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Investigating the antifungal effects of essential oils on *Aspergillus* sp. in strawberry (*Fragaria ananassa* Duchesne) fruit

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ABSTRACT

Purpose: The growing attention and interest in alternatives for chemical preservatives with natural types has led to numerous studies on essential oils and plant extracts. Strawberries, due to their high respiration and metabolic activity, and high water content, are highly sensitive to microbial contamination. **Research Method:** An experiment was conducted to investigate the effect of the essential oils of some medicinal plants on the fungus *Aspergillus* sp. in strawberry fruit in *in vivo* and *in vitro* conditions as a factorial form in a completely randomized design with three replications. The first factor included the type of essential oil: frankincense, ginger, cinnamon, and tarragon essential oils, and the second factor included the concentration of essential oil at five levels (0, 200, 400, 600, and 800 $\mu L.L^{-1}$). **Findings:** *In vitro* results showed that with the increase in the concentration of plant essential oils, their antifungal activity increases. As a result, the lowest fungus colony diameter was obtained from the concentration of 800 $\mu L.L^{-1}$ of essential oil. A comparison of the average type of essential oil showed that cinnamon essential oil had more antifungal activity than other essential oils used, so that at any level (200 to 800 $\mu L.L^{-1}$), it caused a 100% inhibition of *Aspergillus* sp. growth. *In vivo,* results showed that the best appearance of the fruit and the highest soluble solids were recorded from the concentration of 800 $\mu L.L^{-1}$ of essential oil. Cinnamon essential oil treatment resulted in the best fruit appearance, the highest soluble solids, and the highest levels of antioxidants, anthocyanin, and sugar compared to frankincense essential oil. **Research limitations:** There were no limitations. **Originality/Value:** Among the essential oils, cinnamon essential oil showed better antifungal activity against *Aspergillus* sp., which causes strawberry fruit spoilage. Therefore, it can be used as a substitute for chemical fungicides, although other essential oils may also be effective.

INTRODUCTION

Today, the demand for organic fruits is increasing, both domestically and from importing countries. These fruits must not have been exposed to any poisons or chemicals during any stage of their production. After that, some countries that import agricultural products allowed the products to enter their country by of applying non-chemical treatments and ensuring the absence of remaining poisons (Behdad et al., 2013). Today, the global desire to find alternative methods to control post-harvest waste, by prioritizing healthy methods and preventing the adverse effects and side effects of toxins on human health, as well as the existence of resistance to fungicides, reduces the possibility of using chemicals (Hashemi et al., 2008; Kahawattage et al., 2023).

The perennial herbaceous strawberry with the scientific name *Fragaria ananassa* Duchesne belongs to the Rosaceae family (Dris et al., 2003). The strawberry is one of the favorite fruits of consumers in many regions of the world (Fan et al., 2021), and a rich source of antioxidants (Amiri et al., 2022). Due to its respiration, high metabolic activity, and significant water content, strawberry is one of the fruits that are highly susceptible to microbial contamination, against mechanical damage and physiological changes during growth and development, and at postharvest stages during transportation and even in distribution and sales centers; it is consistently exposed to contamination by all kinds of fungi. *Rhizopus*, *Aspergillus*, *Botrytis* and *Penicillium* fungi are the most critical microbial factors that limit the lifespan of strawberries after harvesting (Behnamian & Messiah, 2002). Different postharvest treatments positively affected quality and storage life of various fruits mainly by controlling pre and postharvest pathogens (Dorostkar et al., 2022; Moradinezhad & Ranjbar, 2023). One of the ways to maintain the quality of fruits and vegetables and control decay is using antimicrobial and natural compounds. Due to the increasing concerns about endangering the health of consumers, due to the residual chemical toxins on horticultural products and the increasing resistance of fungi to these toxins, scientists are considering using plant essential oils to control postharvest diseases. This has become a new method and alternative to chemical poisons (Ranjbar et al., 2008).

Plant essential oils include a wide range of secondary metabolites, which in most cases have antimicrobial, allelopathic, and antioxidant properties. Essential oils are chemically complex compounds that contain various types of chemicals, including phenols, alcohols, ketones, aldehydes, esters, ethers, terpenes, and terpenoids (Anthony et al., 2003). Terpenes and terpenoids are the most important compounds that cause the antimicrobial properties of essential oils; there is probably a synergy among the different compounds of essential oils, enhancing the antimicrobial properties of each of the compounds (Lazar et al., 2010). The complexity of the compounds in essential oils has a positive effect on their use in managing plant diseases because the possibility of resistance in the pathogen to such complex compounds will be low. Due to their hydrophobic nature, essential oils can destroy the cell membrane in microorganisms, thereby disrupting the electron transfer chain and the functioning of enzymes, leading to a significant reduction in microbial cell activity (Lanciotti et al., 2004).

