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ORIGINAL ARTICLE

Antifungal effects of chitosan films incorporated with essential oils and control of fungal contamination in peanut kernels

Su Hlaing Chein[1] | Muhammad Bilal Sadiq[2] | Anil Kumar Anal[1]

[1]Department of Food, Agriculture and Bioresources, Asian Institute of Technology, Pathum Thani, Thailand

[2]School of Life Sciences, Forman Christian College (A Chartered University), Lahore, Pakistan

Correspondence
Anil Kumar Anal, Department of Food Agriculture and Bioresources, Asian Institute of Technology, Pathum Thani 12120, Thailand.
Email: anilkumar@ait.asia, anil.anal@gmail.com

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Abstract

The study investigated the effects of chitosan (CS) combined with essential oils (EOs) in controlling the fungal contamination in peanut kernels. The antifungal activities of CS and EOs were evaluated against *Aspergillus flavus* and *Penicillium citrinum*. CS (2%, w/v in 1% v/v acetic acid) packaging films were formulated by incorporating different EOs (4%) separately, that is, thyme (TEOs), cinnamon (CEOs), and lemongrass (LEOs), respectively. CEOs showed lowest minimum inhibitory concentration (MIC) of 40 μl/ml against *A. flavus* and *P. citrinum*. CS films incorporated with CEOs showed high tensile strength and smooth morphology with less fissures in comparison to films incorporated with TEOs and LEOs. CEO-based CS films showed complete inhibition of fungal growth at 28°C and 5°C for 24 days. The combination of CS and CEOs coating restricted the *A. flavus* and *P. citrinum* contamination to 9.8% and 13.4%, respectively, in artificially inoculated peanut kernels at 28°C for 14 days of storage. CS can be used in combination with EOs to control postharvest fungal contamination in peanuts.

Practical applications

CS are well known for the formulation of food packaging films; however, antifungal activity of CS is limited. This study explains the antifungal effects of CS and EOs. The combination of CS and EO can be used to reduce the concentration of EOs as antifungal agents which otherwise might affect the organoleptic attributes of food. The CS films incorporated with EOs are possible to use for shelf life extension and prevention of postharvest fungal contamination of agriculture commodities.

1 | INTRODUCTION

Fungal deterioration of seeds and grains is a main problem in the postharvest storage system (Ekwomadu, Gopane, & Mwanza, 2018; Norlia, Jinap, Nor-Khaizura, Son, & Chin, 2018). Harvested seeds are colonized by various species of fungi, under conditions leading to deterioration and mycotoxin production (Kumari, Jayachandran, & Ghosh, 2019). Peanuts (*Arachis hypogaea*) are one of the most important food and oilseed crops cultivated and utilized in most parts of the world. Peanuts are widely accepted as excellent source of nutrition due to their high protein content, carbohydrates, fatty acids, dietary fibers, vitamins, and minerals (Nakai et al., 2008). The peanut seeds have several applications such as formulation of peanut butter, oil, and other products (Yaw, Richard, Osei, Seth, & Adelaide, 2008). The structure and nutritional composition of peanuts allow the growth of several fungal species, contaminating the crops and seeds during harvesting and storage (Mutegi, Ngugi, Hendriks, & Jones, 2009). The fungal contamination of peanuts can occur during any stage of harvesting and storage. The presence of *Aspergillus flavus* and *Penicillium citrinum* was frequently reported in storage grains (Riba et al., 2010; Roige et al., 2009). Norlia et al. (2018) reported that in *Aspergillus* section *Flavi*, *A. flavus* was dominant species in the contamination of peanuts. Previously it was reported that *Penicillium* spp. and *Aspergillus* spp. were predominant in the contamination of

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peanuts (Guezlane-tebibel, Bouras, Mokrane, Benayad, & Mathieu, 2013).

To overcome the fungal contamination of food in developing countries, the usual practice is to fumigate or treat the stored commodities using different synthetic chemical preservatives. Most of the synthetic antimicrobials are not easily bio-transformed into simpler forms and remain in the food chain for longer periods, causing toxic effects to consumers even in residual concentrations (Moosavy, Basti, & Ali, 2008). The various synthetic preservatives that were previously used to control fungal contamination are now banned due to associated health hazards. Methyl bromide was previously used for control of fungal spores in stored food commodities but it was banned in 2005. Due to increased consumer awareness and food safety preferences, the current postharvest preservation technologies rely on the natural antimicrobials, biodegradable films, microwave heating, ozonation, and modified atmospheric packaging (Aloui et al., 2014).

Essential oils (EOs) are utilized as natural food preservatives and their usage complies with consumers' expectations, due to natural origin and generally recognized as safe status (Aloui et al., 2014; Arrebola, Sivakumar, Bacigalupo, & Korsten, 2010; Hromis et al., 2016). Application of EOs being natural is considered a safe treatment for the control of postharvest decay of fresh produce (Sivakumar & Bautista-Banos, 2014). However, there are possible negative organoleptic effects of EOs, once applied on fresh produce, especially at higher doses for antimicrobial effect (Hyldgaard, Mygind, & Meyer, 2012). To overcome this issue, various studies have investigated the synergistic effects of EOs with other antimicrobial compounds to lower the required concentration of EOs (Sriwattanachai, Sadiq, & Anal, 2018). Cinnamon, thyme, and lemongrass EOs are effective in controlling the growth of *Aspergillus* spp. and *Penicillium* spp. in food commodities (Antunes & Cavaco, 2010). Thyme, cinnamon, and lemongrass EOs are frequently used as natural antimicrobials in food for controlling fungal contamination (Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011). Chitosan (CS) films incorporated with thyme and cinnamon EOs were used as antimicrobial food packaging (Hosseini, Razavi, & Mousavi, 2009).

CS, a linear polysaccharide derived from chitin deacetylation, is considered a nontoxic, biodegradable product with antimicrobial properties (Badawy & Rabea, 2009; Sathivel, Liu, Huang, & Prinyawiwatkul, 2007). Films obtained from a CS dispersion, mixed with other active substances, have been used as a coating material to inhibit the growth of microorganisms in foods (Wang et al., 2011). The incorporation of EOs in CS coating is facilitated by emulsifying properties of CS, which permits the homogeneous distribution of EO droplets in the system, enabling the formation of a thin and translucent coating (Sánchez-Gonzáles, Chafer, Chiralt, & González-Martínez, 2010). CS is a preferred material for edible coating because it is a biodegradable cationic hydrocolloid and possesses antifungal activity in addition to its film-forming ability (Elsabee & Abdou, 2013; Shao et al., 2015).

