Research Article

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# Prevalence of fungi in fresh tomatoes and their control by chitosan and sweet orange (*Citrus sinensis*) peel essential oil coating

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## Abstract

**BACKGROUND: Fungal contamination is a major cause of food spoilage. There is an urgent need to find and characterize natural preservatives. This study evaluates the prevalence of fungi in tomatoes and their control by using essential oil (EO) from sweet orange peel. Essential oils were extracted from dried and fresh sweet orange peels by using n-hexane and ethanol as extraction solvents. Fourier transform infrared spectroscopy (FTIR) and gas chromatography–mass spectrometry (GC–MS) analyses were performed to identify the chemical composition of the EO. A combination of chitosan (CS) and EO was used to control the fungal decay of tomatoes inoculated with *Aspergillus niger* and *Penicillium citrinum*.**

**RESULTS: Tomatoes obtained from local markets and supermarkets showed a high prevalence of *Aspergillus and Penicillium* spp. Essential oils extracted by ethanol from dried peels showed complete inhibition of *A. niger* and *P. citrinum* and hyphal degradation at a minimum inhibitory concentration (MIC) of 100 μL $mL^{-1}$. The combination of EO with chitosan (2%) as a coating, effectively controlled the fungal decay of tomatoes until the eighth day of storage at 25 °C.**

**CONCLUSION: Due to their edible nature, and their antifungal and preservative potential, EO- and CS-based coatings can be used to extend the shelf life of tomatoes and other agriculture commodities. Essential oil- and CS-based coating can be used as alternative to synthetic preservatives, which are associated with various health hazards.**


*Supporting information may be found in the online version of this article.*

**Keywords:** prevalence; essential oils; radial growth inhibition; hyphal degradation; preservation

## INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an annual short–lived herbaceous plant, produced in 144 countries.[1,2] Approximately 171 million metric tons of tomatoes are produced globally.[3] From an economic point of view tomato is one of the most important crops because tomatoes are consumed in fresh form and processed into various food items such as pulp, ketchup, sauces, and pastes.[4] Agricultural products with high moisture content are more susceptible to fungal attack and spoilage if not stored properly.[5] Tomatoes are more susceptible to fungal contamination due to high moisture content and their thin outer layer.[6,7] Due to worldwide consumption of tomatoes, the presence of any harmful mycotoxin or fungal contamination can affect public health. The common tomato-contaminating fungi are *Fusarium* spp., *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp., and *Alternaria* spp.[1,2,4]

Chemical treatments have frequently been used to control the postharvest fungal decay of vegetables and fruit.[7] The continuous use of synthetic fungicides leads to various environmental and health problems due to their teratogenic and carcinogenic effects.[8] Furthermore, due to frequent use, microbial pathogens have developed resistance against these fungicides.[9] Biologically active natural products can be a good alternative to synthetic fungicides.[10] The natural antimicrobials can be obtained from byproducts of food and feed industry.[11] The fruit and vegetable processing industries generate several byproducts and waste materials.[12] Citrus fruit peels are byproducts of fruit processing and can serve as a source of pectin, sugar, and various bioactive compounds such as essential oils (EOs).[13]

Essential oils are present between the crust and the white section, known as the albedo, of citrus peels and contain a variety of bioactive components such as, D-limonene, linalol, citral, $\alpha$- pinene, $\beta$-pinene and camphen.[10,14] The antifungal and antibacterial activities of citrus EO are attributed to the presence of these bioactive compounds.[15] The EOs of orange, mandarin, and grapefruit were reported to exhibit antifungal potential against *Aspergillus* and *Penicillium* spp.[16] The antifungal effect of EO is due their ability to penetrate the cell membranes of microorganisms, which causes ion leakage and disruption of cellular structure.[17] Essential oils also exhibit antioxidant potential due to their ability to neutralize the free radicals.[18] The food and

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agriculture industries are facing tremendous loss because of fungal rotting of fruits and vegetables.[19] There is an urgent need to develop new methods and search for natural antifungal compounds to control food spoilage. Due to its edible nature, EO in combination with certain natural polymers, such as chitosan (CS), can be used to develop preservative coatings for fresh fruits and vegetables. The objective of this study was to find the fungal prevalence in tomatoes and their control by using EO from peels of sweet orange. Furthermore, EO in combination with CS was also used to control the fungal growth in tomatoes to enhance the shelf life of raw tomatoes.

## MATERIALS AND METHODS

### Isolation and morphological identification of fungi

Fresh tomatoes (*Solanum lycopersicum* L.) were obtained from local markets (n = 75) and supermarkets (n = 75) in Lahore, Pakistan, during Fall, 2019. The samples were kept aseptically in a clean chamber at room temperature (25 °C ± 2) for 14 days. The tomato samples with visible fungal contamination were subjected to fungal isolation by removing the contaminated part of tomato (5 mm) aseptically and inoculating the sample on potato dextrose agar (PDA, Oxoid, UK). It was then incubated at 28 °C for 6 days. The fungi were identified morphologically by observing the conidiophores, sporangiophores, and the fruiting bodies of the different molds by using the stereomicroscope (Olympus, Tokyo, Japan).[4,20]

### Extraction of essential oil from sweet orange peel

Sweet oranges (*Citrus sinensis*) commonly known as 'Mosambi' were collected during Fall, 2018 from the local markets of Lahore, Pakistan. The fruits were washed under running tap water to remove dust, and were peeled. The peels were dried in an oven at 48 °C for 4 days and ground into powder. The fresh peels and dried powdered peels were used for extraction of EO by using n-hexane and ethanol as extraction solvents, separately. Essential oils were extracted from sweet orange peels by using Soxhlet extractor.[21,22] The peels (25 g) were added to the extraction solvent (225 mL) and extracted for 6 h at 69 and 78 °C for n-hexane and ethanol, respectively. The solvent was removed by rotary evaporator (Buchi, Flawil, Switzerland) to obtain the EO. The extracted EO were transferred in an airtight vial and stored at 4 °C for further analysis. The EO yield was calculated by dividing the mass of EO obtained by the mass of raw material.