According to recent studies, some essential oils have fungicidal properties against certain important plant pathogenic fungi. The researchers reported the minimum inhibition rate of frankincense essential oil on *A. flavus* fungus as 1.75 $\mu L.L^{-1}$ indicating its higher antifungal efficacy compared to other essential oils like *Zanthoxylum alatum*, *Ocimum gratissimum*, and *Piper betle* (Prakash et al., 2014). The antifungal activity of cinnamon plant essential oil against *A. flavus*, *Rhizopus nigricans*, and *Penicillium expansum* fungi in the culture medium was investigated, and it was found that this essential oil has a high antifungal effect against these plant diseases after harvest (Zhang et al., 2016). Using cinnamon essential oil on strawberry fruit to increase shelf life (Mohammadi et al., 2014), and Shirazi thyme essential oil in controlling fungal diseases at postharvest stage on strawberries (Behdad et al., 2010) are known to be effective. Additionally, using marjoram essential oil on grapes can prevent the growth of *Aspergillus rhizopus* mycelium in storage (de Sousa et al., 2013). Clove essential oil also inhibited the growth of *A. niger* for ten days (Jahani et al., 2020). Considering the promising effect of plant essential oils in controlling fungal diseases of fruits in the post-harvest stage, the sensitivity of strawberries to pathogenic agents, especially *Aspergillus* fungus, and the reduction of desire to use chemicals, the present research aimed to investigate the effect of frankincense, ginger, cinnamon, and tarragon essential oils on *Aspergillus* sp. fungus in strawberry fruit was investigated.

MATERIALS AND METHODS

In this research, the effect of four plant essential oils, including frankincense, ginger, cinnamon, and tarragon essential oils in different concentrations were investigated using a factorial form in a completely randomized design. The study was conducted in two laboratory conditions: the first on the PDA culture medium, and the second on strawberries against *Aspergillus sp.*

Isolation and purification of fungi

Initially, to isolate fungal, infected strawberry fruits that had symptoms, they were collected and transported to the laboratory. Micro-samples were prepared from the infected parts of the fruit, and after disinfection with 1% sodium hypochlorite for 2-3 minutes and three subsequent washes with sterile distilled water, these samples were cultured inside the trays containing potato-dextrose agar culture medium. Subsequently, they were transferred to an incubator with a temperature of 25-28°C. After examining the samples and observing their growth, they were identified based on the fungus's morphology, utilizing microscopic slides with available keys. Water-agar culture medium was employed for purification, using the single sporulation method.

Essential oils used in the experiment

Frankincense, ginger, cinnamon, and tarragon medicinal plant essential oils were obtained from Teb Daru Company, Iran.

Investigation of antifungal activity in laboratory conditions

The antifungal effects of the mentioned essential oils on *Aspergillus* fungus were investigated by mixing essential oils at concentrations of 200, 400, 600, and 800 $\mu L.L^{-1}$ with PDA culture medium. In this method, the PDA culture medium was prepared in one-liter Erlenmeyer flasks and autoclaved. After cooling the environment to 42 to 45°C, essential oils were added at different concentrations to the environment and mixed until a completely uniform emulsion was formed. In order to enhance the solubility of essential oils in a culture medium, Tween 80 was used. The resulting mediums were immediately divided into Petri dishes with a diameter of nine centimeters and allowed to solidify. Also, in all treatments, three Petri dishes of culture medium without added essential oil (zero concentration of essential oil) were considered as the control medium. After freezing the medium, discs with a diameter of five millimeters were removed from the edge of the seven-day-old fungus mycelium with a cork borer and placed upside down on the culture medium (in the center of the Petri dishes containing the culture medium). The fungus was incubated in an incubator at a temperature of

25°C. This experiment was conducted with three repelications for each treatment and control. The vegetative growth rate of the halo of fungi was measured every three days until the surface of the control Petri culture medium was completely occupied by the fungus.

The data related to the average growth were analyzed through the analysis of variance (ANOVA) table, and the test results were investigated and analyzed statistically using SAS 9.4 software in a factorial and completely random design format.

Contamination of fruits with fungus suspension

Initially, the strawberries sourced from a commercial greenhouse in Mashhad were rinsed with sterile distilled water, followed by placement on sterile filter paper for drying. Subsequent experimental procedures were carried out within sterile conditions within a culture room and under a hood. The fruits were subsequently immersed in a suspension of fungal spores (1×10^6 spores per milliliter of sterile distilled water) for three to five minutes, as illustrated in Figure 1A.