The present study evaluated the efficacy of the combined application of CS with different EOs separately, (thyme white oil, lemongrass oil and cinnamon bark oil) to inhibit the postharvest fungal pathogens, *A. flavus* and *P. citrinum* in peanut kernels. The in situ antifungal activity of CS and EOs was observed in peanut kernels during storage at room temperature and cold storage.

2 | MATERIALS AND METHODS

2.1 | Materials

CS of low molecular weight (deacetylation degree) was obtained from Sigma-Aldrich Co. (St. Louis, USA). *A. flavus* TISTR 3041 and *P. citrinum* TISTR 3437 were acquired from Thailand Institute of Scientific and Technological Research (TISTR), Bangkok, Thailand. EOs of cinnamon bark (*Cinnamomum zeylanicum*), lemongrass (*Cymbopogon citratus* L.), and thyme white (*Thymus vulgaris* L.), extracted by hydro-distillation, were provided by Botanic-Essence, Thailand. The major components of EOs provided by Botanic-Essence, Thailand were reported as follows: thyme EO [p-Cymene (26.32%), Thymol (21.31%), γ-Terpinene (19.50%), Linalool (3.02%), Myrcene (2.56%), Terpinen-4-ol (1.78%), and Carvacrol (1.10%)]; lemongrass EO [Geranial (47.46%), Neral (33.34%), Geraniol (5.30%), Citronellal (2.53%), Mycrene (1.10%), Linalool (1.07%), Geranyl acetate (.85%), and Limonene (.35%)]; and cinnamon bark EO [(e)-Cinnamaldehyde (44.46%), Eugenyl acetate (12.94%), Carryophellene (4.18%), Limonene (3.12%), Linalool (3.09%), alpha-cymene (1.67%), Para cymene (1.37%), alpha-Terpinene (.88%), Camphene (.97%), and beta-Pinene (.68%)].

2.2 | Antifungal activity of EOs and CS

The antifungal activities of EOs and CS were estimated by the radial growth inhibition assay (Tian et al., 2011). Potato dextrose agar (PDA, Himedia, India) containing different concentrations of CS (1, 2 and 3%, w/v) and EOs (2.5, 5, 10, 20, 40, and 80 μl/ml) were prepared separately, followed by the addition of Tween 80 (2%, v/v) as emulsifier. The media was inoculated at the center with fungal plug (5 mm), containing actively growing mycelia of *A. flavus* and *P. citrinum*. After sealing with parafilm petri dishes were incubated at 30°C for 7 days in dark. PDA, containing 2% tween 80 without the addition of EOs or CS, was used as a control. The lowest concentration of EOs that showed no visible fungal growth after 7 days was marked as the minimum inhibitory concentration (MIC); the antifungal activities of other concentrations are expressed by following Equation 1 (Tao, Jia, & Zhou, 2014).

$$\text{Inhibition } (\%) = \left[\frac{\text{Dc} - \text{Dt}}{\text{Dc}}\right] \times 10 \tag{1}$$

Dc and Dt indicate the diameters of control and sample, respectively.

2.3 | Effects of CS and EOs on fungal hyphae

The effects of CS, Eos, and combination of CS with EOs on fungal hyphae were determined by the method described by Sriwattanachai

et al. (2018) with slight modifications. Potato dextrose broth (PDB, Himedia, India) 20 ml, containing 2% of Tween 80, was inoculated with fungal spores (10^4) and incubated at 30°C, 180 rpm for 48 hr in an incubator (N-Biotek, South Korea). The hyphae were harvested by centrifugation (Centrikon T-324, Germany) at 4,500× g for 5 min. The fungal cells were further washed twice with 5 ml of phosphate buffer saline (PBS, pH 7.4). The cells were harvested by centrifugation at 4,500× g for 5 min and re-suspended separately in 20 ml of PBS containing 2% Tween 80 (control), with 2% CS, 4% of EOs (separately with EOs of thyme white, lemongrass, and cinnamon bark), a combined mixture of CS (2%) with 4% of each EO. The samples were incubated at 25°C for 24 hr followed by staining of hyphae with lactophenol-cotton blue mounting solution and observed under a light microscope (Olympus, Tokyo, Japan).

2.4 | CS film incorporated with EOs

The CS films incorporated with EOs were prepared by following the method of Wang et al. (2011) with slight modifications. The film-forming solution (FFS) was prepared by dissolving the CS (2%, w/v) in acetic acid solution (1%, v/v), followed by continuously stirring for 24 hr at 25°C. The mixtures were then added with glycerol (1%, v/v) as plasticizer. Before the incorporation of the EOs, CS solution was emulsified with Tween 80 (2% v/v). CS (2%) films enriched with EOs were prepared by adding the different concentrations of each EOs (.25% .5%, 1%, 2%, and 4%, v/v) to the FFS separately and CS film without EOs was used as the control. The FFS was subjected to ultrasonication for removal of air bubbles followed by casting on Plexiglas plates (8 × 8 cm) and dried at 25°C and 50% relative humidity for 48 hr in a humidity chamber (Ningbo Southeast Instrument Co., Ltd., Zhejiang, China). All the films were carefully peeled off and placed in a desiccator at 25°C and 55% RH for 48 hr for further experiments.

2.5 | Film Characterization

2.5.1 | Thickness and tensile strength

Film thickness was measured by micrometer NSK, YAB)2-M (Japan). The mechanical properties of the films were determined as described by Wang et al. (2011) using texture analyzer (TA-XT plus, Stable Microsystems, UK). The tensile strength was calculated by subjecting the films to 10 N load cell at a speed of 50 mm/min.

