA)

First, 10 ml of the fruit extract was mixed with 20 ml of distilled water and the titration process was carried out by placing the electrode of a digital pH meter (pH-MeterCG824) with normal sodium hydroxide from 0.1 to 8.2 pH, based on the amount of sodium hydroxide. During the titration, the amount of acid in the fruit extract was calculated as grams of acid per 100 ml of fruit extract (grams per 100 ml). The amount of titratable acidity was calculated in terms of acidity equivalent (cherries) according to the following equation.

$$\mathbf{TA} = \left(\frac{S \times N \times F \times E}{C}\right) \times \mathbf{100}$$

where TA: amount of organic acids in fruit extract (g/100 ml), S: amount of NaOH used (ml), N: normality of NaOH, F: NaOH factor, C: amount of fruit extract (ml) and E: The desired acidity equivalents are (malic acid). It is necessary to explain that the predominant acid is cherry (malic acid with 67% equivalent weight) and the acidity is obtained according to this acid.

2-9-4- percentage of weight loss of fruits

To determine the amount of fruit weight loss before entering the storage warehouse, they were weighed with a CANDGL300 digital scale and after leaving it on the 15th and 30th days of the experiment, they were weighed again, and the amount of fruit weight loss was measured by measuring the difference between the initial weight and the secondary weight according to The following relationship was calculated [16].

=% reduction in fruit weight

100x (fruit weight before storage / fruit weight at the end of storage - fruit weight before storage)

2-9-5- Determining the firmness of the fruit texture

In order to evaluate the firmness of the fruit texture, the TA-XTPluss texture measuring device made by Stable Microsystem Company of England was used. It was measured with a 6 mm prop and expressed in terms of newtons [17].

2-9-6- Total antioxidant content by DPPH method

To measure the amount of total antioxidant activity by DPPH method[4]First, some of the methanolic extract was diluted 10 times and mixed with 2000 microliters of DPPH solution, then the resulting solution was kept for 30 minutes at the laboratory temperature for 30 minutes, and the absorbance was read at the wavelength of 517 by a spectrophotometer. Blank was also prepared by the above method, with the difference that 80% ethanol was used instead of 50 microliter extract [18].

$$\mathbf{RSA} = \left(\frac{(Abs\ control)t = \mathbf{30}\ min - (Abs\ sample)t = \mathbf{30}\ min}{(Abs\ control)t = \mathbf{30}\ min}\right) \mathbf{x\,100}$$

in which Abs blank: absorption rate of blank and Abs sample: absorption rate of sample.

2-9-7- The amount of fungal decay

As a percentage, the amount of fruit infection with the pathogenic fungus Penicillium expansum. Therefore, they were evaluated by visual observation and expressed as a percentage. Based on the decay on the fruits, in the form of grades 1: fruits without decay (excellent), 2: good (25% decay), 3: average (50% decay), 4: poor (75% decay) and 5 (100% decay). were investigated [16].

2-10- Statistical analysis of data

For the statistical analysis of the data of this research, SAS software was used based on the three-factor factorial test in the form of a completely random basic design. Comparison of mean traits was done based on Duncan's

[4]. Diphenylhydrazyl

multiple range test and Excel 2016 program was used to draw graphs.

3. Results and Discussion

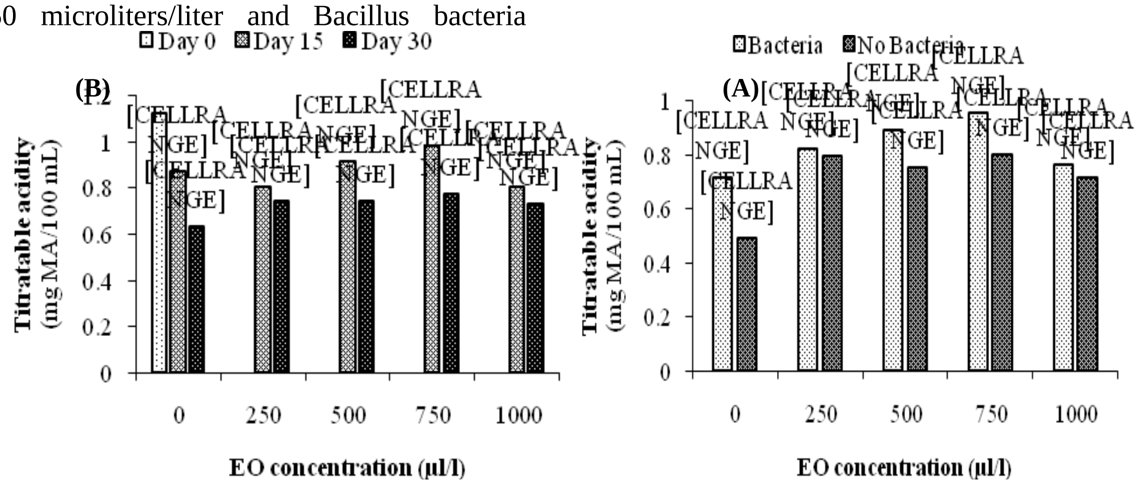
The results of the variance analysis table showed that the simple effect of marjoram essential oil and storage time was significant at the level of 1% probability and the effect of Bacillus bacteria at the level of 5% probability on titratable acidity. Also, the interaction effects of marjoram essential oil and Bacillus bacteria, marjoram essential oil and storage time at the probability level of 1% and the interaction effects of Bacillus bacteria and storage time at the probability level of 5% were significant on titratable acidity (Table 1).