To generate the fungal spore suspension at the specified concentration of 10^6 spores per milliliter, following the method outlined by Asgari Marjanlu et al. (2009), initially, 10 milliliters of sterilized distilled water was gently applied onto the surface of 7-day-old fungus trays. Subsequently, a Pasteur pipette, whose tip had been bent over a flame to form a paddle, was employed to wet the surface of the medium. This action facilitated the scraping of the medium's surface to liberate and collect the spores. A suspension solution of 10^6 spores per milliliter was prepared using a Schomar cell slide and a 200 mL beaker was prepared to dip strawberry fruits and contaminate them. Following the removal of the fruits from the fungal suspension, they were transferred onto filter paper and subsequently placed under a hood for two hours to facilitate the fixation of fungal inoculation. This experimental protocol entailed the utilization of three replicates for each treatment, with each replicate consisting of five experimental units (fruits).

The fruits were then submerged in a beaker containing various concentrations of essential oil. The essential oil solution was formulated by blending essential oil with acetone and tween 80 (at a concentration of 0.05%) to enhance solubility and absorption by the fruit. The immersion duration was three minutes, after which the fruits were allowed to dry on sterile filter paper. Subsequently, they were transferred into disposable containers and stored in a refrigerator at a temperature of 4°C for 12 days. At the end of storage period, various characteristics of the fruits were assessed, as outlined in Figure 1B.

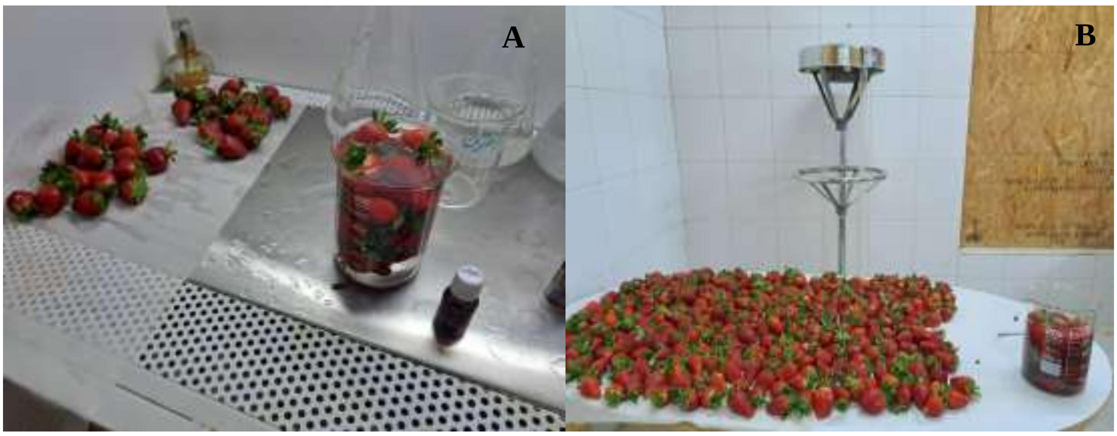

Fig. 1. Immersion of strawberry fruits in the beaker containing fungal spores (A) and immersion of fruits infected with fungus in the beaker containing essential oil solution (B).

The appearance characteristics of the fruit were investigated after 12 days of inoculation of the fungus to the fruits and immersion in the essential oils. The percentage of fruit decay was observed and graded on a scale of five categories: 5= fruits with excellent appearance and had preserved their color and condition. 4= Fruits have a tiny percentage of spoilage below 10%. 3= Fruits were rotting was observed, and about 20-25% were crushed and turned black. 2= Fruits with up to 50% damage in appearance and decay. 1= Fruits with more than 50% crushed and black (Shiri et al., 2013).

Soluble solids content (SSC) were measured using a handheld refractometer (RF10, 0-32° Brix, Extech Co., USA), and fruit acidity was calculated using a digital pH meter (Mettler Toledo, Switzerland). Titratable acidity (TA) was measured by titration with 0.1 Normal Sodium hydroxide until reaching pH 8.2 (AOAC, 1980). Total anthocyanin content was determined using the pH change method (Swain, 1965). The antioxidant capacity of fruit juice was determined by the DPPH (2,2-diphenyl 1-picrylhydrazyl) neutralization method (Turkmen et al., 2005). The Antron method used to measure total sugar (McCready et al., 1950).

RESULTS AND DISCUSION

Colony diameter of fungus *Aspergillus* sp.

