

Application of essential oils and optimizing storage conditions for control of postharvest diseases in apple

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: Postharvest loss in fruit and vegetables accounts for more than one third of the world production. On the other hand, using chemicals has raised food health concerns, and grown the demand for eco-friendly materials. Given the promising results of essential oil use to control and prevent postharvest decay, we conducted this research. In the present study, a two-step statistical method was used to determine and optimize application of essential oils along with parameters of storage conditions. Significant level of essential oils and storage conditions (temperature, ventilation, and relative humidity) were screened by using PBD method and the best concentration were determined by central composite design of response surface method. The results showed that 1000 and 1500 microgram/l ($\mu\text{g/l}$) of basil essential oils, and 1500 $\mu\text{g/l}$ of peppermint essential oils reduced the lesion diameter in apple fruits infected with *Penicillium expansum*. All storage conditions had significant effect on postharvest decay. Based on the CCD of RSM method, the best concentration of essential oils and the optimal level of the storage condition were determined. Furthermore, basil and peppermint treatments reduced rate of the ethylene production during 56 days after treatment. The results of this study revealed and confirmed that basil essential oils as a postharvest treatment under optimized storage conditions can be utilized as a low-cost substrate for controlling postharvest decay in apple.

1. Introduction

It is estimated that more than one third of harvested fruit and vegetables are lost due to pathogen infections in the field or after harvest, resulting in a serious economic loss (Romanazzi *et al.*, 2016). The average loss is about 29% in North America and Europe, and more than 35% in Asia (Romanazzi *et al.*, 2016). Given severity of economic loss worldwide, many researchers have employed various strategies to decrease loss and control the conditions after harvest. The first generation of attempts was to use chemical materials to control diseases, however, health and safety

concerns turned attentions on natural products (Rajestary *et al.*, 2021). Nowadays, essential oils, used as additives, color intensifiers, and antioxidants, are extracted from plant sources have gained attentions because of advantages over synthetic materials such as a good quality, biodegradable, economical characteristics, eco-friendly easy large-scale production, and especially a lack of safety concerns (Sivakumar and Bautista-Baños, 2014). A study suggested that fungal strains could develop a resistant when synthetic fungicide are continuously used, while the different components of essential oils make the process of the resistant more slowly during the application (Sivakumar and Bautista-Baños, 2014).

Recently, many studies have explored the use of essential oils to control postharvest losses. For instance and to mention some of these recent studies, Antonioli *et al.* (2020) reported the use of nano-capsules containing plant essential oil to control bitter rot of apples and showed that the fruits treated with nano-capsules have a smaller lesion. Another study showed that mint and thyme essential oils reduced Rhizopus rot on strawberry and peach fruits. Kontaxakis *et al.* (2020) evaluated several essential oils to assess their effect in preserving fruits during postharvest phase. Many other studies have showed that essential oils are promising to control postharvest decay in fruit and vegetables, however, the question of this study is to (1) find the best concentration in which essential oil could act, and (2) to determine the best environmental conditions of the storage place in which essential oils applied. We think that these points are worth to be explored. Therefore, the objective of the present study was to test efficiency of plant essential oils and optimize storage conditions in apple postharvest period. In this study, two steps statistical method was employed. Moreover, ethylene concentration was evaluated on the optimized conditions.

2. Materials and Methods

Materials and the experiment design

This study was conducted to enhance apple storage through application of plant essential oils as well as optimizing storage conditions. The culture was in submerged ferment and three parameters were assessed to reach an optimal production process. Apples (*Malus domestica*, cv. Red Delicious), harvested from orchards in northern-west Iran, Ardabil, Iran,

were divided in groups of 27 fruits/treatment.

