



ORIGINAL ARTICLE

Investigating the Role of Oregano and Cumin Essential Oils in Curbing *Aspergillus flavus* Growth in Almonds

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KEY WORDS

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ABSTRACT

This study explores the antifungal efficacy of oregano and cumin essential oils against *Aspergillus flavus* in almonds. Our findings revealed that oregano oil at a concentration of 150 $\mu\text{g l}^{-1}$ was significantly more effective in inhibiting *Aspergillus flavus* growth than cumin oil at 300 $\mu\text{g l}^{-1}$. After a 10-day incubation period, almonds treated with oregano oil exhibited an average fungal infection rate of just 5%, compared to 58% for cumin oil-treated almonds, and a high 95% in untreated control samples. This indicates a remarkable 94% reduction in *Aspergillus flavus* growth for oregano oil-treated almonds in comparison to the control group. In contrast, cumin oil showed a 39% decrease in fungal contamination relative to the control, demonstrating its lesser but notable antifungal potential. The study also found that almonds with dry peel had lower mold contamination rates than those with green peel when treated with these essential oils, highlighting the protective role of the hardened endocarp. Thirteen panellists rated almond qualities using a 9-point scale. Cumin oil treated almonds had higher aroma and flavor ratings than oregano oil and control almonds. Cumin offered a spicy aroma and harmonious flavor, while oregano had a medicinal aroma and bitter taste. Cumin effectively inhibited fungal growth and maintained almond quality. Sensory analysis showed a consumer preference for the aroma and taste of cumin oil-treated almonds, despite the lower antifungal efficacy. Overall, this research underscores the potential of oregano and cumin essential oils as sustainable alternatives to synthetic preservatives in controlling *Aspergillus flavus* infection in almonds post-harvest.

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Introduction

Almonds are a type of tree nut native to the Mediterranean climate region of the Middle East but are now grown in various parts of the world, including the United States, particularly in California (Imani *et al.*, 2021). Almonds are a versatile food, used in a variety of culinary applications, including snacks, as a topping for desserts and salads, and even as an ingredient in main dishes. They can be consumed in various forms: raw, roasted, sliced, slivered, or as almond milk, almond flour, almond butter, and almond oil (Kamareh *et al.*, 2015; Firouzbakht *et al.*, 2021). They are a nutritional powerhouse, offering a range of essential nutrients that contribute to overall health and well-being. They are notably abundant in health-beneficial lipids, specifically monounsaturated and polyunsaturated fatty acids. These types of fats have been shown to reduce low-density lipoprotein (LDL) cholesterol levels, thereby potentially enhancing cardiovascular health (Pica *et al.*, 2022). Almonds like other nuts such as walnut are also an excellent source of plant-based protein, making them a useful addition to vegetarian and vegan diets. They are abundant in vitamins and minerals, notably vitamin E, magnesium, and calcium, which have various health benefits including skin health, bone strength, and nerve function. In addition to these, almonds provide a good amount of dietary fiber, aiding in digestion and potentially helping with weight management (DePeters *et al.*, 2020; Sarikhani *et al.*, 2021; Shi *et al.*, 2023). The nut is additionally enriched with antioxidants, predominantly in the guise of vitamin E. These compounds serve to mitigate cellular oxidative stress, which in turn may lower the susceptibility to chronic disease conditions (Jahanbani *et al.*, 2018). Overall, incorporating almonds into your diet can offer a plethora of nutritional benefits (Valdés *et al.*, 2022).

Within the agricultural spectrum of Iraq, almonds occupy a central and strategic position, being only surpassed by oil and carpets in global economic weight. Known colloquially as the "green gold" of

Iraq, almonds are plagued by the persistent issue of aflatoxin contamination, a consequence of *Aspergillus flavus* proliferation. As a result, scholarly efforts are being directed toward assessing the antifungal capacities of natural essential oils in regulating such fungal growth in almonds (Ouzir *et al.*, 2021; Nooraldeen, 2022). There are also some reports of a reduction of aflatoxin production by exposing *Aspergillus flavus* to CO₂ (Mahbobinejad *et al.*, 2019).