Table 1 Analysis of variance of studied traits in cherry fruit

S.O.V.	df	Mean square						
		Titratable acidity	pH	Total soluble solids	Firmness	Weight Loss	DPPH	Fungal decay
Bactria (A)	1	0.016*	0.001ns	0.01ns	0.003**	0.50**	437.9**	25.3**
Essential oil (B)	4	0.02**	0.77**	77.9**	0.011**	0.59**	571.4**	629**
Time (C)	1	0.33**	0.43*	17.1**	0.106**	4.48**	7209**	1000**
A * B	4	0.03**	0.06*	1.4ns	0.0001ns	0.01ns	79.3*	4.22**
A * C	1	0.014*	0.006ns	4.3*	0.0001ns	0.09**	101.8*	8.82**
B * C	4	0.01**	0.11**	2.36*	0.003*	0.14**	157.1**	7.79**
A * B * C	4	0.005ns	0.06*	0.64ns	0.0004ns	0.002ns	14.66ns	0.27ns
Error	40	0.003	0.02	1.10	0.0002	0.011	24.82	1.03

ns, * and **: Non-significant and significant statistical difference at 5% and 1% probability levels, respectively.

A comparison of the mean interaction effects of marjoram essential oil and storage time is presented in Figure 1(A). The highest amount of titratable acidity related to essential oil was 750 microliters/liter on the 15th day. Figure 1(B) shows the comparison of the average mutual effects of marjoram essential oil and Bacillus bacteria. According to that, the combined treatment of marjoram essential oil at 750 microliters/liter and Bacillus bacteria prevented the reduction of titratable acidity. Based on the comparison of the average interactions between Bacillus bacteria and storage time, the amount of organic acids has decreased with increasing time, and this decrease is greater in fruits without bacteria treatment. Bacteria treatment on the 15th day had the greatest effect on maintaining the amount of organic acids (Figure 1). C).



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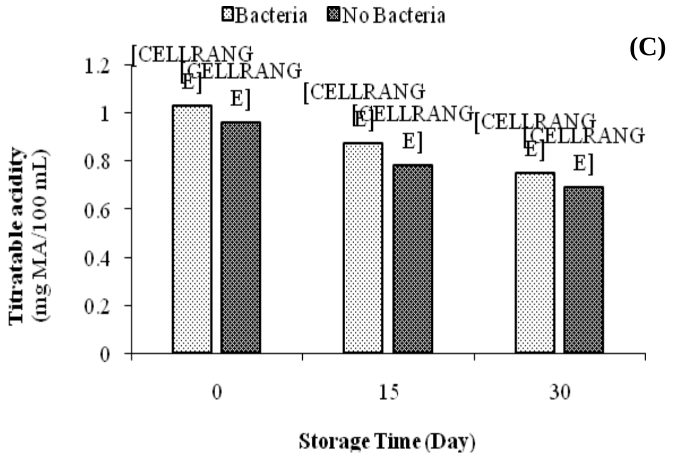

Fig 1 Comparison of the mean titratable acidity of cherry fruit under the effects of EO * storage time (A), EO * B. *subtilis*(B) and B. *subtilis* * storage time (C).
Different letters indicate significant statistical difference (p<0.05).

The simple effects of marjoram essential oil and storage time on the pH of cherry fruit extract were significant, while the effect of Bacillus bacteria was insignificant. Also, the interaction between these three factors was significant at the 5% probability level (Table 1). According to Figure 2, in both cases of bacterial treatment and without it, the pH value has increased with increasing storage time. In the combined treatment of marjoram essential oil and Bacillus bacteria, as well as in the case of no bacterial treatment, the lowest pH was related to the treatment of 750 microliters per liter of essential oil on the 15th day, and the highest value was related to the control treatment on the 30th day. According to the similar results in the bacterial treatment And the treatment without it, it can be seen that the effect of Bacillus bacteria on the pH of the fruit extract was not significant.

