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ORIGINAL ARTICLE / ORIGINALBEITRAG

In Vitro and In Vivo Control of *Alternaria alternata* in Barberry (*Berberis vulgaris*) by Some Essential Oils

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Abstract

In recent years, the use of natural components such as essential oils has been introduced as a new way to control fungal infections. In this study, the effect of the essential oils of anise, chamomile, marjoram, black caraway, and thyme on inhibiting the growth of *Alternaria alternata* fungi in barberry under in vitro and in vivo conditions was tested, using a completely randomized design involving five concentrations (0, 200, 400, 600, and 800 µL L^{-1}) with three repetitions. In vitro results showed that by increasing the concentration of all essential oils, their antifungal activity increased. The best inhibitory effect on *A. alternata* fungi was related to the use of thyme essential oil, followed by black caraway, marjoram, chamomile, and anise, respectively, such that the growth of the fungus was stopped at a concentration of 400 µL L^{-1} for thyme essential oil. In in vivo conditions, the lowest percentage of fruit weight loss was observed with thyme treatment at 600 µL L^{-1} and a 0.02% concentration. The content of total soluble solids was highest with black caraway treatment (200 µL L^{-1}) and was lowest with the control treatment. The highest acidity was obtained in barberry treated with thyme oil at 400 µL L^{-1}, with a pH of 2.10. The highest antioxidant content was observed with chamomile essential oil at 200 µL L^{-1} (80.4 mg L^{-1}), and the lowest was seen with anise essential oil at 600 µL L^{-1} (21.0 mg L^{-1}). The highest phenol content was obtained with marjoram oil at 400 µL L^{-1} (83.18 mg L^{-1}). Treatment with anise (151.10 mg L^{-1}) and marjoram (22.91 mg L^{-1}) essential oils yielded the highest and the lowest anthocyanin content, respectively. Overall, the best results were found with treatment with thyme and black caraway oils (800 µL L^{-1}). The use of essential oils of medicinal plants to prevent the growth of *A. alternata* fungus in laboratory conditions and the effect of these oils on controlling contamination in barberry fruit are new aspects of this project. Recent studies have shown that plant essential oils can be used to control plant diseases in agriculture.

Keywords Alternaria · Biological control · Black caraway · Growth · Thyme

In vitro- und In vivo-Bekämpfung von *Alternaria alternata* mittels ätherischer Öle bei der Berberitze (*Berberis vulgaris*)

Schlüsselwörter Alternaria · Biologischer Pflanzenschutz · Schwarzer Kümmel · Wachstum · Thymian

Introduction

Given the growing population in the world and the need to provide adequate and healthy food based on health standards as well as on the policies of the Food and Agriculture Organization of the United Nations and the U.S. Environmental Protection Agency to reduce the use of chemical pesticides, extensive research has been conducted using secondary plant metabolites to prevent factors blocking production (Abdolmaleki et al. 2011; Alikhani et al. 2009; El-Mohamedy 2017; Wang et al. 2019). One of the most

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important limiting factors in the production and storage of horticultural products concerns pests and diseases. Today, after-harvest care of plant products is of great importance for food security (Calo et al. 2015; Pandey et al. 2017; Wang et al. 2019). Postharvest diseases cause a lot of damage to producers annually, such that 10%–30% of all productive products are not available (Wang et al. 2019). Barberry (*Berberis vulgaris* "Asperma") belongs to the Berberidaceae family, which is grown in Asia and Europe (Kashkooli et al. 2015). Barberry is one of the essential fruits because of its resistance to a wide range of adverse water and soil conditions and also because of its role in industry in Iran. Fresh barberry fruits have a high water content (about 80%) at harvest. Therefore, the central part of the harvested fruit is usually dried to reduce microbial contamination leading to postharvest losses along the supply chain. In this respect, the basic problems for the barberry industry relate to its conventional drying methods and its unsuitable packaging and handling (Kafi et al. 2002). The drawbacks of conventional methods of barberry processing—containment of the extensive decrease in the fruit moisture for its longevity and the long duration of its drying by conventional methods—reduce its postharvest quality and safety. In addition, the demand for small fresh fruits, such as barberry and blueberry, has increased in the market during the last decade because of consumer awareness about the high nutritional value of fresh produce for human well-being. Hence, preharvest and postharvest treatments are essential to minimize microbial spoilage and reduce the risk of pathogen contamination of fresh fruits (Moradinezhad et al. 2018). Therefore, given the economic importance of barberry in Iran, it is important to pay attention to and treat diseases related to it.

Alternaria alternata belongs to the group *Deuteromycetes*, is spread globally, and can be isolated from food, plants, soil, and indoor air. It grows in humid places with a temperature range of 20–30 °C. *A. alternata* is a fungus that has a wide range of plant hosts and can cause diseases in humans. *A. alternata* has emerged as an opportunistic pathogen, particularly in patients with immunosuppression (Anaissie et al. 1989). Economically, it is a very important fungus because of its potential to cause tremendous postharvest losses due to toxin contamination of food and feed (Lee 2015; Moretti et al. 2017).