The test outcomes revealed a significant impact of the singular effect of essential oil concentration on the mean colony diameter of Aspergillus sp (Table 1). Subsequent comparison analysis indicated that the average concentration of 800 µL.L-1 of essential oil exhibited the most minor fungus colony diameter (33 mm). In contrast, the largest fungus colony diameter (59.50 mm) was observed in the control treatment (zero concentration) (Table 2). Furthermore, it was observed that the singular effect of the type of essential oil on the mean diameter of the fungus colony was statistically significant. Specifically, the most minor fungus colony diameter was associated with the cinnamon essence treatment (12 mm), while the largest diameter was recorded in the ginger treatment (52.80 mm) (Table 3).

The interaction effect of the treatments also significantly influenced the average growth of the fungus colony diameter (Table 1), so that the consumption of cinnamon treatment at any level (200 to 800 microliters per liter) caused a 100% inhibition of the fungus growth (Table 4).

In similar studies, cinnamon essential oil demonstrated vigorous, antifungal activity on *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus fumigatus* (Manso et al., 2013). The effectiveness of cinnamon essential oil in preventing the rotting of tomato, orange, and strawberry fruits infected with *B. cinerea*, *P. digitatum*, and *A. niger* fungi showed that strawberry fruit in the vicinity of this essential oil at concentrations of 200, 400, and 600 $\mu L.L^{-1}$ exhibited contamination rates of 45%, 30%, and 11.53%, respectively. For the tomato at the same concentrations, contamination rates were 50.09%, 25.15%, and 7.30%, and for orange fruit, contamination rates were 45%, 20.30%, and 10.10%, respectively (Mousavian et al., 2018). The positive effect of cinnamon essential oil in controlling four fungi, namely *A.niger*, *Penicillium notatum*, *Mucor hiemalis*, and *Fusarium oxysporum* has also been reported (Mousavian et al., 2018). In the bark of the cinnamon plant, there are compounds such as cinnamaldehyde, eugenol, coumarin, benzaldehyde, benzoic acid, cinnamyl acetate, mannitol, linalool, and thymol. By producing benzoic acid, benzaldehyde, and cinnamaldehyde, this plant exerts fungicidal properties on mentioned fungus; therefore, the cinnamon plant possesses numerous fungicidal properties and the compounds in this plant can be used to control plant diseases. Research has shown that phenols show the most antifungal activity compared to alcohols, aldehydes, and others, and it has been determined that the inhibitory properties of the essential oils of cloves, thyme, and cinnamon are related to phenolic compounds (Plaza et al., 2004). Also, Vazifedoost et al., (2022) reported the coating of strawberry samples with *Salvia chorassanica* essential oil nanoemulsion at a concentration of 12.5 µL/mL was able to delay the growth of *R. stolonifera* and *B. cinere* mold spores on the surface of strawberries for up to 9 days. In addition, no mold growth was observed on the surface of the strawberry samples until the end of the 12th day in the samples coated with *Salvia chorassanica* essential oil nanoemulsion with a concentration of 25 µL/mL.

Table 1. Analysis of variance of the concentration factor, essential oil and the interaction effect of concentration and essential oil on the colony diameter of *Aspergillus* sp. fungus.

Sources of variation	df	Radial growth of fungus
Repetition	2	29.26^{ns}
Concentration	4	1439.77**
Essential oil	3	5515/09**
Concentration× Essential oil	12	446.92**
Error	38	21.75
C.V.	-	11.49

ns: non-significant, ** significant at P ≤0.01 probability level, df: degree of freedom.

Table 2. Means comparison of the effect of concentration of essential oils on radial growth for *Aspergillus* sp. fungi treatments in *in vitro* conditions.

Concentration (µL.L^{-1})	0	200	400	600	800
Radial growth of fungus (mm)	59.50^{a}	40.00^{b}	36.83^{bc}	33.50^{c}	33.00^{c}

Similar letter indicates non-significant difference between treatments at 5% levels.

Table 3. Means comparison of the effect of type of essential oils on radial growth for *Aspergillus* sp. fungi treatments in *in vitro* conditions.

Essential oil	Ginger	Cinnamon	Tarragon	Frankincense
Radial growth of fungus (mm)	52.80^{a}	12.00^{c}	47.33^{b}	50.13^{ab}

Similar letter indicates non-significant difference between treatments at 5% levels.

Table 4. Means comparison of the effect of type and concentration of essential oils on radial growth for *Aspergillus* sp. fungi.