### Antifungal effects of EO and CS

The antifungal activity of EO and CS was determined by the radial growth inhibition assay as described by Sriwattanachai *et al*.[23] Potato dextrose agar (Himedia, India) plates containing twofold concentrations (200, 100, 50, 25 µL $mL^{-1}$) of EO and Tween 80 (2%, v/v) were inoculated at the center with a 5 mm fungal plug, containing actively growing mycelia of *P. citrinum* and *A. niger*. Potato dextrose agar containing Tween 80 (2%) was used as control. Similarly, the antifungal effects of CS (Sigma-Aldrich Co. St. Louis, USA) were evaluated at different concentrations (1%, 2%, and 3%). All petri dishes were sealed with parafilm and incubated at 30 °C for 7 days in an incubator. The radial growth of the fungal colonies was measured with a Vernier caliper (Mitutoyo, Kawasaki, Japan). The lowest test concentration of EO that did not show visible growth after 7 days of incubation was marked as minimum inhibitory concentration (MIC) whereas the antifungal activity of other concentrations was expressed in terms of percentage of inhibition by using Eqn (1):

$$\%\text{inhibition} = \left[\frac{dc - dt}{dc}\right] \times 100 \quad (1)$$

where, $d_c$ and $d_t$ represent the diameters of control and sample respectively.

### Effect of EO and CS on fungal hyphae

The effects of EO, CS, and their combined mixture (EO + CS) on fungal hyphae were determined by following the method described by Chein *et al*.[24] Potato dextrose broth (PDB, Himedia, India), 20 mL containing Tween 80 (2%, v/v) was inoculated with fungal spores ($10^4$ spores/ml) and placed in a shaking incubator (Wisecube, Seoul South Korea) at 30 °C and 180 rpm. After 48 h, hyphae were harvested by centrifugation (DLAB D3024R, China) at 4,500× g for 5 min, and were then washed (twice) with phosphate buffer saline (PBS, pH 7.4). Cells were re-suspended separately in 20 mL of PBS containing 2% tween 80 (control); PBS containing 2% Tween 80 and CS (2%); PBS containing 2% Tween 80 and EO (MIC); PBS containing 2% Tween 80 and a combined mixture of CS (2%) with EO (MIC). The samples were further incubated at 30 °C for 24 h followed by staining of hyphae with lactophenol-cotton blue mounting solution, and finally they were observed under a light microscope (Meiji, Japan).

### Fourier transform infrared spectroscopy (FTIR) analysis

Essential oils were analyzed by using an FTIR spectrometer (Agilent Technologies, USA) equipped with a universal attenuator total reflectance (UATR) accessory. The spectra were recorded in the range of 4000–650 $cm^{-1}$ with a resolution of 4 $cm^{-1}$ using absorbance mode.[14]

### Gas chromatography mass spectrometer (GC–MS) analysis

Essential oils were analyzed using GC–MS system (GC-7890A/MS-5975C, Agilent Technologies, Santa Clara, CA, USA) with a HP-5 MS capillary column. Helium was used as a carrier gas (1.0 mL $min^{-1}$) and injector temperature was maintained at 200 °C. The oven temperature was programmed at an initial temperature of 35 °C and gradually increased to 270 °C with an increasing rate of 5 °C per minute followed by a further temperature increase to 320 °C with the rate of 10 °C per minute. All data were acquired by collecting the full-scan mass spectra within the range 50–600 a.m.u. The compounds were identified by using the NIST 05 spectral library (Gaithersburg, MD, USA).[7]

### Application of EO in shelf life extension of tomatoes

The method for the *in situ* fungal activity of EO was adopted from Aloui *et al*.[15] Tomatoes without any fungal contamination and damage were selected based on uniformity of size and color. The tomatoes were washed with sodium hypochlorite solution (0.4%) and dipped for 1 min in spore suspension ($10^6$ spores/mL) of *P. citrinum* and *A. niger* separately and dried for 1 h under laminar air flow hood. Phosphate buffer saline (0.01 mol $L^{-1}$, pH 7.4100 mL) was used to prepare solutions of CS (2%), EO (MIC), and the combination of CS (2%) and EO (MIC), separately. Tween 80 (2%, v/v) was added to formulations containing EO. A set of five tomatoes (previously treated with fungal spore suspension) were dipped in each of these solutions for 1 min and tomatoes dipped only in fungal spore suspensions were used