The first parameter was basil essential oil (Eugenol 10.04%, Linalool 68.52%, a-trans-Bergamotene 6.94%, and b-Cadinol 3.20%). The second parameter was peppermint essential oils (1,8-Cineole 5.89%, Menthofuran 4.59%, Menthol 38.29%, and Menthyl acetate 29.39%). Basil and peppermint essential oil were used in three level (500, 1000, and 1500 microgram/l) as a treatment. The third parameter was storage conditions including temperature (-2, -1, and 0°C), ventilation (3, 4.5, and 6 LPM), and relative humidity (85, 90, and 95%). A 15 percent emulsion (15% essential oil, 83% water and 2% Tween) was prepared from basil and peppermint essential oils. The resultant emulsion was shaken for 45 s before spraying.

A two-step statistical strategy (Embaby *et al.*, 2018) was anticipated to optimize and determine relationship among postharvest control of apple fungal diseases with basil essential oils, peppermint essential oils, and storage conditions (temperature, ventilation, and relative humidity). Plackett-Burman Design (PBD) (Plackett and Burman, 1946) was employed to monitor the linear effect of three parameters using 27 experimental trials designed in 28-run design. PBD is an efficient way to screen the most important variables when there are multiple variables. A three-level optimization (+1, 0, -1) arranged in central composite design (CCD) of response surface methodology (RSM) was used to specify the optimal levels of each key parameters deduced from PBD approach.

Treatments

We used *Penicillium expansum* to create the disease in apples. The fungal spores were inoculated on Potato Dextrose Agar (PDA) slants and incubated for 7 days at 30°C. *Penicillium expansum* was maintained on PDA at 4°C for further use. To prepare a suspension of the fungal spore, spore-inoculated PDA was washed using sterile water and added into seed culture medium. Seed medium was prepared by using modified minimal medium (g/L: yeast extract, NaNO₃ 2, KH₂PO₄ 1, MgSO₄·7H₂O 1, KCl 0.5, and FeSO₄·7H₂O 0.01).

Apple fruits were disinfected in 1% sodium hypochloride dried at room temperature. With sterile and small plastic tip, some wound made on the fruits. Pathogen and essential oil suspensions were dropped into each wound. All samples, control and the inoculated, were stored in chambers with optimized storage conditions. The diameter of the rot around each wound was measured after 18 and 26 days.

Ethylene measurement

Ethylene analysis was performed using HPLC analysis. The analysis was performed using a Waters 244, column: Shoex C18, 4 μ m, 250 mm \times 4.0 mm, temperature: 28°C, injector volume: 20 μ l. Fluorescence detector (Waters 470) set at 331 nm excitation and 500 nm emission wavelength. The mobile phase consisted of methanol/acetonitrile/water (3:3:4 v:v:v), the pH value of the mixture was 2.5, and the flow rate was the 0.15 mLPM. The concentrations of ethylene of samples were calculated by the equation: $C_s \frac{1}{4} C_p A_s = A_p$; where C_p and C_s are the concentration of ethylene solution and sample solution, respectively, A_p and A_s are the area of peaks of ethylene solution and sample solution respectively.

Statistical analysis and calculations

Each experiment was performed in triplicate. To

optimize the production of monascus pigments, the software SPSS Statistics 19.0 was used to generate PBD matrices and carry out multiple linear and non-linear regression analyses. All tables and diagrams were prepared in Microsoft Excel 2010. The effects of the treatments on pigment composition, ethylene, and biomass were analyzed by one-way ANOVA, and tests of significant differences were determined by using Student's t-test at $p < 0.05$.

3. Results

Application of essential oils

To study essential oils (basil and peppermint) and storage conditions (temperature, ventilation, and relative humidity), twenty-four groups were analyzed based on PBD design shown in Table 1. Lesion diameter (mm) ranged from 50.4 to 75.8. Based on confi-