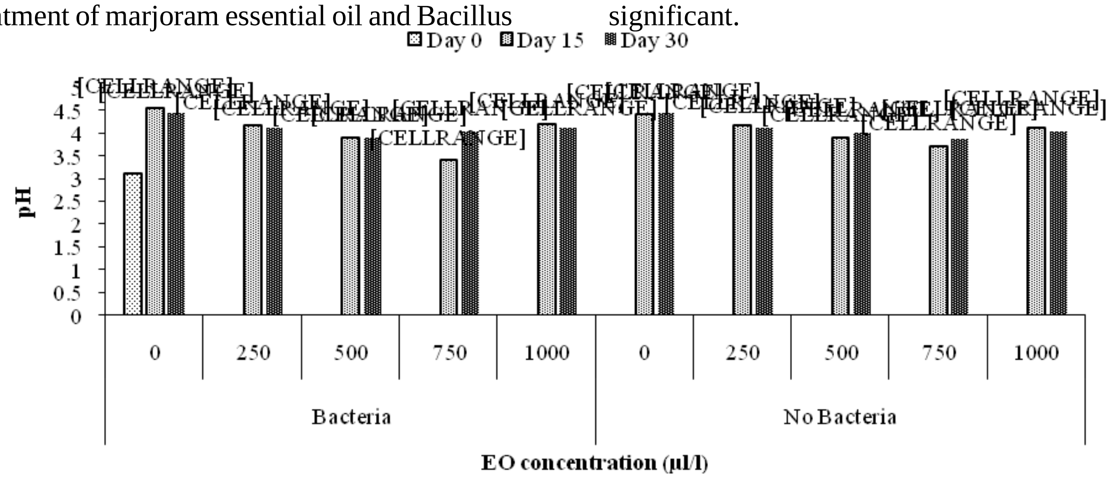

Fig 2 Comparison of the mean pH of cherry fruit under the effect of EO * B. *subtilis* * storage time.
Different letters indicate significant statistical difference (p<0.05).

The results of the variance analysis table show that the simple effects of marjoram essential oil and storage time on the amount of soluble solids were significant at the 1% probability level, but the effect of Bacillus bacteria was not statistically significant. On the other hand, the interaction effects of marjoram essential oil and storage time, bacillus bacteria and storage time were significant at the 5% probability level, but the interaction effects of marjoram essential oil and bacillus bacteria were not statistically significant. In this research, the amount of TSS increased with increasing time. All concentrations of marjoram essential oil prevent the increase of TSS with a significant difference from the control. The lowest amount of dissolved solids was related to the treatment of 750 microliters per liter of essential oil and the highest amount was related to the control data on the 30th day (Figure 3A). Also, our

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results show that Bacillus bacteria treatment on the 15th day has maintained the TSS level better than on the 30th day (Figure 3B).

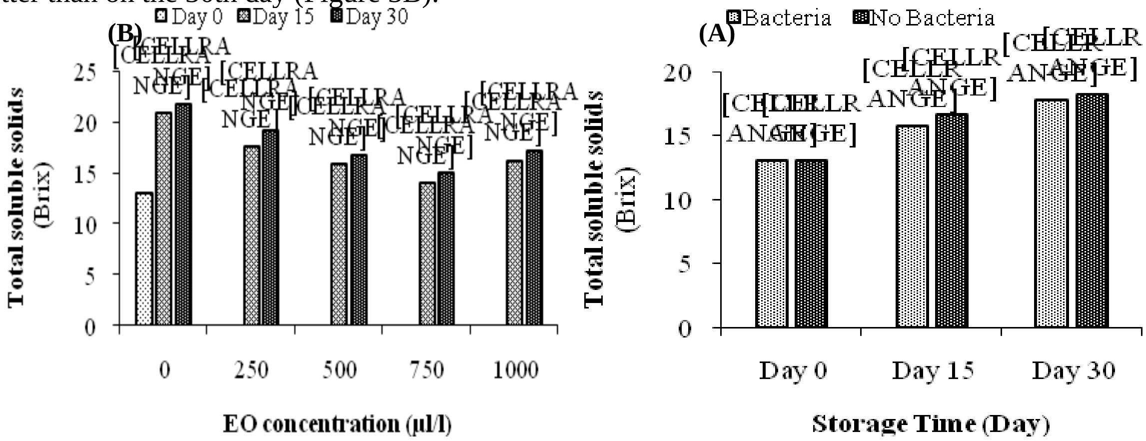

Fig 3 Comparison of the mean total soluble solids of cherry fruit under the effects of EO * storage time (A) and B. *subtilis** storage time (B).

Different letters indicate significant statistical difference (p<0.05).

The simple effect of marjoram essential oil, bacillus bacteria and storage time at the probability level of 1%, as well as the interaction effects of marjoram essential oil × storage time at the probability level of 5% were significant on the firmness of fruit tissue. The interaction effects of Bacillus bacteria and storage time, Bacillus bacteria and marjoram essential oil were not statistically significant (Table 1). According to the comparison of the average mutual effects of essential oil and storage time, we find that with the increase of storage time, the firmness of the fruit texture has decreased. The treatment of 750 µL/L on the 15th day was the most effective treatment in maintaining the firmness of the fruit tissue (Figure 4).