Essential oils are generally extracted from plants by steam distillation and various solvents; these oils are considered a variant complex mixture of volatile compounds, distinguished by low molecular weight and strong odor (Raut and Karuppayil 2014). These volatile compounds have variant ecological activities, acting as antimicrobial materials against herbivores and microorganisms (Bakkali et al. 2008), and they have been traditionally used for centuries in the treatment of such diseases and infections all over the world (Rios and Recio 2005). Essential oils consist of rich ingredients of variant bioactive phytocompounds such as quinines, phenols, tannins, etc., that provide potent antimicrobial activity (Baka and Rashad 2016). The efficacy of different herbal extracts in controlling pathogenic species of the genus *Alternaria* has been reported (Chethana et al. 2012; Dellavalle et al. 2011; Ganie et al. 2013; Raja 2010; Waghmare 2014; Yazgi et al. 2015). The obtained results of antifungal activity of the studied marjoram, camphor, clove, and basil essential oils against *A. alternata* on agar plates determined that all of the essential oils used had various antifungal activity against the selected fungi, and all had proven antifungal activity against many fungi and bacteria infesting heritage objects (Axinte et al. 2011; Bakkali et al. 2008). Keshavarz et al. (2014) have shown that the essential oil of marjoram contains the active ingredients carvacrol and thymol and prevents the growth of fungi. Hadizadeh et al. (2009) described the essential oils of thyme and nettle as being more effective than the essential oils of rute, eucalyptus, and common yarrow against the growth of *A. alternata*.

The objectives of this study were to test and compare the inhibitory effects of the essential oils of anise (*Pimpinella anisum*), chamomile (*Matricaria chamomilla*), marjoram (*Origanum majorana*), black caraway (*Carum carvi*), and thyme (*Thymus vulgaris*) at different concentrations for the postharvest control of *A. alternata* on barberry fruit.

Materials and Methods

Plant Materials and Extraction of Essential Oils

In this study, essential oils of black caraway and thyme were obtained from Mashhad Golfa Shafa Company and those of chamomile and marjoram from the Gorgan Essential Oil Company. Anise air-dried seeds were supplied from agricultural research fields of the University of Birjand, Iran. After the anise seed parts had been authenticated, a 100-g portion of each was subjected to hydrodistillation for 3 h in a Clevenger-type apparatus. The anise oil was dried over anhydrous Na_2SO_4 and protected in sealed vials for future analysis at 4 °C (Fatemi et al. 2013).

Design of Experiments and Treatments

All the experiments (in vitro and in vivo) were carried out in a randomized factorial design with two factors, including five essential oils (anise, chamomile, marjoram, black caraway, and thyme) in five concentrations (0, 200, 400, 600, and 800 μL L^{-1}) with three replications.

In Vitro Experiment

Effect of Essential Oils on Radial Growth of *A. alternata* in In Vitro Conditions

Antifungal action was studied using a contact assay in vitro that produced hyphal growth inhibition. The test had been used before for essential oil treatment on potato dextrose agar (PDA) medium by the solution method (Özden and Bayindirli 2002). In this technique, each essential oil was dissolved in 5% (v/v) Tween 80, and the required amount was added to each 9-cm petri plate containing 20 ml PDA agar at 45 °C. A 0.5-mm disc of *A. alternata* mycelium was placed on the treated PDA medium, and the plate was incubated at 24 °C. Radial mycelial growth was determined each day (up to 10 days). The inhibitory percentage (IP) was determined using the formula:

$$IP = \left[\frac{(dc \times dt)}{dc}\right] \times 100$$

where dc was the mycelium diameter in a control petri dish, and dt was the mycelium diameter in the essential oil–treated petri dish measured daily (Aminifard and Mohammadi 2013a).

In Vivo Experiment

Effect of Essential Oils Postharvest on Some of the Quality Factors of *A. alternata* Inoculation on Barberry Fruit

To perform this part of the experiment, some fresh barberries (*Berberis vulgaris*) were purchased from the Birjand market. First, the barberry was rinsed and placed on sterile paper to dry. The next steps were performed in sterile conditions and in the culture room. The barberry was soaked in sodium hypochlorite (1%) for 5 min. It was then transferred to sterile distilled water and then placed on sterile filter paper for 2 h at room temperature to dry. We then kept the barberry in the fungi spore suspension (spore in milliliters of sterile distilled water) for 3–5 min and then placed it on a filter paper for 2 h to prove the fungal inoculation under the hood. For the preparation of fungi, spores were suspended in the ratio of 10^3 spores ml^{-1} (Asgari Marjanlu et al. 2009). First, 10 ml of sterile distilled water was poured on the surface of the fungi-containing pans for 10 days, and after soaking, the surface of the environment was cut by a pasteurized pipette with its head bent over the flame to release and collect the spores. By hemocytometer, a solution of 10^3 spores ml^{-1} suspension in a beaker containing a 200-ml contaminated sample was prepared. In this experiment, three replicates were used for each treatment and 150 experimental units (fruits) for each replicate. We then put the barberry in a zipper pack and sprayed the essential oils on various concentrations (0, 200, 400, 600, and 800 µL L^{-1}). We prepared the essential oil solution from the essential oil mixed with acetone and Tween 80 (0.05%) for better solubility and uptake by the fruit. Of course, the solvents selected according to the experiments performed with acetone, since it did not affect the growth of the fungus. Samples were placed in disposable containers and refrigerated and stored at 4 °C for 12 days.