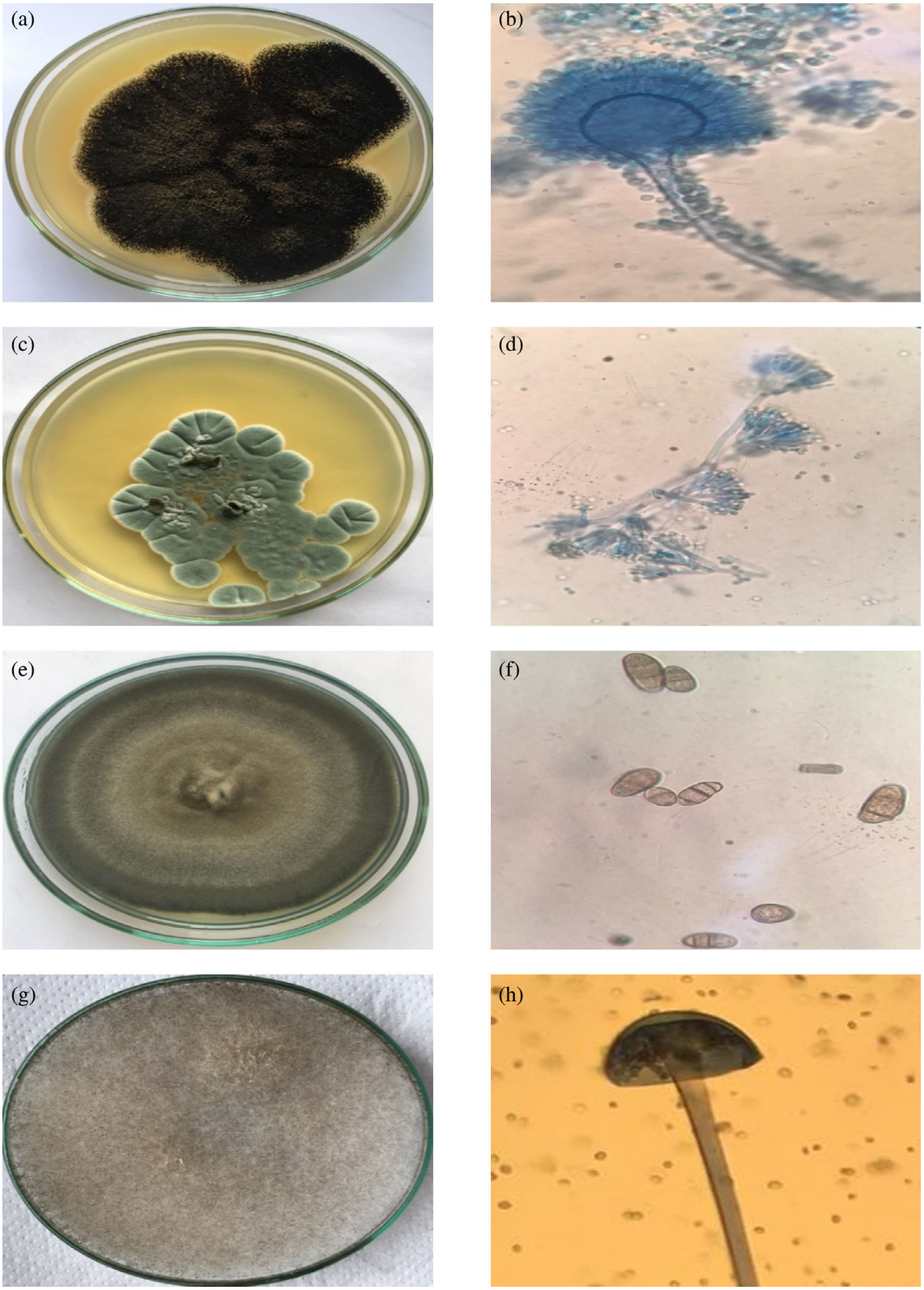


**Figure 1.** Plate morphology and microscopic identification of *Aspergillus* spp. (a and b), *Penicillium* spp. (c and d), *Curvularia* spp. (e and f) and *Rhizopus* spp. (g and h).

as control. All the samples were stored at 25 °C ± 2, 85% RH for 12 days and disease incidence was monitored daily. All experiments were performed in triplicate.

### Statistical analysis

The results for prevalence of fungi in tomatoes were analyzed with chi-squared tests and two-sided Fisher's exact tests. Results



  

**Table 1.** Prevalence (%) of fungi in tomatoes collected from local markets (n = 75) and supermarkets (n = 75)

| | Prevalence (%) | | |
|---|---|---|---|
| Isolate | Local market | Supermarket | *P*-value |
| *Aspergillus* spp. | 69 (92%) | 48 (64%) | 0.001 |
| *Penicillium* spp. | 45 (60%) | 42 (56%) | 0.000 |
| *Curvularia* spp. | 15 (20%) | 18 (24%) | 0.000 |
| *Rhizopus* spp. | 9 (12%) | 18 (24%) | 0.000 |

with $P < 0.05$ were considered statistically significant. For antifungal assays one-way analysis of variance (ANOVA), and Tukey's HSD tests were used to find the significant differences among mean treatments ($P < 0.05$) using an SPSS statistical software package (SPSS, version 23.0, USA).