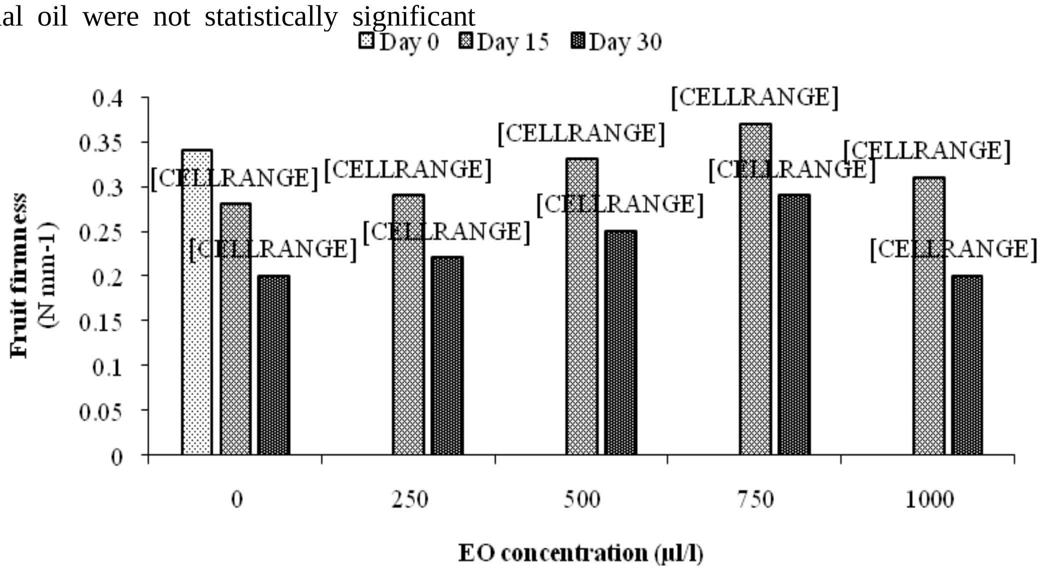

Fig 4 Comparison of the mean firmnessof cherry fruit under the effect of EO * storage time.

Different letters indicate significant statistical difference (p<0.05).

The simple effects of marjoram essential oil, Bacillus bacteria, storage time, as well as the interaction effects of marjoram essential oil × storage time and Bacillus bacteria during storage time were significant at the 1% probability level on weight loss (Table 1). Figure 5 shows the average comparisons of the respective treatments on the amount of weight

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loss, according to which the fruits treated with marjoram essential oil showed less weight loss than the control treatment with increasing storage time. The lowest percentage of weight loss is related to the treatment of 500 microliters per liter on the 15th day, and the highest percentage is related to the control sample on the 30th day. On the other hand, fruits treated with bacteria on the 15th day had the greatest effect in preventing fruit weight loss.

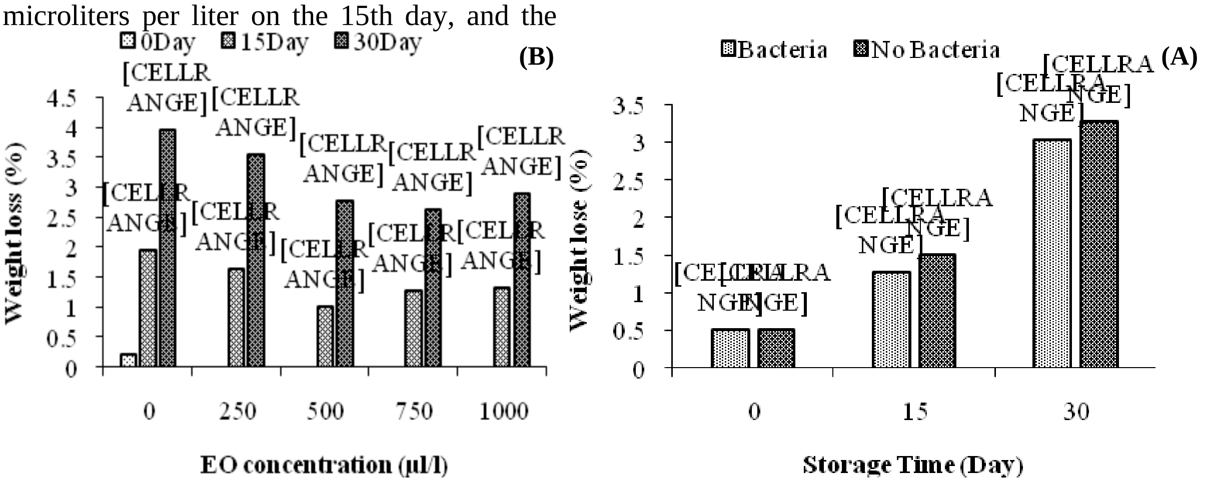
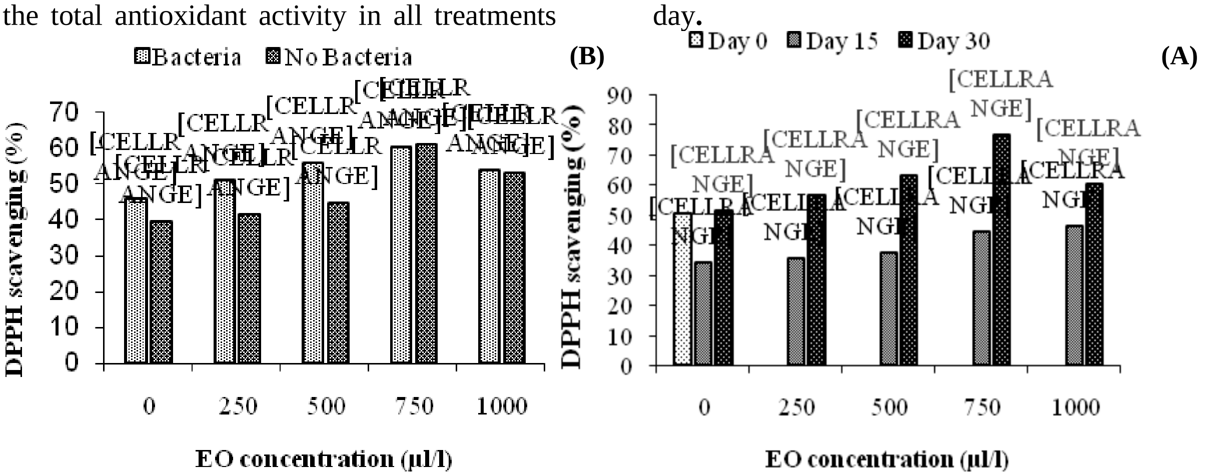