Weight-Loss Percentage

Weight loss was determined by weighing the whole barberry before and after the storage period. Weight loss was expressed as the percentage of loss of weight to the initial weight in the formula (Hosseini and Moradinezhad 2018):

$$WL = \frac{(\text{Initial weight} - \text{Secondary weight})}{(\text{Initial weight}) \times 100}$$

Total Soluble Solids

Total soluble solids (TSS) were determined at 20 °C using a refractometer (RF 10, 0–32° Brix, Extech, USA) and reported as degrees Brix.

pH

The pH of fruit juices was measured at 20 °C using a pH meter (Metro model, Ionenstrasse, 9100 Herisau, Switzerland).

Total Antioxidant Activity Assay

The DPPH method was used to measure the antioxidant activities of barberry fruit (Blois 1958). Briefly, 1 ml of juice was mixed with 2 ml of 0.1 mM DPPH in methanol. The absorbance was measured at 517 nm using a spectrophotometer (Unico 2100, China). Antioxidant activity was expressed as the percentage decline in absorbance relative to the control, corresponding to the percentage of DPPH scavenged (%DPPH), which was calculated according to the following formula (Ranjbari et al. 2016):

$$DPPH\ (\%) = 1 - \frac{A\ \ (\text{sample})}{A\ \ (\text{control})} \times 100$$

Total Phenolic Content Determination

The total phenolic content was defined colorimetrically using Folin–Ciocalteau reagent, as described by Emmons et al. (Emmons et al. 1999) with modifications. The absorbance was measured at 725 nm using a spectrophotometer (Unico 2100, China).

Anthocyanin Contents

Total anthocyanin contents were determined by the differential pH method (Rapisarda et al. 2000). A 1.0-ml aliquot of each barberry fruit extract was diluted to 10 ml with a pH 1.0 solution made from 125 ml of 0.2 M KCl plus 375 ml of 0.2 M HCl. A second 1.0-ml aliquot of fruit extract was diluted to 10 ml with a pH 4.5 solution made from 400 ml of 1 M sodium acetate, 240 ml 1 M HCl, and 360 ml H_2O. The absorbance of each solution was measured at 510 nm using an ultraviolet spectrophotometer (BioQuest CE 2502; Cecil Instruments, Cambridge, UK), and the concentration of anthocyanins was calculated using the equation

$$C_{\text{mg } 100\,\text{g}^{-1}} = \left[\frac{(\text{ApH}_{1.0} - \text{ApH}_{4.5}) \times 484.82 \times 1000}{24{,}825}\right] \times \text{DF}$$

where the period in parentheses was the difference in absorbance at 510 nm between the pH 1.0 and pH 4.5 solutions, 484.82 was the molecular mass of cyanidin-3-glucoside chloride; 24,825 was its molar absorption at 150 nm in the pH 1.0 solution; and DF was the dilution factor (Aminifard and Mohammadi 2013a).

Statistical Analysis

The experiment was conducted in a completely randomized factorial design with three replications consisting of 150 fruits each. Data were analyzed using SAS version 9.1 (SAS Institute, Cary, NC, USA), and means were compared using Duncan's multiple range test at 1% and 5% levels of confidence.

Results and Discussion

In Vitro Experiment: Effect of Essential Oils on Radial Growth of *A. alternata* in In Vitro Conditions

Third Day

The results of the analysis of variance showed that the type and concentration of essential oil and their interactions on the growth rate of *A. alternata* fungi were significant (Table 1).

A comparison of the mean effect of essential oil type showed that there was a significant difference between different essential oils. The maximum amount of fungal growth was observed in anise essential oil, and the minimum was observed in black caraway and thyme essential oils (Table 2).

The effect of essential oil concentration on the growth of *A. alternata* fungi was also significant. Thus, the highest growth rate of the fungus was observed in the control petri dishes, and the lowest growth rate was observed at a concentration of 800 µL L^{-1} (Table 3).

The interaction between the type and concentration of plant essential oil on the growth rate of *A. alternata* fungi showed that the fungus treated with anise essential oil did not differ with different concentrations or with the control treatment, and the essential oil did not affect the growth of fungi (Table 4). The growth rate of *A. alternata* fungi treated with chamomile essential oil at a concentration of 200 µL L^{-1} did not differ from the control treatment, but at higher concentrations, the difference in the control treatment was significant (Table 4). The growth of *A. alternata* fungus treated with marjoram essential oil at a concentration of 200 µL L^{-1} was not significantly different from the control treatment, but at a concentration of 400 µL L^{-1}, a significant difference was observed in the control treatment. At concentrations of 600 µL L^{-1} and 800 µL L^{-1}, fungal growth stopped (Table 4). The growth rate of the *A. alternata* fungus was significantly different in the control treatment and other treatments of black caraway essential oil but did not

Table 1 Analysis of variance for the effect of type and concentration of essential oil on radial growth for *A. alternata* fungi treatments in in vitro conditions

Source of variation	DF	Radial growth of fungus Third day	Radial growth of fungus Sixth day	Radial growth of fungus Tenth day
Type of essential oil	4	914.66*	2807.48*	3433.25*
Concentration of essential oil	4	549.30*	2772.68*	3762.58*
Type of essential oil × concentration of essential oil	16	105.13*	275.03*	375.10*
Error	50	1.40	1.49	1.86
CV	–	10.00	5.6	5.00