2.5.2 | Solubility

The solubility of the films was determined by a method described by Zhong, Song, and Li (2011) with some modification. The films were cut into pieces (3 × 3 cm) and dried at 105°C for 24 hr in an oven to get the initial dry mass (m_1) The films were places in 30 ml of distilled water containing beaker covered with plastic wraps and stored at 25°C for 24 hr. The film pieces were taken out and dried again at 105°C for 24 hr to determine the final dry mass (m_2). The solubility was estimated by using following Equation 2:

$$\text{Film Solubility} = \frac{m_1 - m_2}{m_1} \times 100 \quad (2)$$

2.5.3 | Water vapor permeability (WVP)

The WVP of films was determined by measuring mass changes of Fisher/Payne permeability cups (Fisher Scientific, Pittsburgh, PA) during incubation at room temperature (25°C). Cups were filled with 5.0 g deionized water, sealed with films, and placed in a desiccator with 57% RH. Water vapor permeation ratio (WVPR) was calculated by Equation 3, based on the mass loss (*m*), time (*t*), and effective film area (*A*), whereas WVP was determined using the Equation 4 (Pelissari, Grossmann, Yamashita, & Pineda, 2009).

$$\text{WVPR} = \frac{m}{t \times A} \quad (3)$$

$$\text{WVP} = \frac{\text{WVPR} \times \text{Film thickness}}{\text{sp} \times (\text{RH}_1 - \text{RH}_2)} \quad (4)$$

where sp is the water vapor saturation pressure (Pa), RH1 and RH2 indicate relative humidity inside (100%) and outside (57%) the cup, respectively.

2.5.4 | Color measurement

The color attributes of films were determined by colorimeter (Color Flex, Hunter Lab Reston, VA, USA) method as describe by Ojagh, Rezaei, Razavi, and Hosseini (2010) with slightly modifications. The total color difference (ΔE) was calculated by the Equation 5.

$$\Delta E = \left((L*)^2 + (a*)^2 + (b*)^2 \right)^{1/2} \quad (5)$$

where chromaticity parameters were indicated by *L* = lightness, *a* = red-green, and *b* = yellow-blue.

2.5.5 | Scanning electron microscopy

Microstructural of films was observed by scanning electron microscopy (SEM) (Hitachi SU 8030, Japan). The films were cut and mounted on copper stubs followed by coating with gold. The films were then observed for microstructure at an accelerating voltage of 1.00 kv.

2.5.6 | Fourier transform infrared spectroscopy (FT-IR)

The spectra of all films were recorded by spectrum one FT-IR spectrometer (Perkin Elmer) at wavenumber range of 4,000–6,00 cm^{-1} at resolution of 4 cm^{-1}. The sample was ground to fine powder with potassium bromide (KBr) and the mixture was subjected to compression die before FTIR analysis (Sadiq, Hanpithakpong, Tarning, & Anal, 2015).

2.6 | Antifungal activity of CS films incorporated with EOs in peanut conservation

The CS (2%) films incorporated with 4% EOs (separately with EOs of thyme white, lemongrass and cinnamon bark) were evaluated for the preservation of peanut kernels against the fungal decay. The peanut kernels (n = 30) were packed in CS films (8 × 8 cm) incorporated with EOs by following the method of Iamareerat, Singh, Sadiq, & Anal, 2018 with slight modifications. The CS films without EOs were used as control. EOs (4%) alone were used for dipping (1 min) of peanut kernels to compare with film storage by following the method of Feliziani, Santini, Landi, and Romanazzi (2013). The peanut kernels were selected based on uniformity in size, color, and absence of deformity and treated with above-mentioned treatments. Two temperatures, 28 ± 2°C and 5 ± 2°C, were used to store the peanut kernels for 24 days under controlled relative humidity (RH, 70%–75%). The infected peanut kernels was calculated as the number of contaminated kernels out of the total number of kernels per treatment.

Furthermore, peanut kernels (200 g) previously washed with sodium hypochlorite (.4%) were inoculated separately by dipping for 1 min into conidial suspensions of *A. flavus* and *P. citrinum* at a concentration of 10^4 conidia/ml. After inoculation, the peanut kernels were dried for 1.5 hr at 25°C and then immersed in different FFSs incorporated with EOs and EOs alone as mentioned above. Five replicates of 30 peanut kernels per treatment were placed into the petri dishes. The treated peanut kernels were incubated at 28 ± 2°C for 14 days in an incubator (Memmert, Buchenbach, Germany), while the relative humidity was maintained at 70%–75%. The incidence was expressed as the number of contaminated kernels out of the total number of kernels per treatment (Aloui et al., 2014).

2.7 | Statistical analysis

One-way analysis of variance (ANOVA) and LSD tests were used to find the significant differences among treatments ($p < .05$) using SPSS statistical software package (SPSS, version 23.0, USA).

3 | RESULTS AND DISCUSSION

3.1 | Antifungal activity of EOs and CS

All the test EOs showed concentration-dependent antifungal effects against *A. flavus* and *P. citrinum* (Table 1). Thyme and lemon grass EOs showed no visible growth of fungi at 80 µl/ml; therefore, 80 µl/ml was estimated as MIC of thyme and lemon grass EOs against *A. flavus* and *P. citrinum*. MIC of cinnamon EOs was found 40 µl/ml as there was no visible growth of test fungi at 40 µl/ml after 7 days of incubation. There was a significant ($p < .05$) increase in the inhibition of fungi growth with increase in the concentration of EOs. The antifungal effects of CS also increased with increase in concentration; however, there was no significant difference ($p < .05$) in inhibition at 2 and 3% of CS. The CS at 2 and 3% showed 12.53 and 13.65% *A. flavus* inhibition and 11.90 and 12.54% *P. citrinum* inhibition, respectively.

Šegvić Klarić, Kosalec, Mastelić, Pieckova, and Pepeljnak (2007) reported the MIC values of thyme EOs as 9.85 and 19.17 µl/ml against *Aspergillus* spp and *Penicillium* spp., respectively. Sriwattanachai et

TABLE 1 Antifungal activities of EOs and CS

Sample	Concentration (µl/ml)	Percent inhibition (%)	
		Aspergillus flavus	*Penicillium citrinum*
CS	10	9.16 ± 1.28^a	8.99 ± 1.22^a
	20	12.53 ± 2.16^b	11.90 ± 1.15^b
	30	13.65 ± 3.23^b	12.54 ± 2.53^b
Thyme EOs	10	18.28 ± 3.81^a	19.98 ± 3.59^a
	20	50.47 ± 1.19^b	53.80 ± 1.43^b
	40	72.12 ± 3.44^c	68.58 ± 2.85^c
	80	100.00 ± 0.00^c	100.00 ± 0.00^d
Lemongrass EOs	10	21.99 ± 1.66^a	21.15 ± 1.67^a
	20	52.39 ± 2.59^b	50.39 ± 2.43^b
	40	91.23 ± 4.12^c	92.17 ± 4.31^c
	80	100.00 ± 0.00^c	100.00 ± 0.00^c
Cinnamon bark EOs	2.5	29.30 ± 2.62^a	33.80 ± 1.47^a
	5	59.20 ± 1.97^b	60.20 ± 3.16^b
	10	90.10 ± 3.21^c	87.70 ± 2.37^c
	20	95.10 ± 4.00^c	95.80 ± 4.00^c
	40	100.00 ± 0.00^c	100.00 ± 0.00^c