Fig 5 Comparison of the mean weight lose of cherry fruit under the effects of EO * storage time (A) and B. *subtilis* * storage time (B).

Different letters indicate significant statistical difference ($p<0.05$).

According to the results, all simple effects and two-way interaction effects on the antioxidant content of the whole cherry fruit were significant (Table 1). The mean comparison of these two-way interaction effects is presented in Figure 6. The highest amount of total antioxidant activity was related to the combined treatment of Bacillus bacteria and essential oil concentration of 750 microliters per liter. Also, the total antioxidant activity in all treatments gradually increased with the increase of essential oil concentration in both times, so that the highest amount was related to the treatment of 750 microliters per liter of essential oil, and the lowest amount was related to the control sample and zero time. Finally, with increasing time, the amount of antioxidant activity increased. So that the highest amount was related to fruits treated with bacteria on the 30th day.



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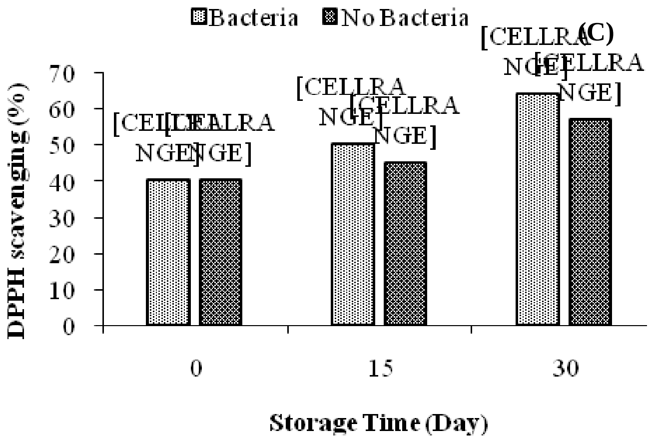
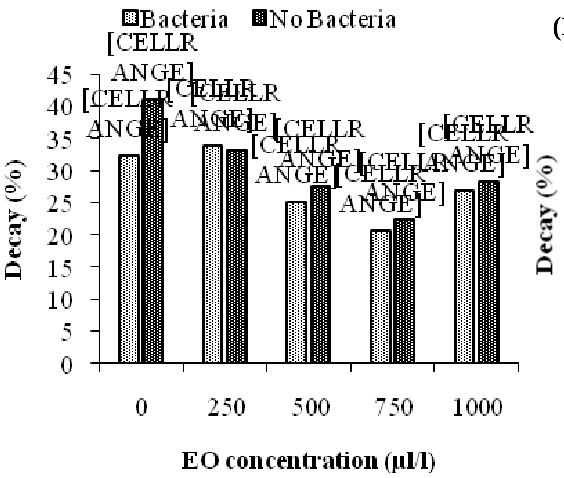
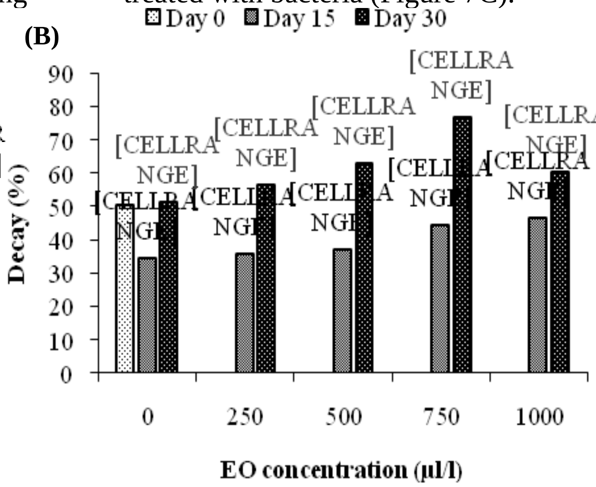


Fig 6 Comparison of the mean DPPH scavenging capacity of cherry fruit under the effects of EO * B. *subtilis* (A), EO * storage time (B) and B. *subtilis* * storage time (C).
Different letters indicate significant statistical difference ($p<0.05$).