DF dilution factor
*Significant at 1% probability level

Table 2 Comparison of means of the effect of essential oil type on radial growth for *A. alternata* fungi treatments in in vitro conditions

Type of essential oil	Radial growth of fungus Third day (mm)	Radial growth of fungus Sixth day (mm)	Radial growth of fungus Tenth day (mm)
Anise	21.67[a]	41.27[a]	46.87[a]
Chamomile	17.67[b]	26.07[b]	31.67[c]
Marjoram	11.00[c]	24.93[c]	34.87[b]
Black caraway	4.33[d]	9.13[d]	13.40[d]
Thyme	4.33[d]	8.33[d]	11.07[e]

Within each column, the same letter indicates no significant difference between treatments at 5% level

differ with different concentrations. No growth was observed at concentrations of 200, 600, 400, or 800 µL L^{-1} (Table 4). The growth rate of *A. alternata* fungus treated with thyme essential oil showed that a significant difference was observed between control growth and other concentrations, as the fungal growth stopped at different concentrations (Table 4).

Sixth Day

According to the analysis of variance table, the effect of type and concentration of essential oil and their interactions on fungal growth were still significant (Table 1).

In the table comparing the average type of essential oil, the minimum growth rate of the fungus was affected by thyme essential oil and also did not differ much with the growth of the fungus in black caraway essential oil. The maximum growth rate was related to the fungus affected by anise essential oil. There was not much difference between marjoram and chamomile essential oils (Table 2).

The results of comparing the mean effect of essential oil concentration showed that the highest growth rate of the fungus in the control treatment and the lowest growth rate were related to the concentration of 800 µL L^{-1} (Table 3).

The results of the interaction of essential oil type and concentration showed that there was a difference between the control treatment and different concentrations of anise essential oil. There was no difference between concentrations of 200 µL L^{-} and 400 µL L^{-1}, but with increasing concentration, the difference in the control treatment increased (Table 4). The growth rate of the fungus decreased from the control to the concentration of 800 µL L^{-1} with chamomile essential oil, and the difference between the different concentrations and the control was significant (Table 4). Fungal growth was observed at a concentration of 600 µL L^{-1} for marjoram essential oil, and there was a significant difference between the control treatment and other concentrations (Table 4). There was a difference between the control and other black caraway essential oil treatments, and no concentration showed any trace of fungal growth (Table 4). No growth was observed at different concentrations of thyme essential oil (Table 4).

Table 3 Comparison of means of the effect of essential oil concentration on radial growth for *A. alternata* fungi treatments in in vitro conditions

Concentration of essential oil (µL L^{-1})	Radial growth of fungus Third day (mm)	Radial growth of fungus Sixth day (mm)	Radial growth of fungus Tenth day (mm)
0	21.67[a]	44.87[a]	53.27[a]
200	13.00[b]	22.20[b]	29.67[b]
400	10.20[c]	18.60[c]	24.93[c]
600	7.47[d]	13.80[d]	17.80[d]
800	6.67[d]	10.27[e]	12.20[e]

Within each column, the same letter indicates no significant difference between treatments at 5% level

Tenth Day

According to the results of the analysis of variance, the effect of the type and concentration of essential oil and their interactions on the growth rate of *A. alternate* fungi were significant (Table 1).

Comparison of the mean effect of essential oil type on the growth rate of *A. alternate* fungi showed that anise essential oil with the highest amount of fungal growth (46.87 mm per day) has the least inhibitory effect, and thyme essential oil with the lowest fungal growth rate (11.07 mm per day) showed the greatest inhibitory effect, followed by black caraway essential oil, with a growth rate of 13.40 mm per day, chamomile at 31.67 mm per day, and marjoram at 34.87 mm per day (Table 2).

The effect of essential oil concentration on the growth of *A. alternata* fungi was also significant. Thus, the maximum growth rate of the fungus was observed with the control treatment, and the minimum was observed at a concentration of 800 µL L^{-1} (Table 3).

The interaction of essential oil type and concentration showed that the highest growth rate was related to the control treatment, and the lowest growth rate was related to the concentration of 800 µL L^{-1} of anise essential oil. Therefore, it was observed that by increasing the concentration of essential oil, the growth rate of the fungus decreased (Table 4). The growth rate of *A. alternata* fungi was significantly dif-

Table 4 Comparison of means of the effect of type and concentration of essential oils on radial growth for *A. alternata* fungi treatments in in vitro conditions