Over the past century, extensive investigations have delved into the antifungal attributes inherent in widely available plant essential oils, revealing promising antimicrobial capabilities exhibited by these natural compounds. For instance, studies have demonstrated that essential oils like cinnamon and clove (0.05-0.10 mg mL⁻¹ and 0.10-0.50 mg mL⁻¹), along with their specific chemical constituents cinnamic aldehyde and eugenol can effectively inhibit the growth and toxin production of *Aspergillus flavus* (Khorasani *et al.*, 2017). Similarly, the use of oregano oil has been conclusively shown to impede the production of toxins by multiple *Aspergillus* species, including *A. versicolor*, *A. ochraceus*, and *A. flavus* (Redondo-Blanco *et al.*, 2020; Chaudhari *et al.*, 2021; Jafarzadeh *et al.*, 2022).

In addition, one study rigorously compared the antifungal properties of 15 different types of spices and two synthetic antifungal agents against eight species of toxin-producing fungi (Albayrak, 2019). The spices examined included commonly used ingredients like cinnamon, oregano, cumin, and basil. The researchers tested the spices at concentrations ranging from 5-15 mg mL⁻¹ against fungi including *Aspergillus flavus*, *Aspergillus parasiticus*, and *Fusarium verticillioides*. Oregano exhibited the strongest overall antifungal potency, completely suppressing fungal growth at 15 mg mL⁻¹. Cinnamon, cumin, and basil also showed significant inhibitory effects, with minimum inhibitory concentrations values under 10 mg mL⁻¹ for most species. Another

investigation focused on assessing the effectiveness of essential oils from a variety of spices including mint, oregano, cumin, thyme, citrus peel, red pepper, bay leaves, and sage in controlling *Aspergillus flavus* and its aflatoxin production (Böhme et al., 2013). Further research empirically validated the antibacterial potential of savory essential oil, confirming its capability to thwart the spread of harmful bacteria (Fratini et al., 2019).

Other studies have zeroed in on the impact of specific essential oils on *Aspergillus flavus*, identifying oregano oil as having the most pronounced antifungal effect when tested in a synthetic medium (Ji et al., 2022). Moreover, research has cataloged spices that inhibit microbial growth, specifying the types of microorganisms affected by each spice (Chakraborty et al., 2020). More recently, studies have extended to examining the efficacy of tropical spice extracts on the growth of various fungi in fruit juices (Suraka et al., 2022), as well as the preventative use of oregano oil on tomatoes (Wu et al., 2023). Another investigation by Tian et al. (2022) looked into the protective effects of eleven different plant essential oils on corn seeds susceptible to *Aspergillus flavus* contamination.

Belonging to the mint family Lamiaceae, oregano, or scientifically known as *Origanum vulgare L.*, is a type of flowering plant. It originally hails from the Mediterranean region but has since become naturalized in various parts of the temperate Northern Hemisphere. This woody perennial plant stands 20–80 cm tall and has opposite leaves that are 1–4 cm long. During the summer, it produces erect spikes of flowers that can be white, pink, or light purple and are 3–4 mm long. On the other hand, cumin, or *Cuminum cyminum L.* as it is scientifically known, is a flowering plant native to the Irano-Turanian Region and belongs to the family Apiaceae. The seeds of this plant, each enclosed within a dried fruit, are a common ingredient in the culinary practices of many cultures, used in both whole and ground forms (Vallverdú-Queralt et al., 2014). The primary objective of our study aligns

with this body of research: to identify effective natural alternatives to synthetic fungicides for the purpose of inhibiting fungal growth in fruits. This aim is twofold: to reduce dependency on chemical solutions and to improve public health through the promotion of more organic and sustainable farming practices.

Material and Methods

In the present research, the natural essential oils of oregano and cumin were purchased from a local garden market.