The results of the variance analysis table showed that the simple effects of marjoram essential oil, Bacillus bacteria and storage time were significant at the 1% probability level on the decay rate, on the other hand, the mutual effects of marjoram essential oil and Bacillus bacteria, marjoram essential oil and storage time, as well as Bacillus bacteria and time Maintenance at the 1% probability level was significant on the decay rate. The rate of decay in different concentrations of essential oil with a significant difference compared to the control treatment increased the control of decay. The treatment with a concentration of 750 microliters/liter of marjoram essential oil along with Bacillus bacteria showed the lowest rate of decay, and the highest rate of decay was related to the control treatment without bacteria treatment. In this experiment, the effect of Bacillus bacteria at zero concentration of essential oil was significant (Figure 7A). As the storage time increased, the rate of decay increased. The lowest amount of decay was related to the treatment of 750 µL/L, and the highest amount of decay was observed on the 30th day in the control treatment (Figure 7B). In addition, with increasing storage time, the rate of decay has increased and the lowest rate of decay was observed on the 15th day in fruits treated with bacteria (Figure 7C).



(B)



(A)

(C)

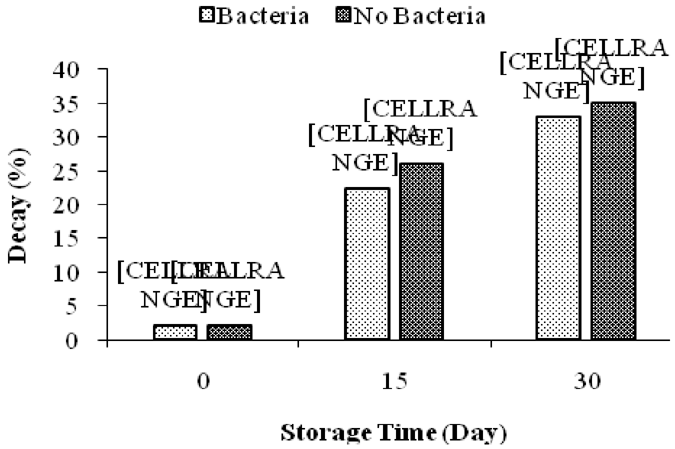


Fig 7 Comparison of the mean decay of cherry fruit under the effects of EO * B. *subtilis* (A), EO * storage time (B) and B. *subtilis* * storage time (C).
Different letters indicate significant statistical difference (p<0.05).

As observed, marjoram essential oil had control effects on the growth of pathogenic fungi. Such ability in marjoram essential oil can be attributed to the activity of its important compounds such as carvacrol. Penicillium species are one of the most destructive phytopathogens that cause post-harvest spoilage, especially in garden products. Pathogenic agents often attack after physical injuries, but there are a number of fungi that attack healthy tissues and smooth the conditions for the development of other pathogenic agents [2]. Regarding the effect of aromatic plants and essential oils extracted from them as antifungal and antiviral compounds, various reports have been presented so far [5 and 19]. The mechanism of the fungal effect of essential oils is related to their permeability in the phospholipids of the membrane of fungal cells and causing disruption in the membrane of the pathogenic agent [20]. In a study investigating the antifungal activity of marjoram and clove essential oil compounds (carvacrol, thymol, agenol) on several Penicillium species that cause citrus rot, they found that the essential oils and their components showed great power in controlling the growth of fungi, and the highest effectiveness was related to thymol. 21 [. Considering that the amount of carvacrol and gamma-terpinene in our research is almost 10 times thymol, so we could not attribute the properties of marjoram essential oil to thymol. Such antifungal effects in marjoram essential oil have also been proven in studies related to increasing the shelf life of food [5]. The results of a research that investigated the antifungal effects of marjoram and cinnamon essential oils in controlling gray mold caused by botrytis fungus, showed that both essential oils in concentrations of 800 and 1000 microliters per liter effectively control the growth of fungi. They also indirectly lead to an increase in phenolic and flavoid compounds and antioxidant activity in the host fruit [22].

Antioxidant activity is one of the important quality factors of fruits and vegetables that affects human health. During this study, more antioxidant activity was observed in the treated fruits than in the control sample, which is probably because the production of free radicals was less in the treated fruits, and as a result, fewer antioxidants were used to remove them. The treatment of plant essential oils during the storage period by preserving the phenolic compounds has preserved the antioxidant activity of the fruit [23]. The presence of phenolic compounds and antioxidant properties in the medicinal plant marjoram has been proven [24]. It seems that the main compounds of marjoram essential oil, including carvacrol, antholes and phenols, increase the antioxidant activity of the host fruit and thus lead to the reduction of aging and the stimulation of defense responses against microbial pathogens [25]. Similar to our results, researchers investigated the effect of medicinal plant essential oils such as garden thyme and