Type of essential oil	Concentration of essential oil (μL L^{-1})	Radial growth of fungus Third day (mm)	Radial growth of fungus Sixth day (mm)	Radial growth of fungus Tenth day (mm)
Anise	0	21.67[a]	45.67[a]	55.67[a]
	200	21.67[a]	41.67[b]	47.67[c]
	400	21.67[a]	41.67[b]	51.67[b]
	600	21.67[a]	39.67[bc]	41.67[d]
	800	21.67[a]	37.67[c]	37.67[e]
Chamomile	0	21.67[a]	45.67[a]	51.67[b]
	200	21.67[a]	31.67[d]	39.67[de]
	400	17.67[b]	21.67[e]	27.67[f]
	600	15.67[c]	17.67[g]	21.67[g]
	800	11.67[d]	13.67[g]	17.67[h]
Marjoram	0	21.67[a]	45.67[a]	49.67[bc]
	200	21.67[a]	37.67[c]	51.67[b]
	400	11.67[d]	29.67[d]	41.67[d]
	600	0.00[e]	11.67[g]	25.67[f]
	800	0.00[e]	0.00[h]	5.67[i]
Black caraway	0	21.67[a]	45.67[a]	57.67[a]
	200	0.00[e]	0.00[h]	5.67[i]
	400	0.00[e]	0.00[h]	3.67[i]
	600	0.00[e]	0.00[h]	0.00[j]
	800	0.00[e]	0.00[h]	0.00[j]
Thyme	0	21.67[a]	41.67[b]	51.67[b]
	200	0.00[e]	0.00[h]	3.67[i]
	400	0.00[e]	0.00[h]	0.00[j]
	600	0.00[e]	0.00[h]	0.00[j]
	800	0.00[e]	0.00[h]	0.00[j]

Within each column, the same letter indicates no significant difference between treatments at 5% level

ferent in the control treatment and other treatments such that with an increasing concentration of chamomile essential oil, the growth rate of the fungus decreased (Table 4). The minimum growth rate of the fungus was observed at a concentration of 800 μL L^{-1} of marjoram essential oil, and a significant difference was observed compared with other concentrations and the control treatment. There was no difference between the control treatment and the concentration of 200 μL L^{-1} on the tenth day. In general, as the concentration of essential oil increased, the growth rate of the fungus decreased (Table 4). At concentrations of 200 μL L^{-1} and 400 μL L^{-1} of black caraway essential oil, a significant difference was observed regarding the control treatment and concentrations of 600 μL L^{-1} and 800 μL L^{-1} such that in concentrations of 600 μL L^{-1} and 800 μL L^{-1} there was no growth, but at concentrations of 200 μL L^{-1} and 400 μL L^{-1}, fungal growth was observed (Table 4). Fungal growth was observed at a concentration of 200 μL L^{-1} of thyme essential oil, which was significantly different from other concentrations and the control treatment, at which the growth of the fungus was zero. Overall, fungal growth stopped at concentrations of 400, 600, and 800 μL L^{-1} (Table 4).

The obtained results were in agreement with those of Aminifard and Mohammadi (2013a), who reported that the growth of grey mold was completely inhibited by the essential oil of black caraway at 400 μL L^{-1}. Similarly, the growth of *B. cinerea* fungi was inhibited by black caraway and fennel oils at concentrations of 400 μL L^{-1} and 600 μL L^{-1}, respectively (Aminifard and Mohammadi 2013b). Also, anise and black caraway oils completely inhibited *P. digitatum* growth at concentrations of 600 μL L^{-1} (Aminifard and Bayat 2018). The amount of essential oil that prevents the growth of fungi depends on the amount of this effective material. Shirazi thyme, due to having the effective material of both thymol and carvacrol, has better antifungal effects than savory essential oil, which contains only carvacrol and a small amount of thymol (Lahooji et al. 2010). A recent study suggested that the antifungal activity of thyme oil was probably due to its major component, carvacrol (Ferhout et al. 1999). In the study of Combrinck et al. (2011), thyme essential oils

Table 5 Analysis of variance of the effect of type and concentration of essential oil on quality factors for *A. alternata* fungi treatments in in vivo conditions

Source of variation	DF	Weight loss	TSS	pH	Total antioxidant activity	Total phenolic	Total anthocyanin
Type of essential oil	4	12.28**	30.72**	1.20**	1742.7**	141.12**	47,779.51**
Concentration of essential oil	4	692.81**	74.86**	1.64**	267.5^{ns}	425.43**	19,600.71**
Type of essential oil × concentration of essential oil	16	1.72**	7.72*	0.37**	499.5**	32.50**	12,918.96**
Error	50	0.23	3.47	0.07	153.00	4.68	24.22
CV	–	12.8	10.7	20.5	21.5	2.8	4.2

DF dilution factor, *ns* not significant, *TSS* total soluble solids
*Significant at 5% probability level
**Significant at 1% probability level

proved to be the most effective inhibitor, totally inhibiting all of the pathogens tested at concentrations of 1000 µL L^{-1}. Numerous reports have shown that several plant extracts may inhibit the mycelial growth of *A. alternata* (Begum et al. 2010; Dellavalle et al. 2011; Kantwa et al. 2014; Rama Devi et al. 2015; Swami and Alane 2013).

In Vivo Experiment

Weight-Loss Percentage

The results obtained from the analysis of variance of barberry data treated with *A. alternata* fungi showed that the type and concentration of essential oil and the interaction of the essential oil type and concentration had a significant effect on the weight-loss percentage of barberry fruit (Table 5).

The effect of type of essential oil on the percentage of weight loss of barberry fruit treated with *A. alternata* fungus showed that the maximum weight loss was related to marjoram essential oil, and the minimum was related to thyme and black caraway essential oils (Table 6).

The effect of plant essential oil concentration on the weight loss of barberry fruit treated with *A. alternata* fungus was that the greatest weight loss was related to the control treatment, and the least was related to 400 µL L^{-} and 800 µL L^{-1} concentrations (Table 7).