First phase

For the purpose of fungal inoculation, a lyophilized ampoule of the aflatoxin-producing *Aspergillus flavus* strain NRRL 3357 was obtained from the Agricultural Research Service (ARS) Culture Collection. This isolate was originally recovered from peanuts in Georgia, USA and has been characterized as an aggressive aflatoxin B1 producer. Following the instructions provided by the ARS, the fungal spores were activated and suspended in a Tween 80 solution to achieve a concentration of 10^6 spores ml^{-1} . For the growth of fungi, a slope of potato dextrose agar was utilized as the ground culture medium, the most suitable medium for the cultivation of fungi. A solution of Tween 80 at a concentration of 0.08% was employed for the purpose of spore washing, while ethyl alcohol was utilized for the dilution of essential oils. Almond samples were gathered from the harvests collected from the almond orchards of Erbil, Iraq. A 3% hypochlorite solution was prepared to sterilize the almond surface, and sterile distilled water was used to eliminate any remaining sodium hypochlorite.

The preparation of Potato dextrose agar and sucrose yeast extract (Fermtech, Merck KGaA, Darmstadt, Germany), was carried out in accordance with the instructions provided by the manufacturer. The mold spore suspension was prepared to a concentration of 10^6 spores ml^{-1} . A sucrose yeast extract culture medium was prepared, containing 75, 150, 300, 450, and 600 $\mu\text{g l}^{-1}$ of oregano and cumin

essential oils, in four replicates (Elgorban *et al.*, 2015; Hu *et al.*, 2019). To these tubes, 0.5 ml of the fungal suspension was added. The samples were then stored in an incubator set at a temperature of 25°C for a period of 10 days. Following the incubation period, the contents of the tubes were filtered using sintered glass strainers. The mass of the micelles was then dried in an oven set at a temperature of 80°C for a duration of 24 hours and subsequently weighed (Ji *et al.*, 2022).

Second phase:

A solution of sodium hypochlorite at a concentration of 2% was prepared. For the treatment of almond samples, 90 almonds with soft skin and 90 almonds with dry skin were selected after washing. To sterilize the surface of the almonds, they were submerged in the sodium hypochlorite solution for a duration of 2 min. Following this, the almonds were immersed in sterile distilled water for 1 min to remove any remaining sodium hypochlorite from their surface. According to the previous results, treatments were carried out in the following three groups:

-Oregano essential oil at a concentration of 150 µg l⁻¹: 20 almonds with soft skin and 20 almonds with dry skin were separately immersed in a solution of oregano essential oil for a duration of 1 min, after which they were drained.

-Cumin essential oil at a concentration of 300 µg l⁻¹: 20 almonds with soft skin and 20 almonds with dry skin were separately immersed in a solution of cumin essential oil for a duration of 1 min.

-Control: 20 almonds with soft skin and 20 almonds with dry skin were transferred to the next stage without undergoing any treatment, serving as control samples.

For the inoculation and incubation process, a spore suspension was sprayed onto each treatment separately, at a concentration of 10⁶ spores ml⁻¹, under a laminar hood. Next, using sterile tweezers and working next to a flame, 20 almonds from each treatment were placed into three Petri dishes. The

almonds were arranged in such a way that they did not touch each other, and the Petri dishes were then covered, labelled, and placed in an incubator set at a temperature of 25°C. The percentage of contamination was recorded at the desired time intervals.

Experimental Design and Statistical Analysis

The almond samples were subjected to three treatments: 1) oregano oil at 150 µg l⁻¹, 2) cumin oil at 300 µg l⁻¹, and 3) control. Each treatment was replicated three times, with each replication containing 20 almonds for fungal inoculation. The effect of essential oil type, almond peel condition (green vs. dry), and incubation time on *A. flavus* growth inhibition were evaluated using a 3×2×4 factorial design. The percentage of fungus-infected nuts was measured at incubation periods of 10-, 20-, and 30-days post-inoculation.

A trained 13-member descriptive sensory panel evaluated the randomized, coded almond samples across 3 treatments for attributes including aroma, taste, texture, and appearance. Panelists were trained over 5 sessions on recognizing and rating key sensory characteristics of almonds using standardized line scales. For aroma, intensity of cumin and oregano was rated on a 15cm line scale from "none" to "extreme". For taste, bitterness, medicinal flavor, and cumin/oregano taste were scored on 10cm scales from "none" to "extreme". Almond color was evaluated for darkness and uniformity on 5cm scales from "light" to "dark" and "uneven" to "even". Texture was rated for crunchiness and hardness on 10cm scales from "soft" to "hard". Panelists evaluated 3 nuts per treatment, with 3 replications, cleansing their palates between samples.