## RESULTS AND DISCUSSION

### Isolation and morphological identification

*Aspergillus* spp., *Penicillium* spp., *Curvularia* spp., and *Rhizopus* spp. were isolated from tomatoes and identified morphologically (Fig. 1). The prevalence of *Aspergillus* spp. was higher in tomatoes collected from local markets (92%, 69 isolates) and supermarkets (64%, 48 isolates) (Table 1). From local markets, the prevalence of *Penicillium, Curvularia*, and *Rhizopus* spp. was 60% (45 isolates), 20% (15 isolates), and 12% (9 isolates), respectively. From supermarkets, the prevalence of *Penicillium, Curvularia*, and *Rhizopus* spp., in tomatoes was 56% (42 isolates), 24% (18 isolates), and 24% (18 isolates), respectively. Previous research studies reported that *Penicillium* spp., *Fusarium* spp., *Aspergillus* spp., *Mucor* spp., and *Rhizopus* spp. were the predominant fungi in the spoilage of tomato fruit.[1,25] Van de Perre *et al.*[4] reported that fresh produce, including tomatoes, was mainly infected by *Penicillium* spp., *Aspergillus* spp., *Fusarium* spp., and *Alternaria* spp. The prevalence of *Aspergillus* spp. and *Penicillium* spp. was higher in tomatoes collected from local markets as compared to supermarkets, which might be due to fact that supermarkets have better food safety management system and storage conditions than the open markets.[26]

### Extraction of EO

With n-hexane, the extraction yield of EO was 9.33 ± 0.3% and 6.12 ± 0.5% for dried peel powder and fresh peels respectively, whereas, when ethanol was used as an extraction solvent, the yield was 10.67 ± 0.17% and 7.08 ± 0.44% for dried peel powder and fresh peels, respectively. The extraction yield of EO was higher when ethanol was used as an extraction solvent. Zhu *et al.*[22] reported that the extraction yield of EO was 40% higher when Soxhlet apparatus was used with ethanol as an extraction solvent, compared with steam distillation. The non-polar solvents like n-hexane favor the extraction of non-polar components from the sample, thus enhancing their percentage in the extracts.[27] Ethanol is a green and safe solvent and can be used to replace n-hexane for extraction of oils from various plant sources, due to toxicity associated with n-hexane.[28]

### Radial growth inhibition assay (RGI)

The EO extracted by ethanol from the dried peels showed antifungal activity against both *A. niger* and *P. citrinum* in a dose-dependent manner and visible growth of fungi was not observed at 100 µL mL$^{-1}$ (supplementary material, Fig. S1). The EO extracted from fresh peels showed significantly lower ($P < 0.05$) antifungal activity compared with the EO from dried peels (Table 2). The particle size of fresh peels was higher than the particle size of dried powder peels, which resulted in lower yield of EO from fresh peels. Furthermore, in fresh peels, high water content restricted the extraction of entrapped oils, which was the reason for the lower antifungal activity of EO extracted from fresh peels.[29] Essential oil extracted from fresh peels by n-hexane as an extraction solvent showed maximum inhibition of 45% and 30%, against *Aspergillus* sp. and *Penicillium* sp., respectively, at the highest test concentration (200 µL mL$^{-1}$). Only the EO extracted by ethanol from dried peels of sweet orange showed complete inhibition of *A. niger* and *P. citrinum* at 100 µL mL$^{-1}$, which was marked as MIC. Essential oils extracted by ethanol showed significantly higher ($P < 0.05$) fungal inhibition than the EO extracted by n-hexane. The reason might be due to the fact that ethanol is amphipathic (contains polar and non-polar ends) so it extracted more compounds contributing to the antifungal effect. The antifungal effects of CS increased in a concentration-dependent manner; however, there were no significant differences ($P < 0.05$) in inhibition at 2% and 3% of CS. The CS at 2% and 3% showed 12.5% and 12.85% *Aspergillus* sp. inhibition and 11.95% and 12.54% *Penicillium* sp. inhibition, respectively. The antifungal effects of EOs are due to their accumulation in fugal cell membranes, which results in damage and destabilization.[30] The formation of hydrogen bonds between the hydroxyl group

**Table 2.** Antifungal activities (% inhibition by radial growth inhibition assay) of essential oils (EO) extracted from fresh and dried peels of sweet orange

| | EO extracted from fresh peels | | | | EO extracted from dried peels | | | |
|---|---|---|---|---|---|---|---|---|
| | *n-Hexane* | | *Ethanol* | | *n-Hexane* | | *Ethanol* | |
| *Conc.* | *Aspergillus* sp. | *Penicillium* sp. | *Aspergillus* sp. | *Penicillium* sp. | *Aspergillus* sp. | *Penicillium* sp. | *Aspergillus* sp. | *Penicillium* sp. |
| 200 µL | $45 \pm 2.5^{dA}$ | $30 \pm 1.7^{efA}$ | $27 \pm 1^{fA}$ | $33 \pm 0.9^{eA}$ | $81 \pm 1.5^{bA}$ | $75.5 \pm 2.5^{cA}$ | $100 \pm 0^{aA}$ | $100 \pm 0^{aA}$ |
| 100 µL | $25 \pm 1.5^{dB}$ | $23.6 \pm 1.1^{dB}$ | $24.6 \pm B\ 0.9^{d}$ | $24.3 \pm 0.1^{dB}$ | $80 \pm 1^{bA}$ | $75.5 \pm 1^{cA}$ | $100 \pm 0^{aA}$ | $100 \pm 0^{aA}$ |
| 50 µL | $19 \pm 0.5^{eC}$ | $10.9 \pm 0.9^{fC}$ | $20.2 \pm 0.7^{deC}$ | $24 \pm 0.5^{cdB}$ | $27 \pm 2^{cB}$ | $73 \pm 1.5^{bA}$ | $72 \pm 4^{bB}$ | $81.33 \pm 1.5^{aB}$ |
| 25 µL | $10.4 \pm 1.5^{dD}$ | $0 \pm 0^{eD}$ | $0 \pm 0^{eD}$ | $19 \pm 1.2^{cC}$ | $0.3 \pm 0.5^{eC}$ | $38.7 \pm 2.7^{aB}$ | $27 \pm 1^{bC}$ | $18.4 \pm 0.6^{cC}$ |

Different small superscript letters (a–e) indicate significant differences among mean observations at same concentration within a row, whereas different capital superscript letters (A–D) indicate significant differences among mean observations at different concentrations within a column.