Table 6 Comparison of means of the effect of essential oil type on quality factors for *A. alternata* fungi treatments in in vivo conditions

Treatment	Weight loss (%)	TSS (mg L^{-1})	pH	Total antioxidant activity (mg L^{-1})	Total phenolic (mg L^{-1})	Total anthocyanin (mg L^{-1})
Anise	3.87^{b}	16.69^{b}	1.451^{b}	41.2^{c}	74.72^{c}	200.10^{a}
Chamomile	3.406^{c}	16.75^{b}	1.227^{c}	70.5^{a}	76.24^{bc}	87.91^{d}
Marjoram	5.364^{a}	15.88^{b}	0.996^{d}	62.8^{ab}	82.39^{a}	49.32^{e}
Black caraway	3.226^{c}	19.43^{a}	1.181^{c}	56.8^{b}	77.68^{b}	136.42^{b}
Thyme	3.214^{c}	18.32^{a}	1.708^{a}	55.9^{b}	75.35^{c}	111.32^{c}

TSS total soluble solids
Within each column, the same letter indicates no significant difference between treatments at 5% level

Table 7 Comparison of means of the effect of essential oil concentration on quality factors for *A. alternata* fungi treatments in in vivo conditions

Concentration of essential oil (µL L^{-1})	Weight loss (%)	TSS (mg L^{-1})	pH	Total antioxidant activity (mg L^{-1})	Total phenolic (mg L^{-1})	Total anthocyanin (mg L^{-1})
0	15.965^{a}	13.98^{d}	1.857^{a}	62.1^{a}	68.43^{d}	96.75^{d}
200	0.916^{bc}	19.84^{a}	1.316^{b}	61.2^{a}	78.54^{bc}	125.18^{c}
400	0.491^{d}	18.19^{b}	1.036^{d}	52.7^{a}	77.52^{c}	139.35^{b}
600	0.583^{cd}	18.44^{b}	1.260^{bc}	57.4^{a}	79.11^{b}	66.08^{e}
800	0.491^{d}	16.62^{c}	1.064^{cd}	53.9^{a}	82.77^{a}	157.71^{a}

TSS total soluble solids
Within each column, the same letter indicates no significant difference between treatments at 5% level

Table 8 Comparison of means of the effect of type and concentration of essential oil on quality factors for *A. alternata* fungi treatments in in vivo conditions

Type of essential oil	Concentration of essential oil (μL L^{-1})	Weight loss (%)	TSS (mg L^{-1})	pH	Total antioxidant activity (mg L^{-1})	Total phenolic (mg L^{-1})	Total anthocyanin (mg L^{-1})
Anise	0	16.211^{a}	13.67^{i}	1.86^{a}	65.1^{abcdef}	75.31^{efg}	94.16^{ij}
	200	1.815^{cd}	19.15^{bcde}	1.75^{ab}	52.3^{cdefg}	75.91^{def}	151.10^{e}
	400	0.082^{f}	17.65^{cdef}	0.84^{d}	46.6^{efg}	69.22^{hi}	333.86^{a}
	600	0.178^{f}	17.00^{efgh}	1.05^{cd}	21.0^{h}	67.88^{hi}	128.88^{f}
	800	1.062^{de}	16.00^{fghi}	1.75^{ab}	21.2^{h}	77.04^{def}	292.48^{b}
Chamomile	0	16.004^{a}	14.00^{hi}	1.9^{a}	59.1^{bcdef}	79.42^{abcd}	99.71^{hi}
	200	0.232^{f}	16.50^{efghi}	1.4^{bc}	80.4^{a}	67.36^{i}	119.02^{g}
	400	0.267^{ef}	16.50^{efghi}	0.7^{d}	62.7^{abcdef}	76.44^{def}	75.27^{k}
	600	0.454^{ef}	18.20^{cdef}	1.4^{bc}	78.3^{ab}	68.29^{hi}	53.33^{lm}
	800	0.075^{f}	18.55^{cdef}	0.7^{d}	72.0^{abc}	71.64^{gh}	92.22^{ij}
Marjoram	0	15.805^{a}	13.90^{i}	1.75^{ab}	60.8^{abcdef}	78.98^{bcde}	103.14^{h}
	200	2.414^{c}	19.30^{bcde}	0.84^{d}	49.1^{defg}	78.90^{bcde}	22.91^{o}
	400	1.992^{c}	18.10^{cdef}	0.7^{d}	66.5^{abcdef}	83.18^{a}	29.44^{o}
	600	2.191^{c}	14.55^{hi}	0.7^{d}	67.4^{abcdef}	82.56^{ab}	41.52^{d}
	800	4.416^{b}	13.55^{i}	0.84^{d}	70.3^{abc}	76.95^{def}	49.58^{mn}
Black caraway	0	15.879^{a}	14.20^{hi}	1.773^{ab}	63.3^{abcdef}	76.89^{def}	90.92^{j}
	200	0.055^{f}	23.55^{a}	0.84^{d}	65.7^{abcdef}	76.51^{def}	150.82^{e}
	400	0.082^{f}	20.15^{bcd}	0.84^{d}	53.2^{cdefg}	78.03^{cde}	111.38^{g}
	600	0.07^{f}	21.75^{ab}	1.4^{bc}	55.8^{cdef}	83.04^{a}	45.69^{mn}
	800	0.045^{f}	17.50^{defg}	1.05^{cd}	46.0^{fg}	81.36^{abc}	283.31^{c}
Thyme	0	15.923^{a}	14.13^{hi}	1.96^{a}	62.3^{abcdef}	79.42^{abcd}	95.83^{hij}
	200	0.062^{f}	20.70^{abc}	1.75^{ab}	58.4^{bcdef}	77.34^{def}	182.07^{d}
	400	0.034^{f}	18.55^{cdef}	2.10^{a}	34.3^{gh}	74.10^{fg}	146.79^{e}
	600	0.022^{f}	20.70^{abc}	1.75^{a}	64.3^{abcdef}	77.61^{cdef}	60.97^{l}
	800	0.027^{f}	17.50^{defg}	0.98^{c}	60.1^{bcdef}	78.38^{cde}	70.97^{k}