Table 1 - Effect of three independent sources including basil essential oil, peppermint essential oils, and storage conditions in postharvest controlling of apple decay caused by *Penicillium expansum* based on PBD. The parameters were Basil and peppermint essential oil in three level (500, 1000, and 1500 microgram/l), temperature (-2, -1, and 0°C), ventilation (3, 4.5, and 6 LPM), and relative humidity (85, 90, and 95%)

| Basil essential oils | Independent variable | | | | Lesion diameter (mm) |
|----------------------|--|------------------|-------------------|-----------------------|----------------------|
| | Peppermint essential oils (μ g/L) | Temperature (°C) | Ventilation (LPM) | Relative humidity (%) | |
| 1500 | 1500 | 0 | 6 | 95 | 69.2 |
| 1500 | 1000 | 0 | 6 | 90 | 72.3 |
| 1500 | 500 | 0 | 6 | 85 | 67.2 |
| 1500 | 1500 | 0 | 4.5 | 95 | 71.2 |
| 1500 | 1000 | 0 | 4.5 | 90 | 75.8 |
| 1500 | 500 | 0 | 3 | 85 | 63.2 |
| 1000 | 1500 | -1 | 6 | 95 | 62.2 |
| 1000 | 1000 | -1 | 6 | 90 | 65.3 |
| 1000 | 500 | -1 | 6 | 85 | 63.7 |
| 1000 | 1500 | -1 | 4.5 | 95 | 67.7 |
| 1000 | 1000 | -1 | 4.5 | 90 | 72.3 |
| 1000 | 500 | -1 | 4.5 | 85 | 56.1 |
| 1000 | 1500 | -1 | 3 | 95 | 64.9 |
| 1000 | 1000 | -1 | 3 | 90 | 70.1 |
| 1000 | 500 | -1 | 3 | 85 | 59.7 |
| 500 | 1500 | -2 | 6 | 95 | 56.5 |
| 500 | 1000 | -2 | 6 | 90 | 59.6 |
| 500 | 500 | -2 | 6 | 85 | 58.3 |
| 500 | 1500 | -2 | 4.5 | 95 | 62.6 |
| 500 | 1000 | -2 | 4.5 | 90 | 66.4 |
| 500 | 500 | -2 | 4.5 | 85 | 50.4 |
| 500 | 1500 | -2 | 3 | 95 | 59.2 |
| 500 | 1000 | -2 | 3 | 90 | 64.3 |
| 500 | 500 | -2 | 3 | 85 | 54.1 |

dence level (>95%), the results showed that basil essential oils have a more significant effect on preventing apple postharvest control of the fungal disease. Of storage conditions, results showed that temperature, ventilation, and relative humidity have a significant effect on controlling the fungal diseases in apple postharvest period. All storage conditions (temperature, ventilation, and relative humidity) were found to have a significant effect (p-value < 0.05). The results of multiple linear regression indicated that basil essential oils as the best treatment under the condition set for storage (intercept effect) have a significant influence on postharvest controlling (Table 2).

When basil essential oils used as treatment, it decreased lesion diameter about 60% and 200%, respectively, compared to those observed in the fruits treated with peppermint essential oils (Fig. 1). A significant percentage of increase 15% and 35%, respectively, was obtained, when basil essential oils used in association with -2°C of temperature, 4.5 LPM of ventilation speed, and 90% of humidity compared to other storage conditions (Fig. 1). With regard to peppermint essential oils, the highest level of postharvest control was achieved in -2°C of temperature, 6 LPM of ventilation speed, and 85% of humidity.

Pertaining to storage conditions, temperature set to -2°C increased postharvest control over the disease through declining lesion diameter, especially when basil essential oils was used as treatment, by

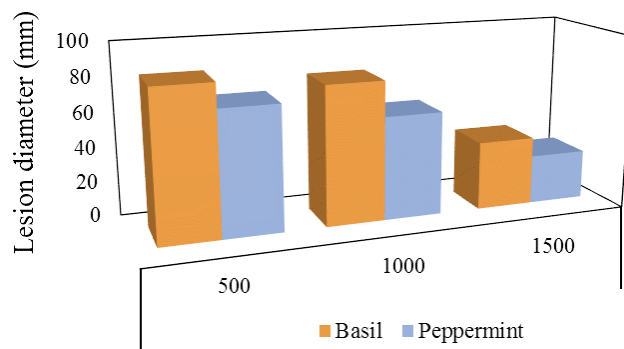


Fig. 1 - Application of basil and peppermint essential oils on postharvest controlling of apple decay caused by *Penicillium expansum* between treated fruits with basil and peppermint essential oils. Values represented in the figure are expressed in percentage (%).

an average decrease of 55% compared to the samples stored at -1 and 0°C (Fig. 2). Based on data of PBD and RSM, sample fruits with application of basil essential oils maintained at -2°C and ventilated with 4.5 LPM speed resulted in significant decrease of lesion diameter (Fig. 3, 4).