Note: Different superscript letters (a–d) within a column indicate significant differences ($p < .05$) among mean observations.

al. (2018) reported the MIC value of thyme EOs as 40 μl/ml against *Penicillium* spp. Ma-in, Aran, and Phongpaichit (2014) reported the MIC values of cinnamon EOs in the range of 2.5–10 μl/ml against *Aspergillus* and *Penicillium* spp. The significant differences between the observed and previously reported MIC of EOs might be due to various factors such as the difference in major components EOs based on extraction technique, cultivation conditions, and different test strain of fungi (Calo, Crandall, O'Bryan, & Ricke, 2015). Pawar and Thaker (2006), reported that antifungal effects of thyme and cinnamon EOs are due to their major constituents of carvacrol and cinnamaldehyde, respectively.

3.2 | Effects of CS and EOs on fungal hyphae

Figure 1 shows the effect of different treatments of EOs (4%) and CS (2%) on *A. flavus*. The control treatment (Figure 1a) showed intact mycelia, then the treatments with EOs and CS. The hyphae treated with EOs (4%) alone showed marked lesions and stained lighter blue than those treat with CS (2%) alone, (Figure 1b–e). Microscopic observations showed coagulation in the fungal cytoplasm characterized by the combination of CS (2%) with EOs (4%) (Figure 1f–h). The cinnamon EOs (4%) alone and in combination with CS (2%) showed prominent antifungal effects compared with other EOs. The prominent antifungal effects of cinnamon EOs were in accordance with the MIC estimation (40 μl/ml), which was found to be lower than other EOs.

The effect of EOs resulted in cytoplasm depletion, lack of integrity, and ultimately mycelial death due to the destruction of cell wall structure (Hua et al., 2014; Tripathi, Sharma, & Sharma, 2009; Xing et al., 2014). Wang et al. (2018) reported that hyphae of *A. ochraceus* showed structural alterations after treatment with cinnamaldehyde in comparison to control treatment. However, the combined effect of EOs and CS indicated higher intensity of antifungal response that was clearly evident from the microscopic examination. Similar results were reported by Sriwattanachai et al. (2018) who studied the effect of combined mixture of thyme EOs and *Lactobacillus* cell-free supernatant on *Penicillium* spp. hyphae and chitin degradation in fungal cell wall. The antifungal effects of CS were reported due to membrane permeabilization and morphological changes on fungal hyphae (Romanazzi, Feliziani, Baños, & Sivakumar, 2015).

3.3 | CS films incorporated with EOs

3.3.1 | Physical properties of film

The physical properties of CS films incorporated with EOs are summarized in Table 2. Thickness of the films varied between .05 and

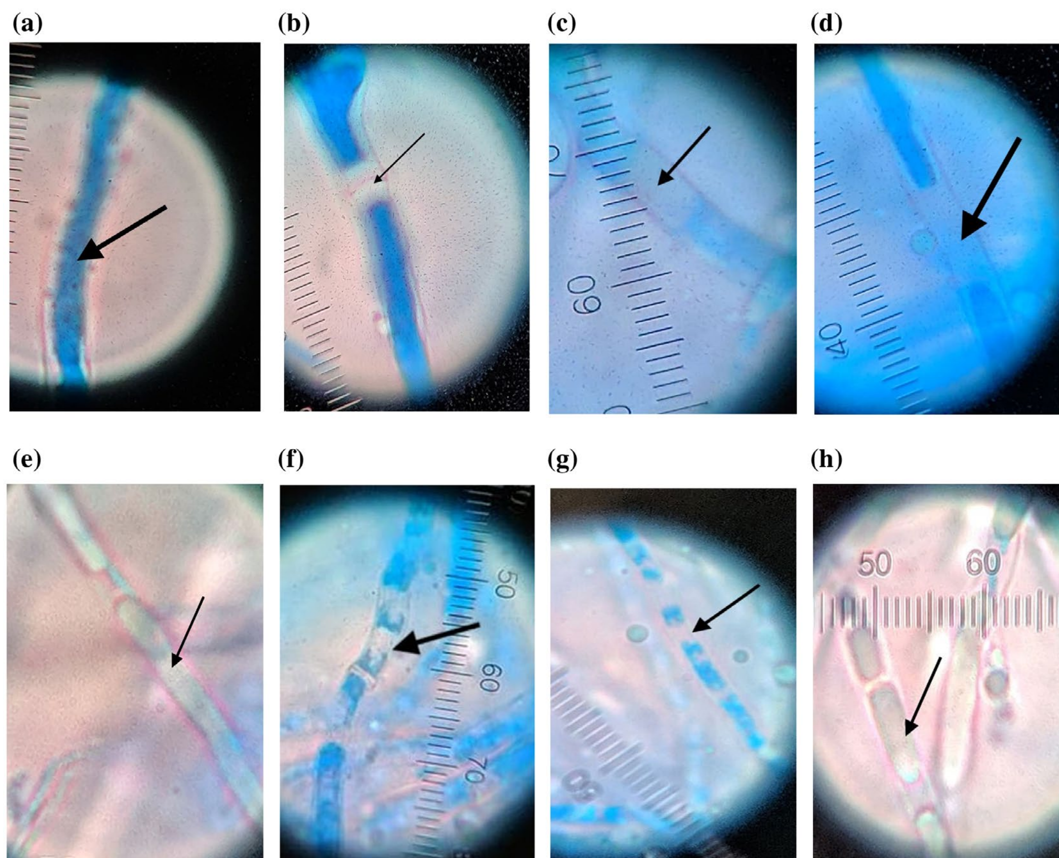

FIGURE 1 Morphological changes in *Aspergillus flavus's* hyphae after the treatment: Control (a), 2% of CS (b), 4% of thyme EOs (c), 4% of lemongrass EOs (d), 4% of cinnamon EOs (e), combined mixture of 2% of CS with 4% of thyme EOs (f), 2% of CS with 4% of lemongrass (g), and 2% of CS with 4% of cinnamon EOs (h)