Within each column, the same letter indicates no significant difference between treatments at 5% level

The interaction of essential oil type and concentration on the percentage of weight loss of barberry treated with *A. alternata* fungus showed that the maximum percentage of weight loss was related to the control treatment, followed by marjoram treatment, and the minimum percentage was related to thyme treatment in the 600 μL L^{-1} concentration (Table 8).

Similar results were reported by Jahani et al. (2020), who found that fruit treated with thyme oil at a concentration of 800 μL L^{-1} had the lowest weight loss in comparison to fruit treated with eucalyptus, galbanum, or clove oil. Also, Fatemi et al. (2013) reported that the weight-loss percentage of essential oil–treated fruit was significantly lower than that of control fruit ($p < 0.01$); fruit treated with oils at a concentration of 800 μL L^{-1} showed the lowest weight-loss percentage (6.18%), while control fruit showed the highest weight-loss percentages. The advantage of decreased weight-loss percentages of cherry, grape, and plum by using natural antifungal compounds such as thymol, eugenol, and menthol vapors have been shown in previous experiments (Aminifard and Mohammadi 2013b; Rattanapitigorn et al. 2006; Serrano et al. 2005). Similarly, in our research, it could be concluded that the tested essential oils, by reducing the respiration rate, had a positive influence on the weight-loss percentage of barberry fruit.

Total Soluble Solids

The data obtained from the analysis of variance of barberry data treated with *A. alternata* fungi showed that the effect of essential oil type and concentration, as well as the interaction of essential oil type and concentration, had a significant effect on the TSS in barberry solution (Table 5).

The results of a mean comparison in barberry treated with *A. alternata* fungus were significantly different between different essential oils regarding the amount of TSS in barberry solution. The highest amount of TSS in barberry treated with black caraway and thyme essential oils and the amount in the other treatments were not much different (Table 6).

The results of the mean comparison in barberry treated with *A. alternata* fungus showed a significant difference

between different concentrations on the total solids content of barberry solution. The maximum amount of TSS was observed at a concentration of 200 µL L^{-1}, and the minimum was observed in the control treatment (Table 7).

The interaction of the type and concentration of essential oil on the TSS of barberry solution treated with *A. alternata* fungus showed that the highest TSS were related to black caraway essential oil at a concentration of 200 µL L^{-1}, and the lowest was related to the control treatment (Table 8).

The obtained results were in agreement whit Asgari Marjanlu et al. (2009), who reported that the TSS of strawberry infected with *B. cinerea* increased with the application of cumin essential oil. Also, Fatemi et al. (2013) showed that fruit treated with black caraway and anise oils did not have significant differences between them, and fruit treated at 800 µL L^{-1} had the highest TSS (15.17 °Brix). Similarly, black caraway oil at 800 µL L^{-1} maintained significantly higher soluble solids contents than all other treatments (Aminifard and Mohammadi 2013a, b).

pH

The results of the analysis of variance data on barberry treated with *A. alternata* fungi showed that the type and concentration of essential oil and the interaction of essential oil type and concentration had a significant effect on the acidity of barberry (Table 5).

The results of the mean comparison in barberry treated with *A. alternata* fungus showed differences among different essential oils regarding barberry acidity. The highest acidity (higher pH) was observed in barberry treated with thyme essential oil, and the lowest acidity (lower pH) was in barberry treated with marjoram essential oil (Table 6).

The results of comparing the average effect of plant essential oil concentration on the acidity of barberry treated with *A. alternata* fungus showed that the highest acidity (high pH) was observed in the control treatment, and the lowest acidity (low pH) was observed at 400 µL L^{-1} (Table 7).

The interaction of essential oil type and concentration on the acidity of barberry treated with *A. alternata* fungus showed that the maximum average acidity occurred with thyme essential oil at a concentration of 400 µL L^{-1}, and the minimum occurred with marjoram treatment at concentrations of 400 µL L^{-1} and 600 µL L^{-1} and chamomile treatment at a concentration of 400 µL L^{-1} (Table 8).

This result follows the same trend as the findings of Mohammadi and Aminifard (2013) in that the pH value of treated tomato juice was significantly different between concentrations of fennel essential oil and control, and fruit treated with a concentration of 800 µL L^{-1} had the lowest pH value, while control fruit had the highest pH value concentrations.