All data were analyzed by analysis of variance (ANOVA) and Duncan's multiple range test using SPSS software version 23.0. Mean separations were performed at a significance level of p<0.05. The data collected from the first phase of the experiment was examined and statistically processed using a full

random factorial method.

Results

The ANOVA of the data is presented in Table 1. According to this table, it can be inferred that the type of essential oil did not have a significant impact on the growth of fungi. The use of varying concentrations

of natural essential oils resulted in statistically significant differences at the 0.05 level in mold growth in the cultivated environment, meaning that altering the concentration of essential oils affected mold growth. The interaction between the type of essential oil and its concentration was statistically significant ($p < 0.05$).

Table 1. Variance analysis of the first phase information

Source of variation	DF	SS	F-Statistic	p-value
Repetition	3	469.25	2.57	-
The effect of essential oil type	1	1450.30	7.97	0.0174
The effect of essential oil dose	8	51212.10	281.44	0.0081
The interaction effect of essential oil type and essential oil dose	8	1830.56	10.06	0.0001
Error	30	172.86	-	0.0001
Total	50	-	-	-

To determine the minimum inhibitory concentration of each essential oil, the partial averages of each concentration and essential oil were calculated. These averages were then compared using Duncan's test (Table 2). By analyzing the table, it is evident that the essential oils of oregano and cumin, at

concentrations of $150 \mu\text{g l}^{-1}$ and $300 \mu\text{g l}^{-1}$ respectively, have completely inhibited the growth of mold in the culture medium. As a result, it is safe to recommend a concentration of $150 \mu\text{g l}^{-1}$ for oregano essential oil and $300 \mu\text{g l}^{-1}$ for cumin essential oil.

Table 2. The effect of different concentrations of essential oils on mold growth

Essential Oil Type	Concentration ($\mu\text{g l}^{-1}$)	p-value	Mycelium weight (mg)
Oregano	0	-	212.36
	75	0.041	98.79
	150	0.002	0
	300	0.001	0
	450	0.001	0
	600	0.001	0
Cumin	0	-	212.36
	75	0.048	94.35
	150	0.028	78.12
	300	0.001	0
	450	0.001	0
	600	0.001	0

The data obtained from the second phase was analyzed using SPSS and the completely randomized factorial method (Table 3). From the table, it can be inferred that the type of almond tested (almond with green skin and almond without green skin) has resulted in a statistically significant difference at the

0.05 level. The type of essential oil used also had a significant effect at the 0.05 level, as did the difference between different times. The interaction effects were also significant, meaning that the use of different essential oil treatments on a specific type of almond had a significant effect on mold growth.

Table 3. Variance analysis of the second phase information

Source of variation	DF	SS	F-Statistic	p-value
Repetition	3	0.081	0.66	<0.01
Effect of almond type	2	6.57	69.35	<0.01
The effect of essential oil type	3	39.45	412.98	<0.01
The interaction effect of almond type and essential oil type	3	0.309	3.07	<0.05
The effect of time	3	17.23	189.46	<0.01
Interaction effect of almond type and time	3	2.91	29.71	<0.01
Interaction effect of essential oil type and time	5	1.54	17.46	<0.01
The mutual effect of almond type, essential oil type and time	5	2.08	25.79	<0.01
Error	31	0.085	-	
Total	58	-	-	

In a particular variety of almonds, applying various essential oil treatments significantly impacted mold development (Fig. 1). In that same almond type, time also had a meaningful influence at the 0.05 significance level (Fig. 2). When using a specific essential oil, time played a notable role in the

treatments (Fig. 3). Moreover, the type of almond examined significantly altered the outcomes; for instance, mold growth was more prevalent in soft-skinned almonds compared to those with dry skin. The choice of essential oil used also significantly influenced the results.