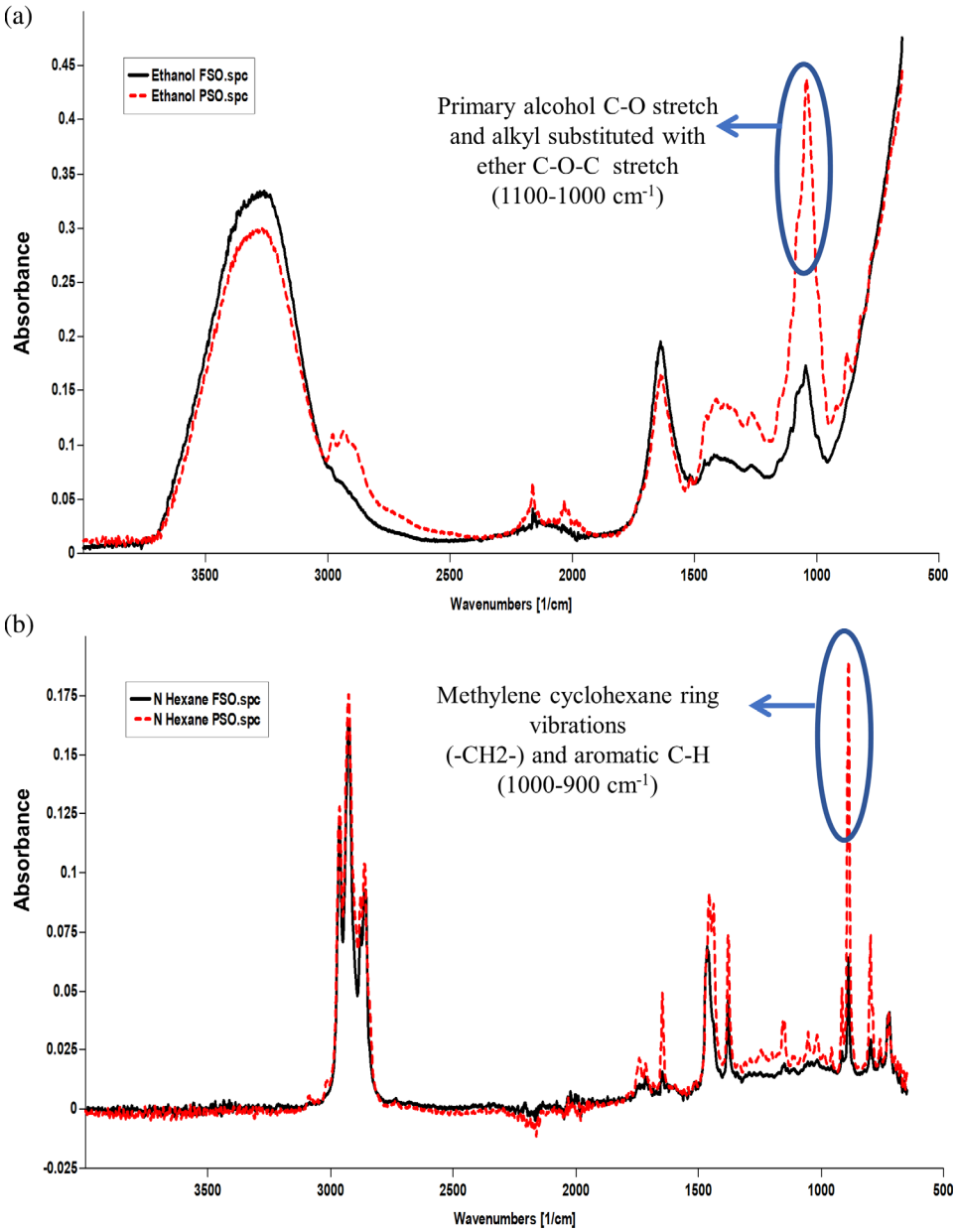


**Figure 2.** Fourier transform infrared analysis of essential oils from dried and fresh peels of sweet orange extracted by ethanol (a) and n-hexane (b), where FSO indicates fresh peels and PSO indicates dried powdered peels.

(-OH) of oil phenolics and active sites of target enzymes induces the antifungal activity of EO.[31]

Viuda-Martos *et al.*[32] reported that orange (*Citrus sinensis* L.) EO inhibited the complete growth of *Aspergillus* spp. and *Penicillium* spp. at a concentration of 9.4 mg $mL^{-1}$. Velázquez-Nuñez *et al.*[33] reported 16 mg $mL^{-1}$ MIC value of orange peel EO against *Aspergillus flavus*. The reported variations in MIC values of EO might be due to the variations in composition of EO due to different extractions methods, cultivation season, different test strains of fungi.[24,34]

### FTIR analysis

Functional groups of EOs from fresh and dried peels of sweet orange were identified by FTIR (Fig. 2 and supplementary material, Table S1). The most intense peaks were observed in the range of 3200–3600 $cm^{-1}$, which were attributed to the O—H alcohol component.[35] The second intense peak was observed in the range of 3000–2900 $cm^{-1}$, which was attributed to CH; $CH_2$ asymmetric and symmetric stretch.[36] The intense peak in the range of 1100–1000 $cm^{-1}$ was predominant in EO extracted from dried peels by ethanol (Fig. 3(a)) and it was attributed to the primary alcohol C—O stretch and alkyl substituted with ether C—O—C stretch.[37] The peak in the range of 1000–900 $cm^{-1}$ was attributed to methylene cyclohexane ring vibrations (—CH2—) and aromatic C—H and it was predominant in EO extracted from dried peels by n-hexane.[36] Peaks at 1700–1600 $cm^{-1}$ were assigned to the C═C allyl group and the C—N amino group.[38] Peaks at 1500–1400 $cm^{-1}$ were attributed to the C—H asymmetric and symmetric bend and C═C olefinic group.[37] Essential oil extracted by ethanol from dried peels showed characteristic bands of high intensity, which explained the predominant antifungal effects of EO from dried peels. After FTIR analysis, EO with predominant antifungal activity was subjected to composition analysis by GC–MS.