Effect of essential oil application on apple ethylene concentration

To determine the effect of optimized storage conditions and essential oil treatment (basil and pepper-

Table 2 - Multiple linear regression of PBD data for postharvest controlling of apple decay caused by *Penicillium expansum* using three independent sources including basil essential oil, peppermint essential oils, and storage conditions

| Model | B-coefficient | | | t-value | | | p-value | | | Confidence (%) | | |
|----------------|---------------|---------|--------|---------|--------|--------|-----------|----------|---------|----------------|--------|--------|
| | A | B | C | A | B | C | A | B | C | A | B | C |
| Intercept | 53.642 | 48.155 | 46.49 | 8.642 | 9.392 | 9.962 | 1.32E-04* | 5.4E-06* | 4.8-01* | 99.99* | 99.99* | 99.9* |
| X ₁ | 30.458 | 24.971 | 23.306 | 4.542 | 5.292 | 5.862 | 0.175001 | 0.80123 | 0.16471 | - | - | - |
| X ₂ | -10.254 | -11.741 | -7.406 | -1.254 | -0.504 | 0.066 | 0.163501 | 0.17999 | 0.29019 | - | - | - |
| X ₃ | -12.429 | -8.916 | -6.581 | -1.429 | -0.679 | -0.109 | 0.192001 | 0.14872 | 0.41567 | - | - | - |
| X ₄ | -5.398 | -6.885 | -8.55 | 4.398 | 5.148 | 5.718 | 0.180501 | 0.29746 | 0.54115 | - | - | - |
| X ₅ | 34.783 | 29.296 | 27.631 | 1.217 | 1.967 | 2.537 | 0.0194* | 0.0362* | 0.0402* | 98.06* | 96.38* | 95.98* |
| X ₆ | 20.854 | 15.367 | 13.702 | 2.146 | 2.896 | 3.466 | 0.0249* | 0.0102* | 0.0313* | 97.51* | 98.98* | 96.87* |
| X ₇ | 15.16 | 9.673 | 8.008 | 2.84 | 3.59 | 4.16 | 0.0342* | 0.0199* | 0.0146* | 96.58* | 98.01* | 98.54* |
| X ₈ | 18.243 | 12.756 | 11.091 | 6.757 | 7.507 | 8.077 | 0.0322* | 0.0168* | 0.0497* | 96.78* | 98.32* | 95.03* |

A= Yellow pigments.
 B= Orange pigments.
 C= Red pigments.

* A = Significant P-value<0.05. R2:066. Adjusted R2: 061. P-value for the model = 0.0038; B = Significant P-value <0.1. R2:071. Adjusted R2: 074. P-value for the model = 0.002; C= Significant P-value <0.1. R2:075. Adjusted R2: 064. P-value for the model = 0.0029.

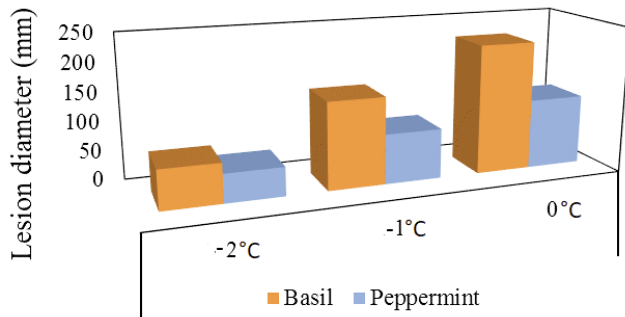


Fig. 2 - Effect of temperature on postharvest controlling of apple decay caused by *Penicillium expansum* between treated fruits with basil and peppermint essential oils. Values represented in the figure are expressed in percentage (%).