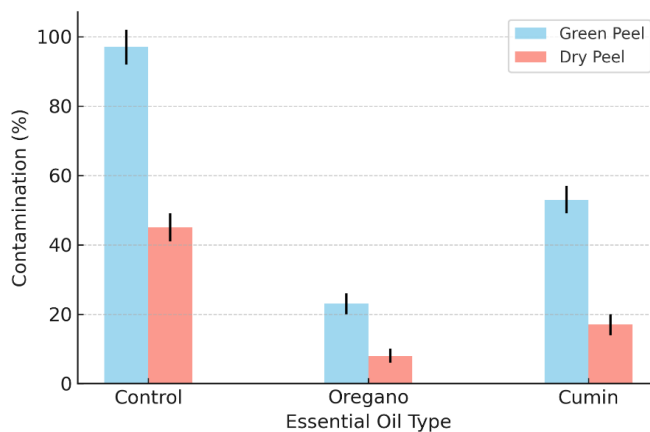


Fig. 1. Interaction effect of essential oil type and almond condition

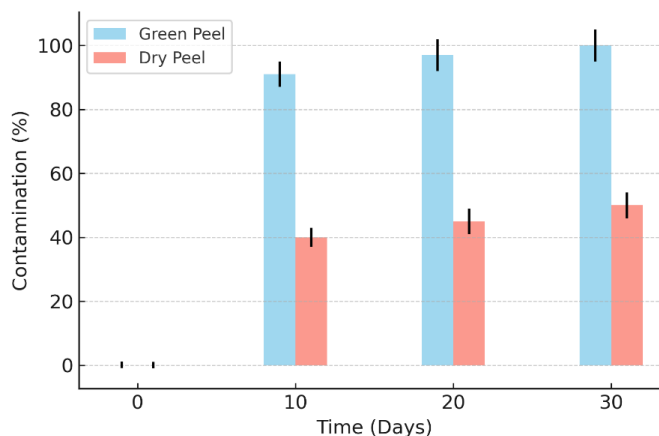


Fig. 2. Interaction effect of almond type and time on almond contamination percentage

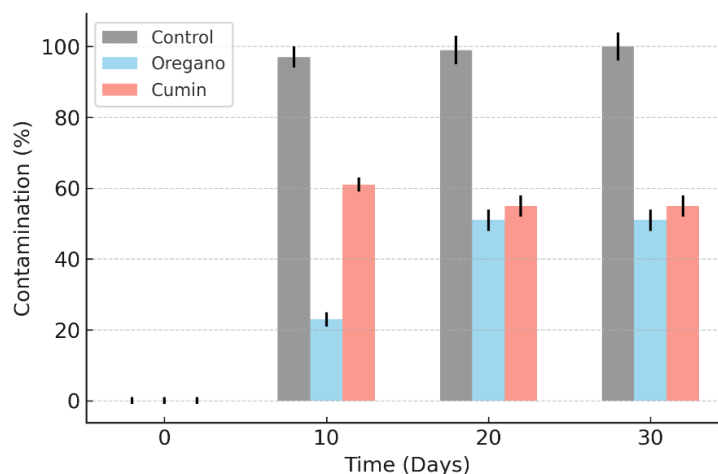


Fig. 3. Interaction effect of essential oil type and time on almond contamination percentage.

To assess if various kinds of almonds had distinct impacts on the growth of mold, Duncan's test was employed to compare the mean mold growth across different almond types. The test findings revealed substantial statistical disparities between the different types of almonds in relation to their mold growth

rates. When the average growth rates were compared, it was evident that mold of dry-skinned almonds exhibited the least growth. This is attributed to less favorable conditions for mold development as compared to molds of green-skinned almonds (Fig. 4).

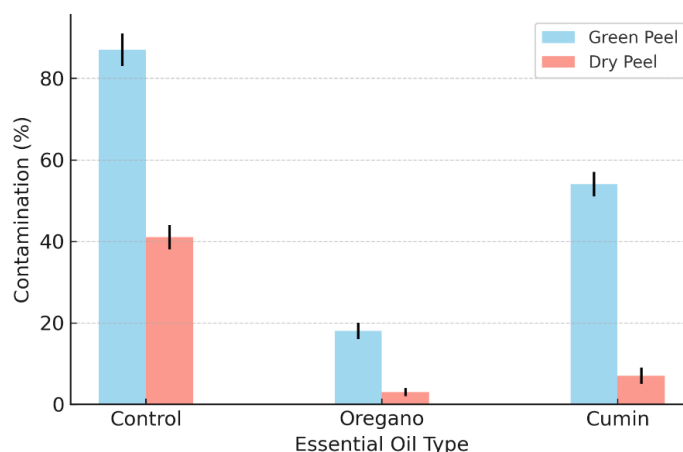


Fig. 4. The effect of almond type on mold growth rate and almond contamination percentage in different treatments.