  

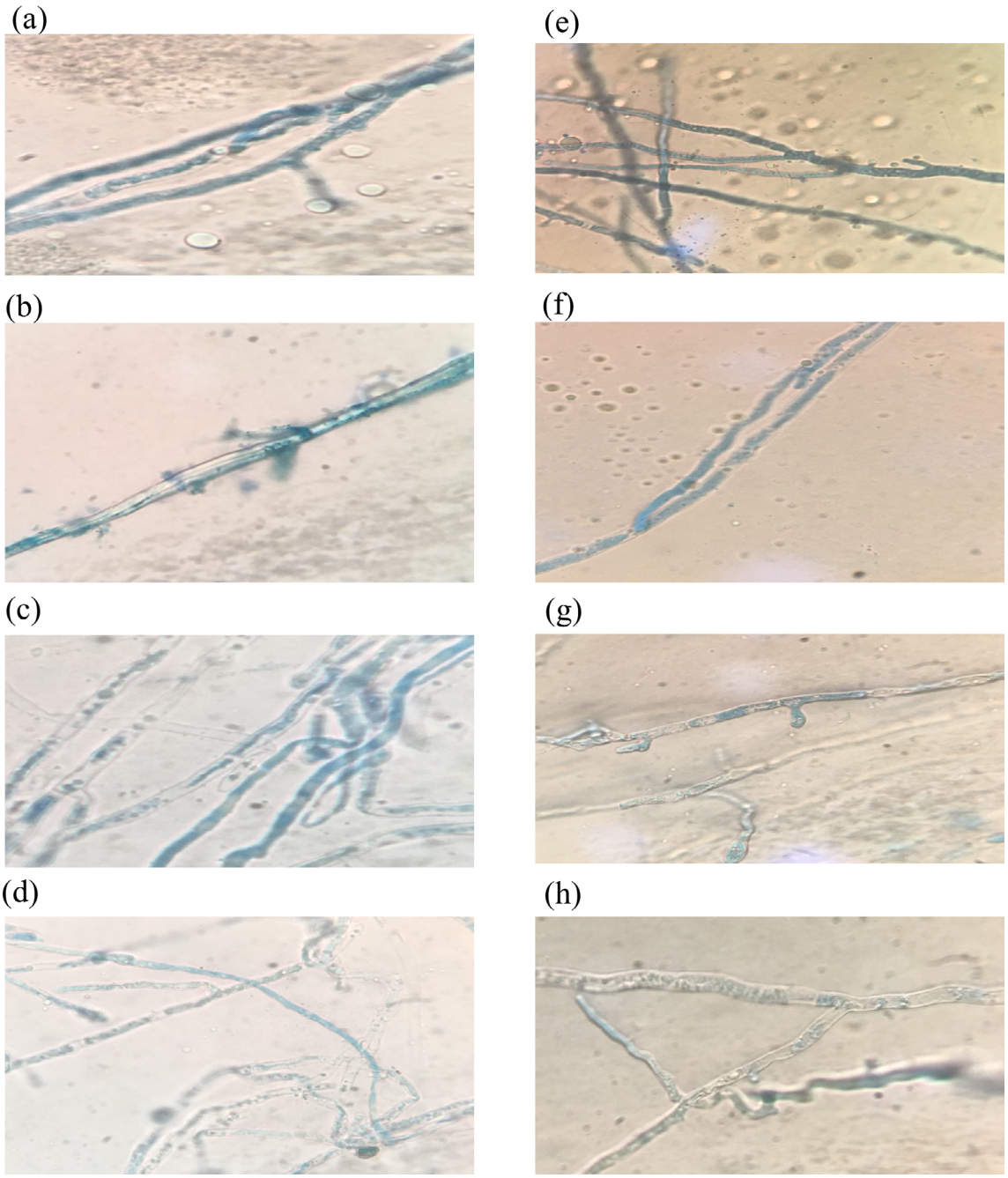


**Figure 3.** Effect of chitosan (CS), sweet orange peel essential oils (CS) and their combined mixture (CS+EO) on hyphal morphology of *Aspergillus* sp. and *Penicillium* sp.; (a) to (d) indicate *Aspergillus* hyphal treatment with control, CS, EO, and CS+EO, respectively, whereas, (e) to (h) indicate *Penicillium* hyphal treatment with control treatment, CS, EO, and CS+EO respectively.