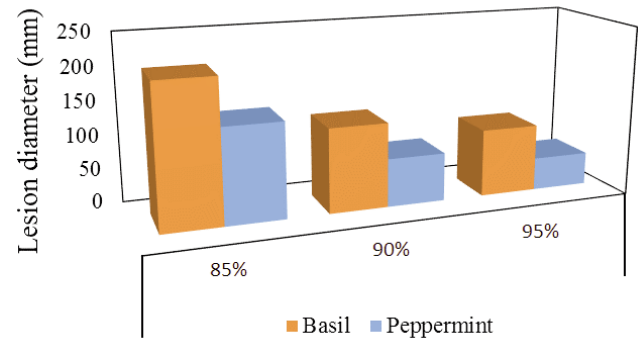


Fig. 4 - Effect of relative humidity on postharvest controlling of apple decay caused by *Penicillium expansum* between treated fruits with basil and peppermint essential oils. Values represented in the figure are expressed in percentage (%).

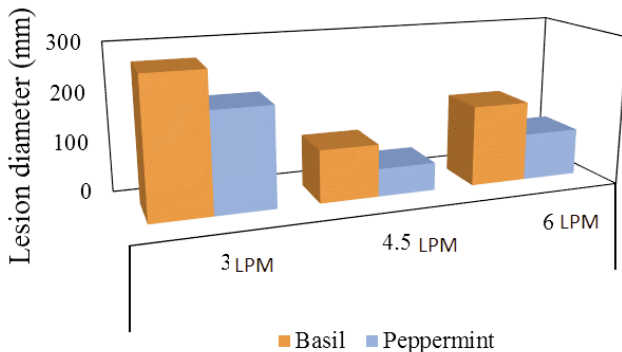


Fig. 3 - Effect of ventilation on postharvest controlling of apple decay caused by *Penicillium expansum* between treated fruits with basil and peppermint essential oils. Values represented in the figure are expressed in percentage (%).

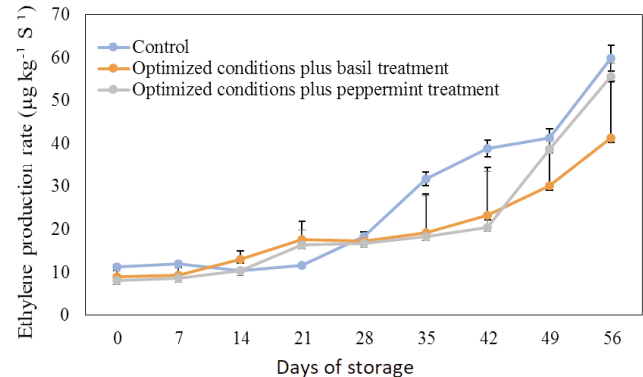


Fig. 5 - Ethylene production rate of apple stored at control and optimized conditions along with the treatment with basil and peppermint essential oils. The control condition was -2°C , 4.5 LPM, and 85% relative humidity. The best levels, determined for basil and peppermint in the previous section of the experiment, were used to assess ethylene production rate during postharvest storage of apple infected with by *Penicillium expansum*.

mint), we analyzed the rate of the ethylene production for 56 days. Every 7 days, the ethylene rate was measured for control, basil, and peppermint treatment. The production rate of ethylene was significantly increased from 28 days and reached its highest level at the day 56 ($p\text{-value} < 0.05$). The fruits treated with peppermint showed significant increase in the ethylene production from the 28th day. At the days 35 and 46, the level of ethylene in treated fruits was significantly low compared to control fruits ($p\text{-value} < 0.05$). Basil treatment reduced significantly rate of the ethylene from 35th day to 56th day ($p\text{-value} < 0.05$). Fruits under the optimized conditions plus basil essential oil showed the lowest amount of the ethylene (Fig. 5).