Based on the findings of this study, there is promising potential for mitigating aflatoxin contamination in almonds through the inhibition of *Aspergillus flavus* growth. This can be achieved by employing specific concentrations of natural essential oils, namely oregano and cumin, as effective anti-fungal agents.

To assess the impact of varying essential oil treatments, average outcomes were analyzed using Duncan's technique. The analysis revealed that the three different treatments tested had statistically significant effects when compared to each other.

Upon scrutinizing the average results, it was observed that although cumin essential oil had a higher concentration of carvacrol, the treatment using 150 $\mu\text{g l}^{-1}$ oregano essential oil led to the least mold growth. This phenomenon could potentially be attributed to the synergistic interactions of other constituents present in oregano essential oil.

To assess the influence of various essential oils on the gustatory perception of almonds, an organoleptic test was employed, given the minimal concentrations of the oils and the intricate interplay of compounds affecting human olfactory and gustatory systems. The

data gleaned from these organoleptic experiments were subjected to statistical analysis using a completely randomized factorial design. To further scrutinize the impact of different essential oil treatments on the flavor profile of almonds, Duncan's test for mean comparison was applied. Based on Fig. 5, it can be inferred that almonds treated with cumin essential oil were more favorably rated by the panellists in comparison to other treatments. Based on

the metrics evaluated by the sensory panel, the essential oil treatments appear to have primarily affected the aroma and taste attributes of the almond samples. Texture and appearance may have been indirectly impacted. However, it should be noted that the taste distinction between the cumin essential oil-treated almonds and the control group was not statistically significant (Fig. 5).

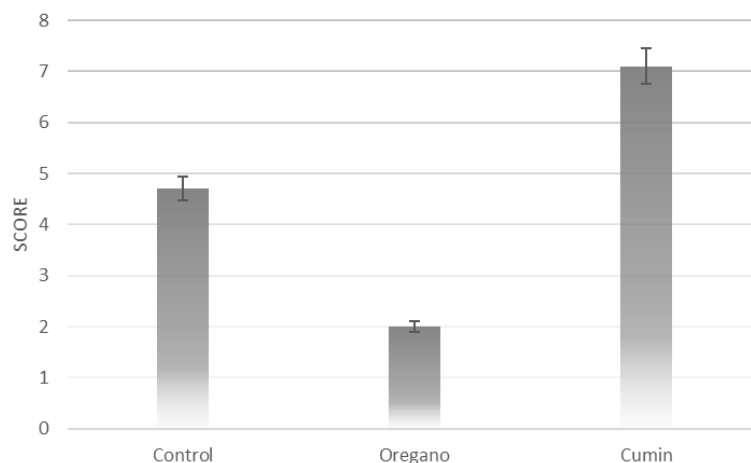


Fig. 5. The effect of different essential oils on almond taste

Discussion

The key findings of this study demonstrate the potent antifungal properties of oregano and cumin essential oils against *Aspergillus flavus* proliferation and subsequent aflatoxin production in almonds. Specifically, oregano oil at $150 \mu\text{g l}^{-1}$ was found to be more effective at suppressing fungal growth compared to cumin oil, even at a higher concentration of $300 \mu\text{g l}^{-1}$. This was an unexpected result considering cumin contains higher levels of the phenolic compound carvacrol, which is attributed to have strong antimicrobial effects (Vallverdú-Queralt *et al.*, 2014). A potential explanation is the synergistic interaction between carvacrol and other bioactive components present in oregano oil, like thymol and rosmarinic acid, contributing to enhanced antifungal action (Al-Mariri and Safi, 2014).