### GC–MS analysis of essential oils

Gas chromatography–mass spectrometry analysis revealed that the EOs of sweet orange dried peel were mainly comprised of terpenes and D-limonene major components (Table 3 and supplementary Fig. S2). Other than limonene, various terpenes, and organic compounds were also detected in EO: 5-hydroxymethylfurfural, ascaridole epoxide, $\gamma$-elemene, 9-octodecen-12-ynoic acid, methyl ester, bis (2-ethylhexyl) phthalate, vitamin E, and $\beta$-sitosterol. D-Limonene was reported to exhibit chemotherapeutic activity and an anti-cancer effect.[29] Miranda *et al.*[39] reported limonene as a main component in the dry peel of sweet orange. Limonene was reported to be the main component in EO extracted from peel of mandarin orange (46.7%) and sweet lemon peel (41.79%).[40,41] Limonene was reported as a main component in citrus EO; however, various factors may contribute to variations in the limonene content such as geographical distribution, season, environment, soil type, climate, genetic variations, different varieties, extraction method, and part of the plant used.[42]

### Effect of EO on fungal hyphae

Fungal hyphae treated with EO and CS showed lesions whereas the control treatment (without EO) showed intact mycelia



  

**Table 3.** Gas chromatography–mass spectrometry analysis of essential oils extracted by ethanol from sweet orange dried peels

| Serial # | Retention time | % of total | Compounds | Molecular formula |
|---|---|---|---|---|
| 1 | 5.006 | 41.4.4 | D-Limonene | $C_{10}H_{16}$ |
| 2 | 7.734 | 4.552 | 4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl | $C_6H_8O_4$ |
| 3 | 9.354 | 6.339 | 5-Hydroxymethylfurfural | $C_6H_6O_3$ |
| 4 | 9.558 | 5.159 | Ascaridole epoxide | $C_{10}H_{16}O_3$ |
| 5 | 10.628 | 1.181 | $\gamma$- Elemene | $C_{15}H_{24}$ |
| 6 | 11.426 | 4.726 | Naphthalene, 1,2,3,4,5,6,7,8,8a-octahydro-1, 8a-dimethyl-7-(1-methylethenyl)-, [1R-(1$\alpha$, 7$\beta$, 8a$\alpha$] | $C_{15}H_{24}$ |
| 7 | 12.760 | 1.494 | Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl- | $C_{14}H_{24}O_3$ |
| 8 | 15.307 | 1.040 | 9-Octodecen-12-ynoic acid, methyl ester | $C_{19}H_{32}O_2$ |
| 9 | 16.709 | 5.161 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ |
| 10 | 18.314 | 8.999 | 9,12-Octadecadienoic acid (Z, Z)- | $C_{18}H_{32}O_2$ |
| 11 | 21.509 | 6.979 | Bis (2-ethylhexyl) phthalate | $C_{24}H_{38}O_4$ |
| 12 | 25.698 | 0.916 | Vitamin E | $C_{29}H_{50}O_2$ |
| 13 | 27.454 | 1.080 | $\beta$-Sitosterol | $C_{29}H_{50}O$ |
| 14 | 27.914 | 5.078 | 17-(1,5-Dimethylhexyl)-2, 3-dihydroxy-10, 13-dimethyl-10, 13-dimethyl-1, 2,3,7,8,9,10,11,12,13,14,15,16,17 tetra | $C_{27}H_{44}O_3$ |
| 15 | 28.366 | 5.892 | (22R)-6$\alpha$,11 $\beta$,21-trihydroxy-16$\alpha$, 17$\alpha$ propylmethylenedioxypregna-1, 4-diene-3, 20 dione | $C_{25}H_{34}O_7$ |

(Fig. 3). Hyphae treated with EO (MIC) alone showed marked lesions compared with hyphae treated with CS (%). However, microscopic observations showed that hyphae treated with combination of CS and EO showed prominent antifungal effects in comparison to EO treatment only, and hyphae were observed as pitted and colorless. The combined treatment (2% CS + MIC of EO) resulted in the breakage of the fungal chitin cell wall, which was evident from the microscopic colorless appearance of hyphae even after staining with lactophenol cotton blue mounting solution, a dye that stains chitin on the fungal cell wall and appears blue under a light microscope.[23] The antifungal effects of EO are characterized by their ability to change membrane permeability and cell-wall breakage.[43,44]

### Effect of EO on shelf life of tomatoes

The EOs of sweet orange peels inhibited the growth of *A. niger* and *P. citrinum* in artificially inoculated tomatoes (Fig. 4). In dipping treatment, the infection level by *Penicillium* sp. and *Aspergillus* sp. increased gradually in the case of the control and CS treatments as compared with the tomatoes dipped in EO and a combined mixture of EO with CS. The rate of infection level was significantly delayed when the combined treatment of EO and CS was applied. Sweet orange peel EO restricted the growth of *Aspergillus* sp. in tomatoes until the sixth day of storage, whereas a decay incidence of 66.7% and 26.7% was observed in control and CS-coated tomatoes, respectively. Tomatoes treated with combined mixture of CS + EO restricted the growth of *Aspergillus* sp. until the eighth day and decay incidence of 66.7% was observed on the ninth day of storage. Similarly, a combined mixture of CS+EO restricted the growth of *Penicillium* sp. in tomatoes and showed a decay incidence of 46.6% on the ninth day of storage. Due to their edible nature, CS and EO based coatings can be used as an alternative to synthetic preservatives to extend the shelf life of fresh tomatoes and other agriculture commodities. Chein *et al.*[24] reported that the combined mixture of CS and cinnamon EO inhibited the growth of *A. flavus* and *P. citrinum* in peanut kernels and the inhibition was significantly higher than the treatments of peanut kernels with EO and CS separately. According to another study, the combined mixture of CS and oregano EO showed high antifungal activity and affected the morphology of spores and mycelia of *R. stolonifer* and *A. niger*.[17] Chitosan coating was reported to be effective against postharvest *A. flavus* infections; furthermore, the incorporation of EO in CS coating was reported to control postharvest fungal contamination in sweet pepper, strawberry, and banana.[15,45,46] Tzortzakis *et al.*[47] reported that *Aloe vera* gel and sage EO based edible coating was able to delay the mycelial growth in fresh tomatoes and maintained the quality attributes during the 14-day storage period in comparison to uncoated tomatoes. However, the contamination symptoms were observed after 7 days of storage in tomatoes coated with *Aloe vera* and sage EO. Chitosan, beeswax, and lime EO-based edible coatings were reported to control the growth of *Escherichia coli* and *Rhizopus stolonifer* in fresh tomatoes; however, the disease incidence was observed after 4 days of storage (12 °C and 25 °C) in tomato wounds inoculated by *Rhizopus*.[48] In this study, CS and sweet orange peel EO based coating was able to restrict the growth of *Aspergillus* and *Penicillium* sp. in tomatoes until the eighth day of storage at 25 °C. Chitosan and sweet orange peel EO-based coating enhanced the