Essential oil	Concentration (µL.L^{-1})	Radial growth of fungus (mm)
Ginger	0	60^{a}
	200	57.33^{ab}
	400	53.33^{abcd}
	600	45.33^{ef}
	800	48^{cdef}
Cinnamon	0	60^{a}
	200	0^{h}
	400	0^{h}
	600	0^{h}
	800	0^{h}
Tarragon	0	60^{a}
	200	47.33^{def}
	400	44^{ef}
	600	48^{cdef}
	800	53.33^{abcd}
Frankincense	0	58^{a}
	200	55.33^{abc}
	400	50^{bcde}
	600	40.66^{f}
	800	30.66^{g}

Similar letter indicates non-significant difference between treatments at 5% levels.

Fruit appearance

The simple effect of concentration and type of essential oil as well as the interaction effect of treatments had a significant effect on the appearance of strawberry fruits (Table 5). It was observed that with 800 µL.L^{-1}, the best appearance of the fruit was obtained and the lowest index was obtained in the control treatment (Table 6). Regarding the essential oil type factor, the best fruit appearance was obtained in strawberries impregnated with cinnamon essential oil, and the least favorable appearance was observed in ginger essential oil (Table 6). The results of the interaction of the treatments showed that the consumption of all concentrations of cinnamon essential oil resulted in the best appearance of the fruit (Table 7).

Consistent with our findings, previous studies have also documented the efficacy of cinnamon essential oil in preserving and enhancing the visual quality of fruits, as well as inhibiting fungal growth on strawberries throughout the storage duration (Mohammadi et al., 2014; Tzortzakis, 2007). Xing et al. (2010) also stated that cinnamon essential oil reduced the activity of *Penicillium expansum*, *Aspergillus flavus*, and *Rhizopus nigricans* on jujube and orange fruits. The decrease in the decay rate corresponds to the antibacterial and antifungal properties of the essential oil. The antimicrobial activity of essential oils can be related to an aromatic nucleus and OH group, which can affect the hydrogen bonds of enzymes in microorganisms (Sharma & Tripathi, 2008). The antifungal activity of cinnamon is due to the presence of cinnamaldehyde which is an aromatic aldehyde that prevents the activity of amino acid decarboxylase and is highly electronegative. Such compounds interfere with biological processes associated with electron transfer and react with nitrogen-containing compounds such as proteins and nucleic acids, which prevent the growth of microorganisms (Gupta et al., 2008).

Total soluble solids

The outcomes derived from the analysis of variance revealed a notable impact of the singular effects of both essential oil concentration and type of essential oil on total soluble solids. However, the interaction effect among the treatments was found to be statistically non-significant (Table 5). The concentration effect on the amount of total soluble solids showed that the highest amount of this trait (4.61) was obtained at concentrations of 600 and 800 µL.L^{-1} (Table 6). Among the essential oils used in the experiment, cinnamon essential oil showed the highest amount of total soluble solids (Table 6).

In this regard, the researchers announced in their experiments on bananas, papayas, and strawberries that the fruits treated with the tested essential oils during the storage period lost the amount of soluble solids less than the control. Our results are consistent with increasing the concentration of essential oil, a smaller decrease was observed compared to the control (Maqbool et al., 2011). In similar findings, Tzortzakis (2007) reported an increase in soluble solids in strawberry fruit during treatment with cinnamon essential oil. Also, the results of Mohammadi and Aminifard (2011) also showed that the fruits treated with cinnamon essence had a higher percentage of soluble solids than the control fruits, which is consistent with the results of the present study. Aminifard and Bayat (2018) also reported the highest amount of soluble solids in orange fruit from a concentration of 800 µL.L^{-1} of anise and black cumin essential oil. The amount of soluble solids is one of the critical indicators that has a direct relationship with the edible quality of the fruit at the time of ripening, and consumers have an excellent desire for ripe fruits with a high TSS level. Soluble solids increase at the beginning of storage due to biochemical changes and then decrease drastically due to fruit tissue respiration or fungal contamination and fruit decay. Essential oils play a role in reducing fungal contamination and fruit rot, preventing excessive respiration, and consequently, reducing soluble solids (Sharafi et al., 2011). Abbasi et al. (2021) also showed that the lowest amount of soluble solids in lemon fruit with 5.58% Brix was obtained in the treatment of 0.2% garden thyme essential oil.