The inhibitory activity exhibited by oregano and cumin oils aligns with previous studies demonstrating the efficacy of plant-derived compounds as natural antifungal agents against toxigenic fungi (Perczak *et al.*, 2019a, 2019b). For instance, Redondo-Blanco *et*

al. (2020) reported the effectiveness of oregano oil in suppressing aflatoxin secretion by *Aspergillus flavus*, validating the current findings. Our results are also consistent with research by Albayrak (2019) that compared the antifungal potential of various spices, with oregano displaying the strongest inhibition of multiple fungal species including *Aspergillus flavus*.

Moreover, this investigation revealed lower *Aspergillus flavus* contamination rates in dry-peel almonds compared to green-peel nuts when treated with essential oils. The hardened endocarp of mature, dry almonds likely provides a more effective barrier against fungal infiltration and subsequent toxin production (Rodrigues *et al.*, 2012a, 2012b, 2013). Sensory analysis indicated consumer preference for cumin oil-treated almonds, which can inform the development of acceptable essential oil-based formulations to protect almonds from fungal decay while maintaining desirable organoleptic qualities.

Some limitations of this study include the examination of antifungal effects under laboratory

conditions, which may not directly translate to field efficacy. Additionally, only single concentrations of the essential oils were tested based on in vitro optimization, whereas dose-dependent trials could provide further insights. The inclusion of more almond varieties and fungal strains would also help generalize the results. Future directions involve investigating the stability and release kinetics of essential oil nano-emulsions as a practical antifungal treatment strategy for almonds and other nuts susceptible to aflatoxin contamination.

Overall, this work adds to existing literature supporting the potential of plant essential oils as sustainable antifungal alternatives to mitigate food losses and safety issues driven by undesirable mold growth. In particular, oregano and cumin oils exhibit promising inhibitory effects against *Aspergillus flavus* proliferation and associated mycotoxin production in almonds, addressing concerns facing the nut industry. Practical applications would require determining optimal application methods and dosages to achieve sufficient antifungal efficacy while preserving sensory attributes. Further research in this domain can facilitate the transition towards more organic, eco-friendly postharvest protection of valuable agricultural commodities.

Conclusions

The present study investigated the antifungal potential of oregano and cumin essential oils to inhibit the growth of aflatoxin-producing *Aspergillus flavus* in almonds. Based on the results, oregano oil at 150 $\mu\text{g l}^{-1}$ demonstrated stronger antifungal properties compared to cumin oil at 300 $\mu\text{g l}^{-1}$, despite the higher carvacrol content in cumin. Specifically, after 10 days of incubation, oregano oil treated almonds showed an average of 5% fungal infection versus 58% in cumin oil treated nuts and 95% in the untreated control. The potent inhibitory activity of oregano oil led to a 94% reduction in *Aspergillus flavus* growth compared to control almonds. While cumin oil exhibited activity, its impact was lower with a 39% decrease in fungal

contamination relative to control. This highlights the significant antifungal potential of oregano oil at improving almond preservation. The synergistic effect of various bioactive components in oregano oil likely contributed to its pronounced inhibitory activity. Additionally, dry-peel almonds showed lower mold contamination rates compared to green-peel nuts when treated with essential oils, indicating the protective role of the hardened endocarp. Organoleptic analysis revealed consumer preference for cumin oil-treated almonds from a sensory perspective. Overall, this research validates the efficacy of plant-derived essential oils as natural antifungal agents to curb fungal proliferation and associated mycotoxin secretion in agricultural commodities. In particular, oregano and cumin oils exhibit promising potential as sustainable alternatives to synthetic preservatives for controlling *Aspergillus flavus* infection in almonds post-harvest. Further studies can help optimize the application of essential oil-based formulations to maximize antifungal potency while maintaining acceptable sensory attributes. Adopting effective bio-fumigation strategies using plant essential oils can significantly mitigate crop losses due to fungal decay and promote food safety. The findings of this investigation provide impetus for expanding research on plant-based antifungals to better address the pressing challenges of food security, safety, and sustainability in the modern era.

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Conflict of interests

The authors declare no conflict of interest.

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