TABLE 2 Physical and mechanical properties of the films

Film	Thickness (mm)	Solubility (%)	WVP (10^{-9} g/m.s.Pa)	Tensile strength (Mpa)
2% CS	0.05 ± 0.01^{a}	13.99 ± 0.09^{a}	2.04 ± 0.11^{a}	2.93 ± 0.22^{a}
2% CS + 4% TEOs	0.06 ± 0.02^{a}	9.84 ± 0.07^{b}	0.97 ± 0.04^{c}	1.25 ± 0.28^{c}
2% CS + 4% LEOs	0.07 ± 0.01^{a}	3.59 ± 0.04^{d}	1.09 ± 0.18^{c}	1.84 ± 0.35^{b}
2% CS + 4% CEOs	0.06 ± 0.01^{a}	5.49 ± 0.05^{c}	1.61 ± 0.03^{b}	2.14 ± 0.27^{b}

Note: Different superscript letters (a–d) within a column indicate significant differences ($p < .05$) among mean observations.
Abbreviations: CEOs, cinnamon essential oil; CS, chitosan; LEOs, lemongrass essential oil; TEOs, thyme essential oil.

TABLE 3 Color measurement of the films

Color	L^*	a^*	b^*	ΔE
CHs	46.31 ± 0.36^{a}	1.08 ± 0.23^{a}	0.43 ± 0.16^{a}	47.03 ± 0.37^{a}
2% CHs + 4% TEO	35.70 ± 0.77^{b}	0.65 ± 0.31^{ab}	1.96 ± 0.42^{b}	57.63 ± 0.76^{b}
2% CHs + 4% LEO	33.60 ± 0.11^{c}	0.57 ± 0.26^{ab}	2.13 ± 0.24^{b}	59.75 ± 0.10^{c}
2% CHs + 4% CEO	31.69 ± 0.09^{d}	0.72 ± 0.20^{b}	4.42 ± 0.27^{c}	61.72 ± 0.10^{d}

Note: Different superscript letters (a–d) within a column indicate significant differences ($p < .05$) among mean observations.

.07 mm; however, there was no significant difference ($p < .05$) in thickness of CS films incorporated with EOs. WVP of control films was significantly ($p < .05$) decreased with the incorporation of EOs. WVP of CS film was decreased from 2.042 to .97 (10^{-9} gm^{-1} s^{-1} Pa^{-1}) after incorporation of thyme EOs. WVP of CS films incorporated with cinnamon EOs was significantly higher than the films incorporated with thyme and lemongrass EOs. EOs as lipid compounds are known to enhance the water barrier properties of polymer-based films by interfering with the hydrophilic/hydrophobic characteristics of the films because of their hydrophobic nature (Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2009). The solubility of CS film was significantly decreased from 13.99 to lowest value of 3.59% after the addition lemongrass EOs. The solubility of films is associated with water diffusion, ionization of amino or carboxyl groups, dissociation of hydrogen and ionic bonds, and polymer relaxation (Mathew, Brahmakumar, & Abraham, 2006). The decrease in solubility could be attributed to the increasing cross-linking interactions between CS and hydrophobic EOs.

The addition of EOs significantly ($p < .05$) decreased tensile strength of CS films from 2.93 to lowest value of 1.25 Mpa after incorporation of thyme EOs. However, tensile strength of CS films incorporated with cinnamon (2.14 Mpa) and lemongrass (1.84 Mpa) EOs was significantly higher than the CS films incorporated with thyme EOs. The mechanical characteristics of films depend on the nature of components present in the composite films (Vieira, Silva, Santos, & Beppu, 2011). The variations in the tensile strength of biopolymeric films are linked with structural configuration of hydrophobic phase in the film matrix. The incorporation of EOs results in structural discontinuities that could explain the variation in tensile strength (Chen & Liu, 2016). The nature and composition of EOs can variably influence the tensile strength of the films.

Similar results were previously reported by various studies indicating that addition of EOs decreased the tensile strength of CS films and CS-cassava starch composite films (Pelissari et al., 2009; Sriwattanachai et al., 2018). Sánchez-Gonzáles et al. (2010) reported that the tensile strength of CS film decreased after incorporation of bergamot EOs. However, Ojagh et al. (2010) reported an increase in tensile strength after introducing cinnamon EOs into CS films. Tensile strength is usually related to the film network microstructure and the intermolecular force (Atarés, Bonilla, & Chiralt, 2010).

3.3.2 | Color attributes of films

Color of the film is important parameter in terms of general appearance and consumer preferences. The rectangular coordinates (L^*, a^* and b^*) and total color difference (ΔE) of CS films incorporated with EOs are presented in Table 3. The addition of EOs resulted in decreased L^* (lightness) value of CS films significantly ($p < .05$). The total color difference (ΔE) could be ascribed to the natural yellow color of EOs (Atarés et al., 2010). The difference in color of the film was related to the nature of the EOs and the internal structure developed during film drying (Villalobos, Chanona, Hernández, Gutiérrez, & Chiralt, 2005).

3.3.3 | SEM analysis of the films

The microstructure of CS films was influenced by the structural arrangement of the different components in the matrix. The addition of EOs increased the roughness of the cross-section matrix of CS films. This implied that flocculation and coalescence occurred during film drying. Characteristic SEM images of surface and cross-sections