pH of fruit juice

The simple effect of concentration, type of essential oil, and their mutual effect on the pH of fruit juice infected with fungus was significant (Table 5). According to the results of the comparison of the average of mutual effects, it was observed that the infected fruits treated with tarragon essential oil at a concentration of 400 $\mu L.L^{-1}$ with a pH of 3.74 had the highest pH (Table 7). Sharafi et al. (2011) stated that the use of plant essential oils had a significant effect on the pH of apple fruit. The pH value reflects the acidity level of the fruit extract; the higher the amount of organic acids in the fruit, the lower the pH will be, so the decrease in pH due to essential oil treatments compared to the control indicates the preservation of organic acids in the fruit during the storage period (Alikhani et al., 2009). Essential oils, similar to edible coatings, establish a semi-permeable barrier around the fruit, thereby diminishing the ingress and egress of gases and consequently retarding the respiration process. This delay in respiration curtails the ripening process of the fruit and the metabolism of organic acids, thus contributing to the maintenance of the fruit's pH level. Moreover, the presence of phenols in essential oils plays a pivotal role in pH maintenance by attenuating ethylene production and moderating the pace of metabolic processes (Nasrullah Zade Asl, 2013). Mohammadi and Aminifard (2013) in different results reported the highest pH value in the control treatment and the lowest pH value of tomato juice with a concentration of 800 $\mu L.L^{-1}$ of essential oil.

Table 5. Analysis of variance of the investigated qualitative traits of strawberry fruits infected with *Aspergillus* sp. fungus.

Sources of variation	df	Appearance of the fruit	TSS	pH	Titratable acidity	Antioxidant capacity	Total anthocyanin content	Total sugar
Repetition	2	0.35^{ns}	0.018^{ns}	0.009^{ns}	0.004^{ns}	136.2^{ns}	3409^{ns}	8.11^{ns}
Concentration	4	9.80^{**}	0.99^{**}	0.033^{**}	0.108^{**}	205.02^{ns}	20980^{**}	342.3^{**}
Essential oil	3	14.19^{**}	0.95^{*}	0.146^{**}	0.473^{**}	667.1^{**}	42.70^{ns}	705.1^{**}
Concentration× Essential oil	12	0.87^{**}	0.19^{ns}	0.016^{**}	0.051^{**}	372.6^{**}	3914^{*}	121.8^{**}
Error	38	0.17	0.22	0.004	0.005	117.4	1685	24.99
C.V.	-	11.44	10.77	1.88	8.44	28.62	29.39	9.91

ns: non-significant, * significant at P ≤0.05 and ** significant at P ≤0.01 probability level, df: degree of freedom.

Table 6. Means comparison of the effect of concentration and essential oil on the investigated qualitative traits of strawberry fruits infected with *Aspergillus* sp. fungus.

Treatments	Appearance of the fruit	TSS (%)	pH	Titratable acidity (%)	Antioxidant capacity (%)	Total anthocyanin content ($mg.g^{-1}$)	Total sugar ($mg.gfw^{-1}$)
Concentration ($\mu L.L^{-1}$)							
0	2.16^{d}	4.12^{bc}	3.45^{b}	1.02^{a}	32.33^{a}	206.1^{a}	55.86^{a}
200	3.66^{c}	4.00^{c}	3.55^{a}	0.98^{a}	34.89^{a}	112.7^{c}	53.87^{ab}
400	3.75^{bc}	4.50^{ab}	3.58^{a}	0.91^{b}	39.66^{a}	153.0^{b}	51.10^{bc}
600	4.08^{b}	4.61^{a}	3.55^{a}	0.84^{c}	39.89^{a}	124.7^{bc}	49.17^{c}
800	4.58^{a}	4.61^{a}	3.56^{a}	0.79^{c}	42.50^{a}	101.5^{c}	42.02^{d}
Essential oil							
Ginger	2.26^{c}	4.34^{b}	3.39^{c}	1.13^{a}	30.69^{c}	138.4^{a}	45.98^{c}
Cinnamon	4.53^{a}	4.74^{a}	3.56^{b}	0.77^{c}	33.98^{bc}	138.0^{a}	43.40^{c}
Frankincense	4.00^{b}	4.18^{b}	3.58^{ab}	0.77^{c}	45.13^{a}	141.5^{a}	57.98^{a}
Tarragon	3.80^{b}	4.22^{b}	3.62^{a}	0.97^{b}	41.62^{ab}	140.6^{a}	54.26^{b}

The same letter indicates no significant difference between treatments at 5% levels.