  

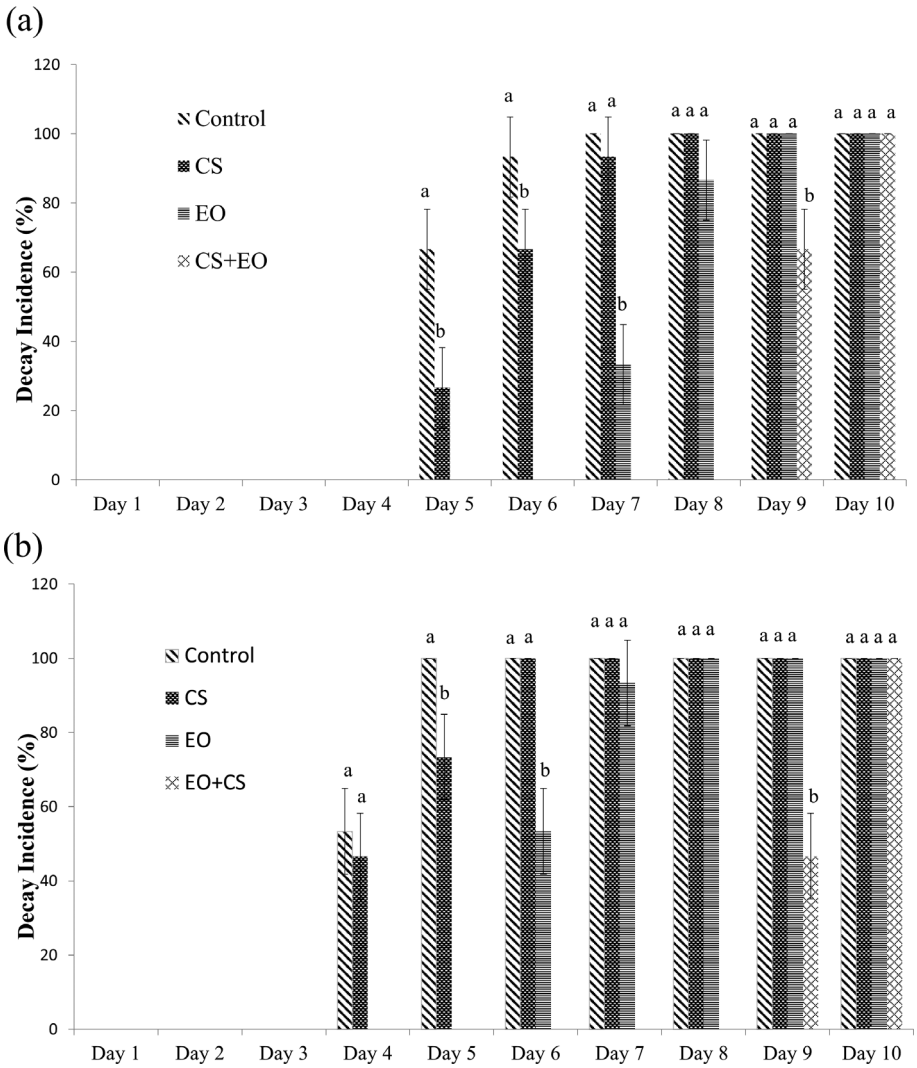


**Figure 4.** The preservation effect of essential oils (EO) and chitosan (CS)-based coatings on tomatoes inoculated with Aspergillus sp. (a) and *Penicillium* sp. (b). Different superscript letters (a–c) indicate significant differences ($P < 0.05$) among mean observations of different treatments at a given day.

shelf life of tomatoes inoculated with fungi and could be used further for the preservation of various fresh food and agriculture commodities by controlling the microbial contamination.

## CONCLUSION

Postharvest fungal contamination of food and agriculture commodities is a global concern that results in significant food loss. Sweet orange peel EO and CS-based coating showed an antifungal effect and restricted the growth of fungi in fresh tomatoes inoculated with *Aspergillus* and *Penicillium*. Due to its antifungal effect, its edible nature, and the low-cost raw material, sweet orange peel EO can be used in combination with CS to formulate a natural preservative coating to enhance the shelf life and reduce the post-harvest loss of tomatoes and other agriculture commodities.

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Ethical approval was not applicable for this research.

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## DATA AVAILABILITY STATEMENT

Data available on request from the authors.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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