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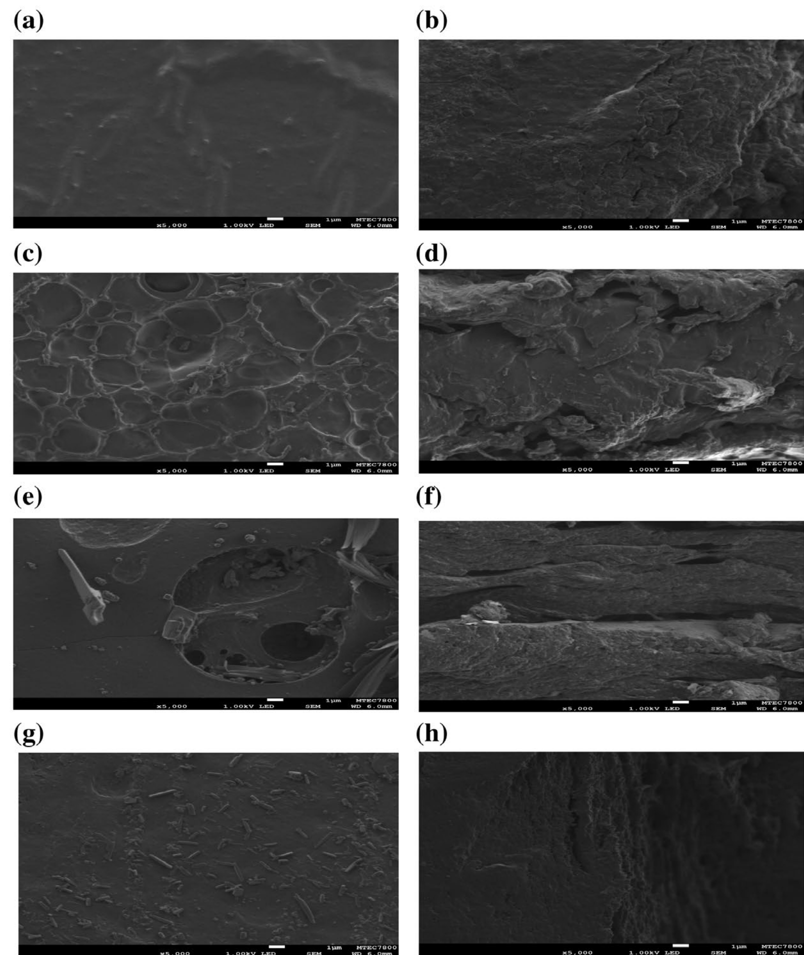

FIGURE 2 SEM images of CS–EOs films. (a) and (b) surface and cross-section morphology of CS film (2%), respectively, (c) and (d) surface and cross-section morphology of CS-thyme EOs film, (e) and (f) surface and cross-section morphology of CS-lemongrass EOs film, (g) and (h) surface and cross-section morphology of CS-cinnamon EOs film

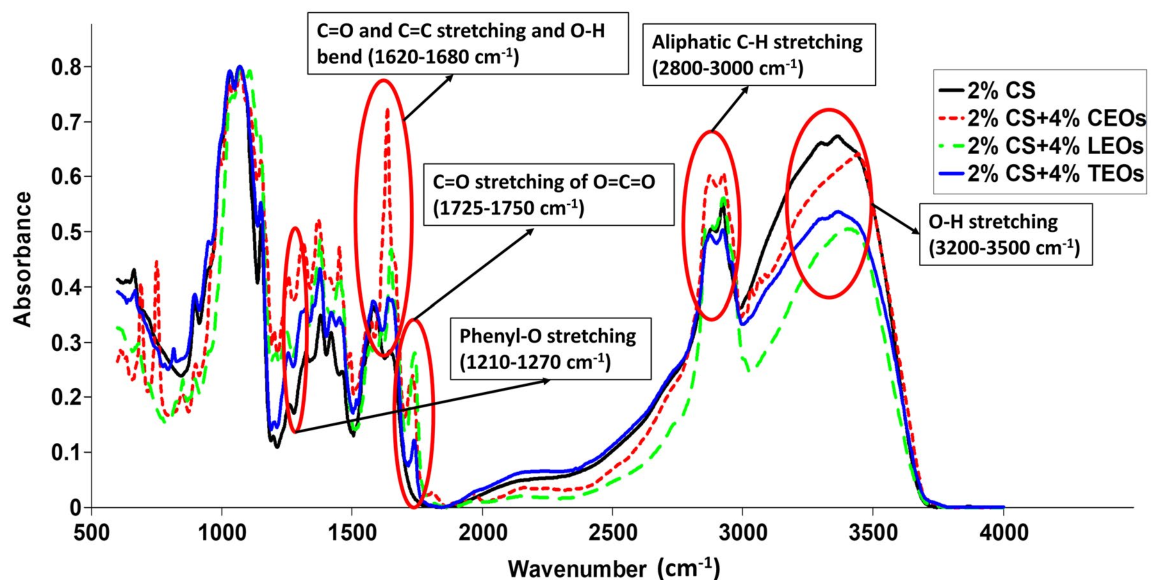

FIGURE 3 FT-IR spectra of CS films incorporated with essential oils. CEO, cinnamon essential oil; CS, chitosan; LEO, lemongrass essential oil; TEO, thyme essential oil

of different films are shown in Figure 2. The CS (2%) film showed a smooth and plane surface without any discontinuities. The uniformity of the film was compromised and a comparatively nonuniform surface morphology was observed in all CS-EOs films. The cinnamon EO-based CS films showed comparatively smooth and morphology with less fissures compared to thyme and lemongrass-based films. Similar results were observed by Sánchez-González et al. (2009) when tea tree EO was added to the hydroxypropyl methylcellulose film.

3.3.4 | FTIR of CS films incorporated with EOs

The characteristic differences in the absorption peaks of CS films incorporated with EOs are presented in Figure 3 and Table S1. The broad brands in the range of 3,200–3,500 cm^{-1} were attributed to O–H stretching of hydroxyl group. The sharp bands at 2,800–3,000 cm^{-1} were assigned to aliphatic C–H stretching (Sadiq et al., 2015). The characteristic bands in the ranges of 1,725–1,750 cm^{-1}, 1,620–1,680 cm^{-1}, and 1,210–1,270 cm^{-1} were assigned to C=O stretching of O=C=O, olefinic C=C stretching, and phenyl-O