Titratable acidity (TA)

According to the results of this experiment, it was evident that both the individual effects of concentration and type of essential oil, as well as their combined effect, exerted a significant influence on TA. (Table 5). Fruits infected with *Aspergillus* fungus not treated with essential oil (control) had the highest amount of TA (1.02 %), which had no statistically significant difference with a concentration of 200 µL.L^{-1}, and the lowest amount. This index (0.79 %) was obtained from a concentration of 800 µL.L^{-1} (Table 6). The fruits treated with ginger essential oil had the highest TA (Table 6). Additionally, mutual effects indicated that ginger essential oil at a concentration of 400 µL.L^{-1} had the highest total acidity (Table 7). Organic acids play a crucial energy storage source in fruit tissue. During the ripening process, a considerable portion of these organic acids undergo decomposition due to respiration. (Wills et al., 1998). Organic acids possess a higher oxygen-to-carbon ratio compared to carbohydrates or fatty acids. This characteristic renders them more readily utilized as a source of energy during respiration. Along with the results of this experiment, the results of Ghafouri (2013) on pomegranate fruit showed that the lowest amount of total acidity in cinnamon essence is 500 mg.L^{-1} (0.9), and the highest amount of total acidity was observed in the control treatment (1.2). Vesal Talab and Gholami (2012) also reported that clove essential oil gradually reduced the amount of total acidity in treated grapes during the post-harvest period. Similar findings have also been documented by other researchers in studies conducted on grapes and strawberries (de Sousa et al., 2013; Hernández-Muñoz et al., 2006).

Antioxidant capacity

The results indicate that both the effect of essential oil and the interaction between concentration and essential oil had a significant impact on antioxidant percentage. However, the effect of concentration alone did not exhibit a significant influence on this index (Table 5). The results showed that the infected fruits treated with tarragon essential oil with a concentration of 800 µL.L^{-1} had the highest antioxidant (52.18%) (Table 7). Antioxidants are crucial in mitigating physiological damage and enhancing tissue resistance against stress and microbial contamination by neutralizing free radicals and diminishing oxidative stress (Wang & Lin, 2000).

The results of the current investigation reported by Sazvar et al. (2022) stated that the effect of concentration on the antioxidant percentage of barberry fruits infected with *Alternaria* fungus was not significant, however, the interaction of concentration and essential oil confirmed that the highest antioxidant percentage was obtained from the concentration of 200 µL.L^{-1} of chamomile essential oil. Raspberries treated with plant essential oils had higher levels of phenolics, anthocyanin content, and stronger antioxidant activity than untreated raspberries (Jin et al., 2012), aligning with the findings of this research.

Total anthocyanin content

The concentration and the interaction effect of concentration and essential oil on the amount of anthocyanin in strawberries infected with fungus were significant (Table 5). The highest amount of anthocyanin was observed in the control treatment (zero), while the concentration of 800 µL.L^{-1} had the lowest amount (Table 6). The results of the mutual effects of the treatments showed that the fruits infected with the fungus without the addition of essential oil had the highest amount of anthocyanin. In contrast, the fruits infected with fungus treated with ginger essential oil with a concentration of 800 µL.L^{-1} had the lowest amount of this index (Table 7).

Table 7. Means comparison of the interaction effect of concentration and essential oil on the investigated qualitative traits of strawberry fruits infected with *Aspergillus* sp.

Essential oil	Concentration (μL.L^{-1})	Appearance of the fruit	pH	Titratable acidity (%)	Antioxidant capacity (%)	Total anthocyanin content (mg.g^{-1})	Total sugar (mg.g^{-1})
Ginger	0	2.00^{ef}	3.47^{cde}	0.96^{ef}	30.97^{f}	236^{a}	54.97^{abc}
	200	1.66^{f}	3.41^{ef}	1.25^{ab}	42.06^{abcd}	143^{cde}	52.26^{abcd}
	400	2.00^{ef}	3.35^{f}	1.27^{a}	19.00^{fg}	155^{bcde}	48.41^{cde}
	600	2.33^{ef}	3.36^{ef}	1.16^{abc}	33.87^{bcdef}	103^{defg}	40.95^{efg}
	800	3.33^{cd}	3.38^{ef}	1.05^{cde}	27.57^{defg}	53.8g	33.29^{gh}
Cinnamon	0	2.66^{de}	3.44^{ef}	1.05^{cde}	33.69^{bcdef}	203^{abc}	60.22^{a}
	200	5.00^{a}	3.57^{bc}	0.71^{ijk}	12.33^{g}	97.33^{efg}	47.23^{cde}
	400	5.00^{a}	3.63^{b}	0.75^{hij}	41.87^{abcd}	128^{def}	42.43^{ef}
	600	5.00^{a}	3.60^{b}	0.64^{jk}	37.97^{abcde}	142^{cde}	36.03^{gh}
	800	5.00^{a}	3.57^{bcd}	0.69^{jk}	44.03^{abcd}	118^{defg}	31.08^{h}
Frankincense	0	2.33^{ef}	3.46^{def}	1.02^{de}	42.30^{abcd}	214^{ab}	56.68^{ab}
	200	4.00^{bc}	3.61^{b}	0.84^{fgh}	50.57^{ab}	142^{cde}	57.51^{ab}
	400	4.00^{bc}	3.60^{b}	0.67^{jk}	50.87^{ab}	162^{bcde}	59.17^{ab}
	600	4.66^{ab}	3.60^{b}	0.69^{jk}	35.66^{abcdf}	118^{defg}	59.42^{ab}
	800	5.00^{a}	3.63^{ab}	0.62^{k}	46.24^{abc}	69.55^{fg}	57.13^{ab}
Tarragon	0	1.66^{f}	3.41^{ef}	1.07^{cde}	22.36^{efg}	169^{abcd}	51.58^{cd}
	200	4.00^{bc}	3.62^{b}	1.13^{bcd}	36.60^{abcdef}	68.25^{fg}	58.47^{ab}
	400	4.00^{bc}	3.74^{a}	0.96^{ef}	46.90^{abc}	166^{bcd}	54.39^{abcd}
	600	4.33^{ab}	3.66^{ab}	0.89^{fg}	52.06^{a}	135^{def}	60.30^{a}
	800	5.00^{a}	3.66^{ab}	0.82^{ghi}	52.18^{a}	163^{bcde}	46.58^{de}