4. Discussion and Conclusions

In this study, basil essential oil in three levels as well peppermint essential oil in three levels were evaluated to find the best treatment for postharvest control of apple decay caused by *Penicillium expansum* using statistical methods, then the best treatments were investigated under various storage conditions (temperature, ventilation and relative humidity, each in three levels). The aim was to optimize and

enhance postharvest control of apple using cost-effective, safe, and eco-friendly plant materials. There is a few report that showed effect of basil and peppermint essential oil for controlling postharvest decay (Domínguez-Espinosa and Webb, 2003; Lopez-Reyes *et al.*, 2010). In the context of studying the effect of basil essential oil as controller of postharvest decay in apple, a study by Mohammadi *et al.* (2021) showed that basil essential oil can be utilized as a low-cost material for preventing button mushroom (*Agaricus bisporus* L.) during postharvest period. Lopez-Reyes *et al.* (2013) reported the efficiency of use of peppermint essential oil in controlling rot in stone fruits. Recently, the optimization and a combination of several factors to control postharvest decay, has been the object of several study. For instance, essential oils of Citrus species as mandarine (*Citrus reticulata*, Blanco), lemon (*Citrus aurantifolia* (Christm.) Swingle), and orange (*Citrus sinensis* L) have been reported as an effective inhibitor against some bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*) and yeast (*Candida albican*) (Fisher and Phillipps, 2008).

Herein, controlling apple decay after harvest was subjected to two-step statistical method (PBD and CCD of RSM) in order to reduce capital cost of postharvest control, maximizing quality of apple and minimize safety concerns. It has been indicated that statistical approaches are a reliable method towards enhancing and optimization of the yield in various biological processes (Jirasatid *et al.*, 2013; Embaby *et al.*, 2018). In this regard, the optimal level of each essential oils from basil and peppermint influencing postharvest control of decay in apple was determined to reduce decay effectively.

With regard to essential oils, the highest level of controlling and the lowest decay observed in samples upon using basil essential oils as the treatment. Basil essential oils enhanced inhabitation of the decay in apple more than the peppermint essential oil (Fig. 1). This might be attributed to the nature of basil essential oil component in degrading fungus-caused diseases (Grande-Tovar *et al.*, 2018) compared to peppermint essential oil. Action of the mono- and sesquiterpenes and mono- and sesquiterpene hydrocarbons in basil essential oils could inhibit fungal diseases (Caccioni and Guizzardi, 1994). Reports regarding essential oils as pesticides and antimicrobial agent have shown that the inhibitory effect of essential oils to prevent fungal diseases during postharvest period strongly associated with the monoterpenic

phenols, notably thymol, carvacrol and eugenol in the oils (Antunes *et al.*, 2010). Hence, the rich compounds of basil essential oil would be enhance fungistatic activity and as a result the effective control over the fungal disease (Prakash *et al.*, 2015; Namiota and Bonikowski, 2021). It seems that essential oil content of peppermint has a lower level of fungicidal activity compared to basil, although it reduces the growth of the disease when relatively high amount of essential oil used as treatment.

Pertaining to peppermint essential oils, this study showed that peppermint essential oil could reduce decay caused by the fungal disease in apple samples (Fig. 2). Previous studies have revealed the effect of peppermint essential oils as postharvest treatment in reducing decay and maintaining quality of fruits and vegetables (Sellamuthu *et al.*, 2013; Qu *et al.*, 2020). Since menthol is the main constituent of peppermint essential oil, this compound could be mainly responsible for the effects. Furthermore, several studies suggest that the effect of peppermint might be indirect and through raising the activity level of superoxide dismutase (Jin *et al.*, 2009).

The results of the study showed that the best level of basil essential oil as treatment is 1000 and 1500 µg/l, while the best level for peppermint was 1500 (Fig. 1). The difference between 1000 and 1500 µg/l of basil was not significant. Lopez-Reyes *et al.* (2010) reported that 10% emulsion of essential oils could be more effective than 1% of the emulsion in controlling decay. However, scientific evidences are limit in the field of concentration effects on the inhibitory ability of essential oils during postharvest phase. Here, our findings suggest that 1000 and 1500 µg/l essential oil decrease the lesion diameter in apple compared to 500 µg/l, while the effect of basil essential oil with 1000 and 1500 µg/l is similar. In the case of peppermint, there is significant difference between 500, 1000, and 1500 µg/l. This might result from the nature of essential oil constitutes. More study is needed to support the power of constitutes in controlling postharvest diseases.