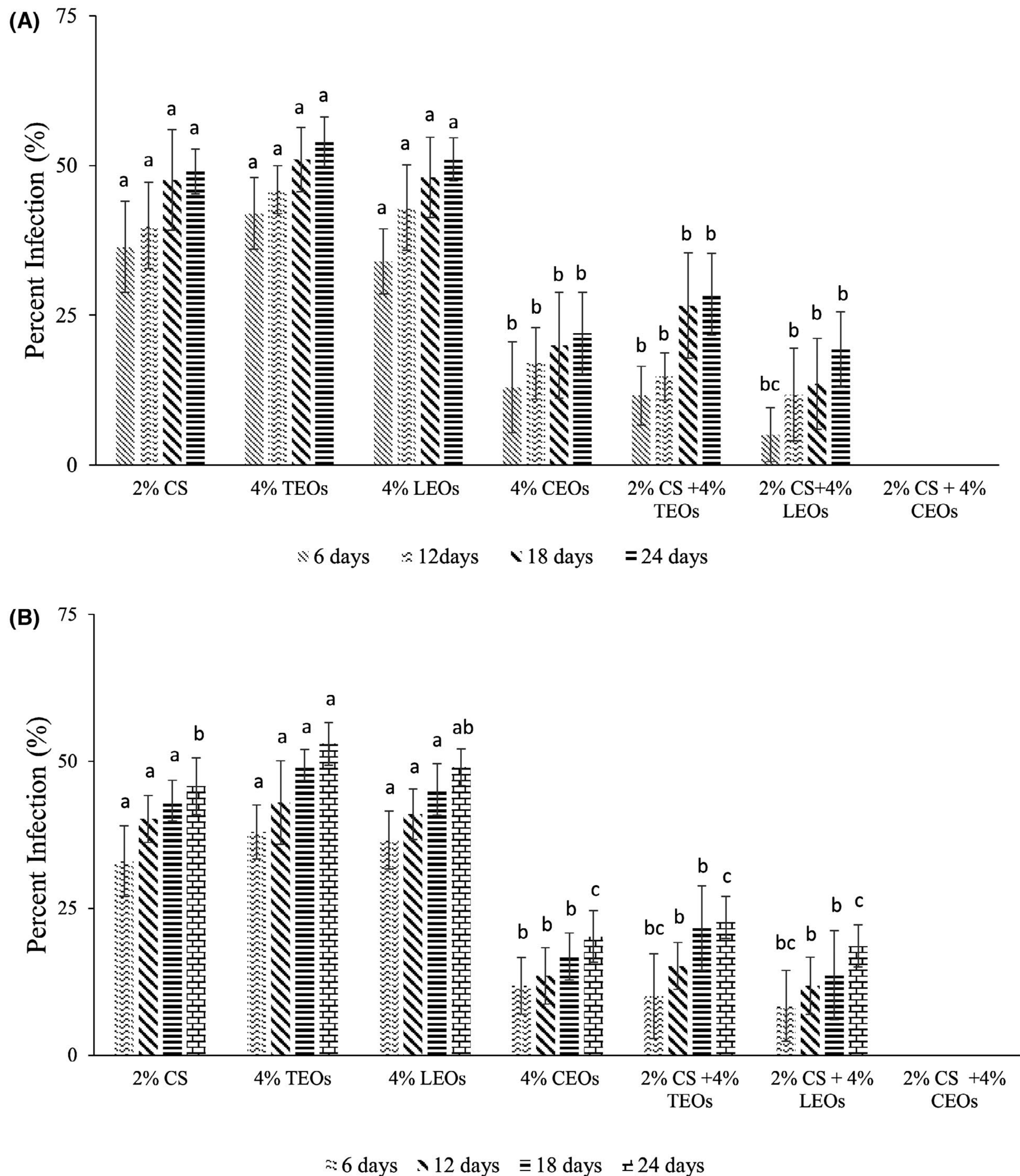

FIGURE 4 Effect of CS films and essential oils in inhibiting the fungal infection in peanut kernels stored at 28 ± 2°C (A) and 5 ± 2°C (B). 2% CS, CS film; 2% CS + CEOs, CS films incorporated cinnamon essential oils; 2% CS + 4% LEOs, CS films incorporated lemongrass essential oils; 2% CS + 4% TEOs, CS films incorporated thyme essential oils; CEOs, cinnamon essential oils; LEOs, lemongrass essential oil; TEOs, thyme essential oil. Different letters (a–c) above the bars indicate significant differences ($p < .05$) among different treatment groups at each time interval

stretching, respectively. The films incorporated with cinnamon EOs showed sharp bands compared to other CS films. Moreover, the shift in the peaks of CS film after incorporation of EOs demonstrates the existence of interactions among the chemical constituents of the film-forming solution (Wen et al., 2016).

3.4 | Antifungal effects of CS films incorporated with EOs in peanut conservation

The peanut kernels stored in 2% CS films showed higher contamination index as compared to storage in 2% CS films incorporated with 4% EOs (separately with EOs of thyme white, lemongrass, and cinnamon bark) (Figure 4a,b). The results indicated that the percentage of infected peanut kernels was significantly ($p < .05$) reduced by incorporation of EOs into 2% CS film at both storage temperatures, that is, 28 ± 2°C and 5 ± 2°C. The peanut kernels treated with 4% of TEOs and LEOs (dipping technique) showed significantly ($p < .05$) higher % of infected kernels compared with the peanut kernels treated with 4% CEOs. The peanut kernels packed in 2% CS films incorporated with 4% CEOs showed complete inhibition of fungal growth at both storage temperatures after 24 days of storage compared with all other treatments. The CS films incorporated with EOs showed significantly ($p < .05$) higher fungal growth inhibition in peanut kernels than the treatments with EOs alone. After 6 days of storage at 28 ± 2°C peanut kernels treated with TEOs, LEOs and CEOs showed 42, 34, and 13% fungal infection, respectively; however, the CS films incorporated with TEOs and LEOs showed 11.6 and 5.1% of fungal infection. The CS films incorporated with CEOs showed no evidence of fungal infection at end of storage period (24 days) in comparison to control CS films (without EOs) that showed 49% of fungal infection in peanuts after 24 days. There was a gradual increase in fungal contamination of peanut kernels during storage at 28 ± 2°C, and after 24 days, fungal infection was found to be 54%, 51%, 22%, 28.5%, and 19.3% for TEOs, LEOs, CEOs, CS films incorporated with TEOs, and LEOs, respectively. Similar trends were observed for different treatments of peanut kernels stored at 5 ± 2°C (Figure 4b).

FFS of CS (2%) combined with EOs (4%) inhibited the growth of all target decay causing fungi in artificially contaminated peanut kernels during 14 days of storage at 28 ± 2°C (Figure 5). The artificially infected peanut kernels coated with TEOs, LEOs, and CEOs showed higher % of infection than the CS (2%) FFS incorporated with EOs. In comparison to all treatments, growth of *A. flavus* and *P. citrinum* was significantly ($p < .05$) inhibited in artificially inoculated peanut kernels coated with CEOs (4%) and combination of CEOs (4%) with CS (2%) after 14 days of storage at 28 ± 2°C. The combination of CS and CEOs coating restricted *A. flavus* and *P. citrinum* infection to 11.6 and 15%, respectively, in artificially inoculated peanut kernels after 14 days of storage at 28 ± 2°C.