Similar letter indicates non-significant difference between treatments at 5% levels.

In line with this experiment, the results of Amiri et al. (2019) research showed that control strawberry fruits inoculated with fungus showed the highest amount of anthocyanin and the same treatment also demonstrated the highest reduction in fruit weight. Additionally, Jahani et al. (2020) also showed that grape fruits infected with *Penicillium* fungus untreated with essential oil had the highest amount of anthocyanin. The increase in the amount of anthocyanin during the harvest period is related to the weight loss and moisture loss of the fruit, and as a result, the concentration of anthocyanin content (Meighani et al., 2018). Anthocyanins, a class of phenolic compounds, contribute to the red-blue hues observed in various vegetables and fruits, and are known for their significant impact on human health. The findings imply that the synthesis of these compounds continues even after harvest (Wang & Gao, 2013).

Total sugar

According to the results of this experiment, the simple effect of the concentration, the type of essential oil, and the interaction effect of the treatments on the sugar content of the treated strawberries were significant (Table 5). By increasing the concentration of essential oils, the amount of sugar in the treated fruits decreased. The control treatment exhibited the highest amount of 55.86 mg.g^{-1} fresh weights, while the fruits treated with a concentration of 800 μL.L^{-1} of essential oil had the lowest amount of sugar with 42.02 mg.g^{-1} fresh weight (Table 6). Statistical comparison of the averages revealed that the frankincense essential oil treatment exhibited the highest sugar content, whereas the cinnamon essential oil treatment displayed the lowest sugar content (Table 6). The results of the interaction of the treatments showed that the fruits treated with tarragon essential oil with a concentration of 600 μL.L^{-1} had the highest amount of sugar at the end of the experiment (Table 7). Hatfi et al. (2013) reported that the highest amount of sugar was observed in the coated samples compared to the control. By reducing the amount of respiration, the coating delays the consumption of sugar in the respiratory enzymatic reactions. Soluble sugars act as a defense mechanism, limiting the penetration of pollutants into the fruit tissue. Additionally, higher sugar levels in the fruit tissue correlate with reduced water content, resulting in less water loss due to evaporation and transpiration.This, in turn, contributes to the increased shelf life of strawberry fruit (Amal et al., 2010).

CONCLUSION

In general, this research demonstrated that essential oils have antifungal properties in controlling postharvest fungal diseases. *In-vitro* tests showed that ginger essential oil had a shallow fungicidal effect, while cinnamon essential oil had the most fungicidal effect in controlling *Aspergillus* sp. Antifungal properties increased with increasing the concentration of essential oil, but the antifungal property of cinnamon essential oil was 100% in all concentrations used. Under natural conditions, the use of essential oils had a significant effect on maintaining the appearance characteristics and preserving soluble solids during the storage period of strawberry fruit. Among the essential oils, cinnamon essential oil showed commendable antifungal activity against the fungus *Aspergillus* sp. a common cause of strawberry fruit spoilage. This suggests its potential as an alternative to chemical fungicides, although other essential oils also possess such potential. Considering the biodegradability and low toxicity of plant essential oils, it is recommended to consider using cinnamon essential oil to enhance the shelf life of food products to increase the shelf life of food products.

Conflict of interest

The authors declare that there is no conflict of interest to report.

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