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Shirazi, cloves and cinnamon in order to control the rot caused by green mold in lemon fruit; The results showed that plant essential oils had a significant effect on maintaining antioxidant activity compared to the control sample [26]. On the other hand, microbial antagonists reduce oxidative damage in the host by stimulating antioxidant systems and expressing resistant genes [27]. By examining the mechanisms of bacterial control more closely*Bacillus subtilis JK-14* against post-harvest peach fruit diseases, it was concluded that Bacillus bacteria treatment leads to an increase in antioxidant activity [28], which confirmed our results. Also by examining volatile organic compounds produced by bacteria*Bacillus subtilis CF-3* Against pathogenic fungi*C. gloeosporioides* On lychee fruit, they reported that the volatile compounds produced by reducing the activity of plant tissue-decomposing enzymes produced by pathogenic fungi cause the destruction of reactive oxygen species and reduce cell damage [29]. Titratable acidity decreases during storage in fruits and leads to the breakdown of acids into sugar during respiration, and as a result, the amount of fruit acidity decreases, which has a direct relationship with metabolic activities. In this study, the amount of acidity decreased with time, but this process was slower in the treated samples. Essential oils prevent the reduction of the acidity of the whole fruit by controlling the oxidation activity due to the presence of antioxidant phenolic compounds [30]. In this regard, the vapor of thyme essential oil in cherry fruit has led to an increase in titratable acidity compared to the control [31]. Also, the results of the present research showed that Bacillus bacteria caused a better preservation of organic acids in both storage times. Researchers have proven that in the treatment of lychee fruits with bacteria*Bacillus amyloliquefaciens* LY-1 titratable acidity in all treatments decreased with increasing time, but this decrease was more in control fruits [32].

An increase in the pH of fruit juice can indicate the consumption of organic acids during storage. The low pH of the fruits treated with marjoram essential oil can be explained by the positive role of the essential oil due to the phenolic compounds present in reducing metabolic processes, especially respiration and ethylene, and as a result reducing the hydrolysis of carbohydrates and ultimately maintaining the pH [33]. Maintenance, the pH was maintained at lower levels in all treatments compared to the control with a significant difference; The lowest pH level was related to the concentration treatment of 750 microliters per liter. The results of this study were consistent with the results of other researchers on peach fruit [30] and pomegranate fruit [34]. It was observed that the application of marjoram essential oil and Bacillus bacteria reduced the amount of soluble solids in the fruits. Thus, TSS in the treated fruits was much lower than the control sample. The increase in TSS in untreated fruits is due to the breakdown of carbohydrates, hydrolysis of polysaccharides and pectin materials of the cell wall. Plant essential oils are like an insulator around the fruit by preventing water loss, which leads to an increase in sugar concentration in the fruit, and also prevent cell destruction and reduce the TSS of the fruit during storage [35]. In agreement with the findings of this research, the researchers have proven the effect of the essential oil of the medicinal plant Khoshariza on reducing the amount of TSS during the storage of strawberries [36]. On the other hand, with the use of bacteria*Bacillus amyloliquefaciens* LY-1 on lychee fruit, it was reported that although the level of soluble solids increases at the end of the storage period, TSS in the treated fruits was lower than the control [32]. It seems that the reduction of TSS is due to the activity of cell wall digesting enzymes and the conversion of pectin into soluble galactronic acid [32]. The firmness of the fruit is one of the important factors in attracting the consumer's opinion. The softening of the fruit tissue during storage is caused by the increase in the activity of decomposing enzymes. In the present study, the firmness of the fruits decreased over time, so the fruits treated with marjoram essential oil were able to maintain the firmness of the fruit better than the control. Plant essential oils as edible coatings prevent water loss and softening. On the other hand, the essential oils of medicinal plants increase the

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antioxidant activity of the host plant due to the presence of compounds such as carvacrol and other phenolic compounds and lead to a decrease in the rate of aging and softening of fruits [37]. One of the reasons that essential oils reduce weight loss is related to their fatty properties that cover the surface of the fruit, which reduces the rate of respiration and weight loss, and then preserves the firmness of the fruit. Our study showed that the concentration of 1000 microliters/liter of marjoram essential oil, both in combination with bacteria and alone, had less effect on maintaining the firmness of the fruit tissue, which may be because the high concentration of essential oil acts as stress and increases cell metabolism. While in combined treatments with bacteria, it has prevented the growth of bacteria and its effect. In a study that was conducted with the aim of controlling the Penicillium fungus, it was concluded that the stiffness of the tissue in Japanese parsnip fruits treated with *Bacillus amyloliquefaciens B4*It decreases compared to control samples [38]. Similar to this effect of the antagonistic bacterial strain*Pseudomonas putida BP25*In order to increase the storage life of mango fruit, it was investigated and the results showed that the bacterial strain maintained and increased firmness and reduced the weight loss process of the fruit [39].