Total Antioxidant Activity Assay

The results obtained from the analysis of variance of the data related to barberry treated with *A. alternata* fungi showed that the type of essential oil and the interaction of the type and concentration of essential oil had a significant effect on the antioxidant content of barberry fruit (Table 5).

The results of comparing the average effect of essential oils on the content of antioxidants in barberry treated with *A. alternata* fungus showed that the highest content of antioxidants was related to chamomile essential oil, and the lowest content was related to anise essential oil (Table 6).

No difference was observed between different concentrations (Table 7).

The results of the interaction of the type and concentration of essential oil showed that the maximum average antioxidant content in barberry treated with *A. alternata* fungus was related to the 200 µL L^{-1} concentration of chamomile essential oil, and the minimum was related to anise essential oil at concentrations of 600 µL L^{-1} and 800 µL L^{-1} (Table 8).

These results are not in accordance with those observed by Aminifard and Bayat (2018), in whose research the fruit treated with black caraway and anise essential oils had higher antioxidant contents than the control fruit, and their best treatments were seen with the oils at 800 µL L^{-1}.

Total Phenolic Content Determination

Analysis of variance data on barberry treated with *A. alternata* fungi showed that the type and concentration of essential oil and the interaction of type and concentration of essential oil had a significant effect on the content of barberry phenol (Table 5).

The highest amount of phenol content was related to marjoram essential oil, and the lowest was related to anise essential oil (Table 6).

Comparing the average effect of essential oil concentrations on barberry treated with *A. alternata* fungus, the maximum phenol content was observed at 800 µL L^{-1}, and the minimum was observed in the control treatment (Table 7).

The interaction of the type and concentration of essential oil on the phenol content of barberry phenol treated with *A. alternata* fungus showed that the highest phenol content occurred with black caraway essential oil at 600 µL L^{-1} and marjoram at 400 µL L^{-1}, and the lowest was related to treatment with chamomile at a concentration of 200 µL L^{-1} (Table 8).

The cytotoxic activity of essential oils is mainly due to the presence of phenols, aldehydes, and alcohol (Bakkali et al. 2008; Bruni et al. 2004; El-Mohamedy 2017). Essential oils, including the major phenolic compounds, are most active against microorganisms (Vesaltalab and Gho-

lami 2012). Also, phenolic essential oil compounds are made in the phospholipid layer of plant cell membranes, and the higher the content of phenolic substances in the essential oil, the higher its antimicrobial properties (Moreira et al. 2005).

Anthocyanin Contents

Analysis of variance data for barberry treated with *A. alternata* fungi showed that the essential oils, their concentrations, and their interaction had a significant effect on the barberry anthocyanin contents (Table 5).

A comparison of the mean effect of plant essential oils on barberry treated with *A. alternata* fungus was significant between different types of essential oils on the amount of barberry anthocyanin. The highest amount was found in barberry treated with anise essential oil, and the lowest was related to marjoram essential oil (Table 6).

The results of a mean comparison in barberry treated with *A. alternata* fungus showed a significant difference between different concentrations on barberry anthocyanin such that the maximum amount of anthocyanin was observed at 800 µL L^{-1} and the minimum at 600 µL L^{-1} (Table 7).

The interaction of the type and concentration of essential oils showed that the highest content of anthocyanin in barberry treated with *A. alternata* fungus was related to anise essential oil at a concentration of 400 µL L^{-1}, and the lowest was related to treatment with marjoram at concentrations of 200 µL L^{-1} and 400 µL L^{-1} (Table 8).

These results confirm that fruit treated with black caraway and anise essential oils had higher anthocyanin contents than the control fruit, and their best treatments occurred with the oils at 800 µL L^{-1} (Aminifard and Bayat 2018). The results are in disagreement with those of Fatemi et al. (2013), who demonstrated that the highest anthocyanin levels were found at 800 µL L^{-1} for fruit treated with black caraway and anise oils (54 mg per 100 g fresh weight) and the lowest amount of anthocyanin is related to control fruit (20 mg per 100 g fresh weight), respectively.

Conclusions

Because chemical fungicides cause considerable biological pollution, and the level of pollution in nature increases with the creation of resistant species, the use of natural compounds is an urgent need for human societies. The results of this study showed that in laboratory conditions, different essential oils have different effects on *A. alternata* fungus. The effect of herbal essential oils on different concentrations of *A. alternata* fungus was different, and in general, all the essential oils had an antifungal effect on *A. alternata* fungus such that their inhibitory effect increased with increasing concentration of the essential oil. The most effective essential oils on *A. alternata* fungus were thyme, black caraway, marjoram, chamomile, and anise, respectively. In the second concentration of thyme essential oil (400 µL L^{-1}) and the third concentration of black caraway essential oil (600 µL L^{-1}), fungal growth stopped altogether, and the treatment was most effective. In in vivo conditions, in all treatments—except for the treatment with marjoram essential oil—the inhibitory effect of the essential oil in controlling barberry fruit rot was evident. According to the in vivo results, the most desirable results were related to treatment with thyme and black caraway essential oils and were observed at concentrations of 800 µL L^{-1}.

Conflict of interest E. Sazvar, M. Jahani, M.H. Aminifard, and S.A. Hosseini declare that they have no competing interests.

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