Temperature of the storage place is one of the main factor for optimal maintain of fruit and vegetables during postharvest phase (Agboyibor *et al.*, 2018; Patrovsky *et al.*, 2019; Silbir and Goksungur, 2019; Liu *et al.*, 2020). Several study have already showed that different isolates of fungal diseases grow best under an optimum temperature range of 30°C to 37°C (Mannaa and Kim, 2017; Liu *et al.*, 2018). These findings support the results of this study

where higher temperature showed a higher lesion diameter. Alternatively, an optimum relative humidity of the storage place is the point of a discrepancy. A study on some strains of fungal-cause diseases revealed that relative humidity of 85% promotes the fungus growth and thereby a higher decay (Nabi *et al.*, 2017). Another study reported that the greatest diameter of lesions achieved at relative humidity of 95 to 98% (Arah *et al.*, 2015). In our study, sole effect of relative humidity was not significant between groups. However, it seems that high temperature trigger relative humidity effect on the growth the fungal disease. According to our results, in the absent of essential oil, fruits stored at high temperature and relative humidity showed higher lesion diameter. Regarding ventilation, Partridge-Hinckley *et al.* (2009) reported the highest occurrence of the decay in fruits stored in the poor ventilation conditions. Moreover, Carmona-Hernandez *et al.* (2019) showed that aeration in terms of agitation at 150 rpm promotes the growth and distribution of fungal diseases.

With regard to ethylene production rate ($\mu\text{g kg}^{-1} \text{S}^{-1}$) in the control and optimized storage conditions, results indicated that the level of ethylene in the control increases and reaches its highest level at 56 days after treatment. The same pattern meets for optimized conditions (Fig. 5). On the other hand, the finding suggests that basil and peppermint treatment significantly decrease the ethylene production rate at 32 and 42 days. After the day 42, basil treatment continues its inhibitory effects compared to peppermint treatment, although the ethylene production rate is significantly high and damage apple storage. Our results is consistent with those findings that have shown application of exogenous material such as melatonin, polyamines, and calcium limits rate of ethylene production (Wang *et al.*, 1993; Bulens *et al.*, 2012; Onik *et al.*, 2021). Jhalegar *et al.* (2015) showed that essential oil treatment could lower ethylene rate in fruits affected with fungal diseases during postharvest storage. Ethylene production is the main factor in deterring postharvest life of any fruits, especially apple. Data from various studies have shown that there is almost linear correlation between the ethylene production and fruit damage during storage phase (Cristescu *et al.*, 2002). Meanwhile, fruits affected with postharvest diseases show the higher level of the ethylene, thereby higher decay and damage (Jhalegar *et al.*, 2012). There are limited scientific reports on application of essential

oils to control ethylene production during the postharvest period. Two studies by Moline and Locke (1993) and Sharafi *et al.* (2011) showed the decrease in the ethylene production in fruits treated with plan essential oils. Here, we represented that basil and peppermint decrease the ethylene rate in apple, as well as we optimized storage conditions with the treatment.

This study used the comprehensive combine of treatments along with well-designed experiment to show an efficiency of plan essential oils to control apple decay during postharvest phase, and mainly to find the best treatment and storage conditions. It is well understood that postharvest conditions depend on several factors and we showed that treatments need to be aligned with storage condition. Thereby, we showed that 1000 $\mu\text{g/l}$ of basil essential oil is significantly effective under -1 and 0°C , 4.5 LPM, and 90% of relative humidity. While 1500 $\mu\text{g/l}$ of peppermint need to be under -1°C , 6 LPM, and 85% humidity to be effective. Furthermore, basil essential oil decreased the ethylene production rate in longer days after treatment. The findings suggest the basil essential oils as a cost-effective and eco-friendly substance that could be more applicable in a large scale.

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