This study demonstrated that combination of CS with CEOs significantly ($p < .05$) reduced the fungal contamination of both *A. flavus* and *P. citrinum* in peanut kernels and hence can be used for

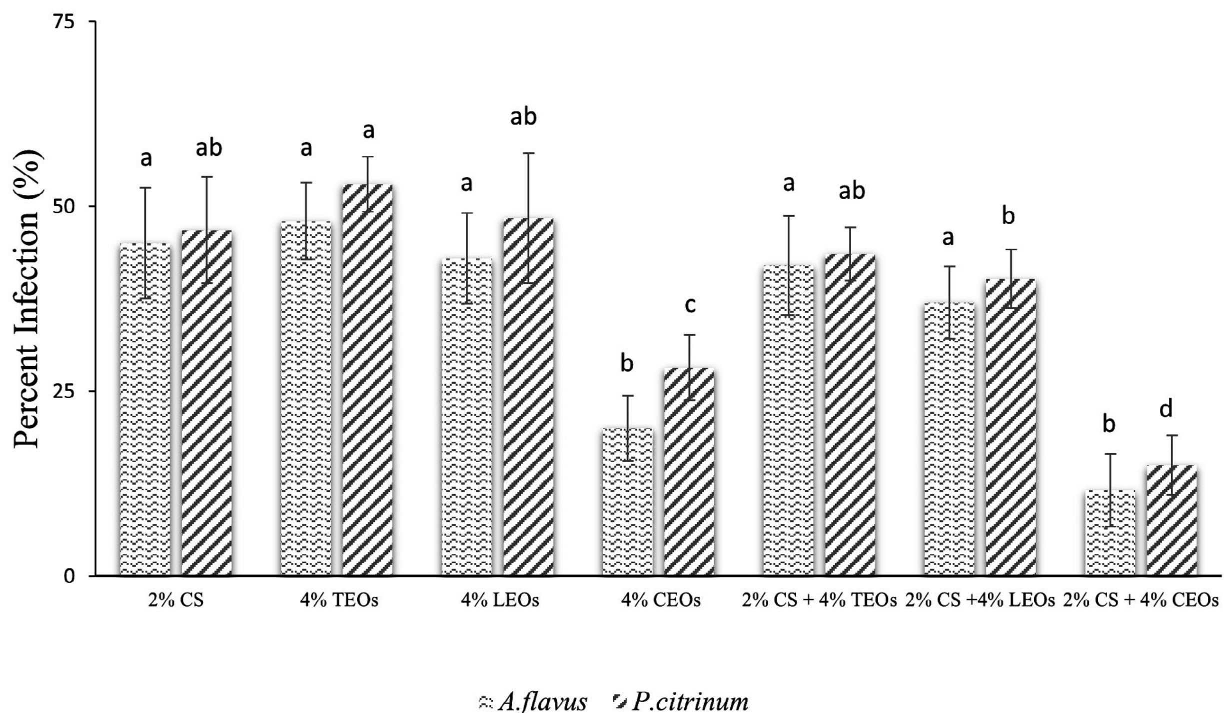

FIGURE 5 Effect of CS and EO coatings in inhibition of fungal infection in peanut kernels artificially inoculated with *Aspergillus flavus* and *Penicillium citrinum*. The different coatings used were: 2% CS, chitosan; 2% CS + CEOs, CS coating enriched with cinnamon essential oils; 2% CS + 4% LEOs, CS coating enriched with lemongrass essential oils; 2% CS + 4% TEOs, CS coating enriched with thyme essential oils; CEOs, cinnamon essential oils; LEOs, lemongrass essential oil; TEOs, thyme essential oil. Different letters (a–c) above the bars indicate significant differences ($p < .05$) among different treatment groups for each fungal strain

storage of other perishable food products. CS coating can be used as a carrier to incorporate different EOs or extracts with antifungal activities (Romanazzi et al., 2015). After coating with combination of CS and CEOs, the disease incidence was decreased in jujube that was artificially inoculated with *P. citrinum* (Xing, Li, et al., 2011). CS as a coating or solution was reported to be effective against postharvest infections by *A. flavus* (Aloui et al., 2014). The CS coating enriched with CEOs was reported to be effective in controlling the postharvest fungal infection in sweet pepper (Xing, Li, et al., 2011), strawberry (Perdones, Vargas, Atares, & Chiralt, 2014), crown rot disease of banana (Win, Jitareerat, Kanlayanarat, & Sangchote, 2007), and gray mold disease of strawberries artificially infected by (*Botrytis cinerea* (Mohammadi, Hashemi, & Hosseini, 2015). The addition of lemon EOs enhanced the antifungal effects of CS in strawberries inoculated with a spore suspension of *B. cinerea* (Perdones, Sanchez-Gonzalez, Chiralt, & Vargas, 2012).

TEOs were reported to inhibit postharvest decay in table grapes (Valverde et al., 2005), strawberries (Wang, Wang, & Chen, 2008), and kiwi fruit (Shirzad et al., 2011). However, there is no information regarding the combined inhibitory effect of CS coating and EOs in controlling fungal growth in peanut during postharvest storage.

The combination of CS and *C. zeylanicum* EOs as a coating was effective in reducing the microbial deterioration of sweet peppers throughout 35 days of storage at room temperature (Xing, Xu, Li, Che, & Yun, 2012). In general, studies have shown that the CS exhibits more consistent antimicrobial activity against bacteria, while the effect on filamentous fungi and yeast has been variable (Sánchez-Gonzáles et al., 2010). This study clearly demonstrated that CS films incorporated with EOs inhibited the postharvest fungal growth in peanut kernels at ambient and cold storage.

4 | CONCLUSION

The combination of CS with different EOs was used for the conservation of peanut kernels. The incorporation of EOs in CS films resulted a decrease in solubility and WVP of films; however, CEO-based CS films showed better characteristics in terms of tensile strength and surface morphology compared to CS films incorporated with TEOs and LEOs. The peanut kernels packed in CS films incorporated with CEOs exhibited low incidence of fungal infection during storage at ambient and cold temperatures. Furthermore, the combination of CS and CEOs was observed as the most effective coating treatment in controlling the growth of *A. flavus* and *P. citrinum* in artificially inoculated peanuts. These findings revealed that CS in combination with CEOs can be used as an effective coating material for controlling fungal growth in agricultural commodities. The CS films incorporated with CEOs can be used to enhance the shelf life of food products as a preferable packaging due to biodegradable nature.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

ORCID

Anil Kumar Anal https://orcid.org/0000-0002-8201-112X

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SUPPORTING INFORMATION

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