The results of this study showed that the treatment of 500 microliters per liter of marjoram essential oil was the most effective treatment in preventing cherry fruit weight loss.**The findings of this research with the test results** Mostofi, Dehestani-Ardakani **And**]40[**It is consistent with the effect of thyme essential oil and chitosan edible coating on grape fruit.**The increase in the percentage of weight loss during the storage period is the result of the evaporation of water from the surface of the fruits. In general, evaporation and transpiration and breathing of harvested products cause weight loss. With the passage of time, the weight loss of fruit increased in different treatments, but the intensity of weight loss was observed less in the treated fruits, the amount of weight loss is correlated with the increase of fungal spoilage. Most likely, the inhibitory effect of plant essential oils against the growth of fungi and other microorganisms can reduce weight loss and water loss in treated fruits. Plant essential oils by limiting the exchange of O_2، CO_2 and water and creating a changed atmosphere around the fruit cells minimize the rate of respiration and ethylene production [41]. Regarding the effect of Bacillus bacteria application, researchers have reported that the trend of weight loss in blueberry fruits treated with Bacillus strain*Bacillus subtilis* KLBC BS6 is slower during retention time than the control. The reason for this is related to the growth of bacteria on the surface of the fruit and the formation of biofilm, which is like a barrier to the exchange of O_2 و CO_2 which reduces the rate of respiration and the wastage of fruit juice, thereby reducing the weight loss of the fruit [42]. Control effect*Bacillus subtilis L1-21* It has been reported on the growth of Penicillium fungus in orange fruit [43], which is consistent with the results of the present study. In another study, biological control of bacteria*Bacillus licheniformis HG03* It was investigated against pathogenic agents of peach fruit after harvest and the results showed that bacterial treatment induces resistance and defense responses, including increasing the activity of defense enzymes and antioxidant capacity [44].

4- General conclusion

According to the results of the present study, it can be concluded that the antioxidant activity of cherry fruit is enhanced by increasing the concentration of marjoram essential oil. Such an application of marjoram medicinal plant essence, especially at a concentration of 750 microliters and Bacillus bacteria, has acceptable effects in maintaining the quality characteristics of cherry fruit and controlling the growth of Penicillium fungus on cherry fruit. Also, the application of marjoram essential oil and Bacillus bacteria kept the firmness of the fruit and reduced the amount of soluble solids in them. The concentration of 1000 microliters per liter of marjoram essential oil had a lesser effect on improving some quality parameters of the fruit, which is necessary to be careful in choosing the appropriate concentration. Finally, considering

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the adverse effects of chemicals on human health and the environment, as well as being more cost-effective Natural compounds, the essence of medicinal plants can be proposed as a potential and suitable alternative to chemical compounds in order to increase the storage time of fruit products after harvest. In this regard, it is necessary to increase future research in this field.

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مقاله علمی_پژوهشی

تأثیر مهارکنندگی اسانس مرزنجوش(*Origanum vulgare* L.) و باکتری آنتاگونیست *Basillus subtilis* روی کپک آبی(*Penicillium expensum*) و پارامترهای کیفی پس از برداشت گیلاس

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چکیده

پوسیدگی ناشی از قارچ بیماری‌زای پنی‌سیلیوم از مهم‌ترین عوامل کاهش عمر پس از برداشت محصولات میوه‌ای در سراسر دنیا می‌باشد. در تحقیق حاضر، اثر اسانس گیاه دارویی مرزنجوش (*Origanum vulgare L.*) و باکتری باسیلوس در کنترل پوسیدگی ناشی از کپک آبی میوه گیلاس (*Prunus avium* L.) در آزمایشگاه باغبانی دانشگاه ارومیه بررسی شد. اسانس مرزنجوش در مرحله گل‌دهی با دستگاه کلونجر و به روش تقطیر با آب استخراج شد. ابتدا میوه‌های گیلاس با سوسپانسیون قارچ به غلظت 10^5 هاگ در میلی‌لیتر آلوده شدند. سپستیمارهای آزمایشی شامل سوسپانسیون با غلظت 10^8سویه منتخب باکتری آنتاگونیست و اسانس مرزنجوش در ۵ سطح غلظت (۰، ۲۵۰، ۵۰۰، ۷۵۰ و ۱۰۰۰ میکرولیتر در لیتر) اعمال گردیدند. میوه‌های تیمارشده در سردخانه با دمای صفر درجه سلسیوس و رطوبت نسبی ۹۵-۹۰ درصد به مدت ۳۰ روز نگهداری و در نهایت صفات مختلف کیفی شامل اسیدیته قابل تیتراسیون، **pH**، مواد جامد محلول، سفتی بافت، میزان کاهش وزن، فعالیت آنتی‌اکسیدانی با روش جاروب‌کنندگی رادیکال **DPPH**و پوسیدگی قارچ طی سه زمان (۰، ۱۵ و ۳۰ روز) و در سه تکرار مورد اندازه‌گیری قرار گرفتند. تیمار اسانس مرزنجوش در غلظت ۷۵۰ میکرولیتر در لیتر و باکتری باسیلوس مؤثرترین تیمار در حفظ اسیدیته قابل تیتراسیون، کاهش **pH** و مواد جامد محلول در روز۱۵ام بود. همچنین فعالیت آنتی‌اکسیدانی با افزایش غلظت اسانس تقویت گردید. کمترین میزان کاهش وزن میوه مربوط به غلظت ۵۰۰ میکرولیتر در لیتر اسانس طی روز۱۵ام مشاهده شد. طبق یافته‌های ما، ترکیبات اسانس مرزنجوش به‌ویژه کارواکرول و نیز باکتری باسیلوس به‌عنوان مواد طبیعی و تضمین‌کننده سلامت انسان می‌توانند جایگزین ترکیبات شیمیایی در کنترل قارچ‌های بیمارگر محصولات میوه‌ای پیشنهاد گردند.