Food and Chemical Toxicology 134 (2019) 110821

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Comparison of antifungal activity of essential oils from different plants against three fungi

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ARTICLE INFO

Keywords:
Essential oil
Antifungal
Bread spoilage
Aspergillus spp

ABSTRACT

The antifungal activity of plant essential oils (EOs) extracted by steam distillation from seven different species (Cinnamon, Anise, Clove, Citronella, Peppermint, Pepper, and Camphor) was investigated. Three common fungi were isolated from moldy wheat bread, which were identified as *Aspergillus niger*, *A. oryzae*, and *A. ochraceus*. The antifungal activity of anise, peppermint, clove, cinnamon, pepper, citronella, and camphor EOs from seven different spices was confirmed by agar diffusion assay against three fungi. Among all the EOs, the cinnamon EO showed the highest antifungal activity for all the fungi strains with the largest inhibition zone at the concentration of 800 mg/mL and lowest MIC ranging from 0.0625 to 0.125 mg/mL, followed by clove EO. The remaining EOs exerted moderate inhibitory effects. Further research indicated the substantial inhibitory activities of cinnamon and clove EOs on mycelial growth and spore germination in a dose-dependent manner. Further, the *in vivo* inhibitory activity of selected EOs on naturally infected bread demonstrated that cinnamon and clove EOs can as be used as natural antifungal agents.

1. Introduction

Fungal contamination in food, feed, and other agricultural products leads to enormous deterioration and a series of food safety problems. The extensive food spoilage caused by fungal contamination is responsible for approximately 30% of the annual food losses worldwide and considerable economic loss (Saladino et al., 2016). In addition, the mycotoxins produced by some fungi in the process of infecting food are potentially toxic with a high risk to elevate human ergot poisoning, leukocyte deficiency, aflatoxicosis, and other diseases (Matasyoh et al., 2011; Pawlowska et al., 2012).Whole wheat bread is an ideal food for mold growth on the account of its rich nutrients and high moisture content. In the past decades, to extend the shelf-life of wheat bread, several preservatives are used such as calcium propionate, potassium sorbate, and sodium dehydroacetate in order to control the incidence of mold in the past decades. However, in recent years, many chemical preservatives were reported to be carcinogenic with residual toxicity to human health (Suhr and Nielsen, 2003). Meanwhile, the extensive application of chemically synthesized additives in foods has faced controversies for the generation and development of resistant problems (Rapp, 2004). Therefore, based on the intensified demand of consumers for safe foods, the use of natural plant antimicrobial agents to control the fungal contamination and mycotoxins residue is promoted for considerable application.

Plant essential oil (EO), a kind of volatile oil extracted from aromatic plants is reported to possess widespread antimicrobial, insecticidal, anti-aflatoxigenic, and antioxidant properties (Hu et al., 2017; Pas et al., 2018; Smeriglio et al., 2017; Lv et al., 2011). Many researchers have highlighted the antifungal activities of EOs derived from various plant species, which can prolong the storage time of foods, ensure their quality, and also provide a promising substitute to chemically synthetic additives (Burt, 2004; Basak and Guha, 2018; Debonne et al., 2018; Dianez et al., 2018). Unlike chemical preservatives, plant EOs as natural products acquire a broad spectrum of antifungal properties, diverse modes of action, and limited side effects on environment or human health. Currently, a wide variety of plant EOs have been studied and applied in food or food packaging to control the fungi invasion. For instance, *Allium sativum* EO had shown significant

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https://doi.org/10.1016/j.fct.2019.110821
Received 8 August 2019; Received in revised form 12 September 2019; Accepted 14 September 2019
Available online 15 September 2019
0278-6915/

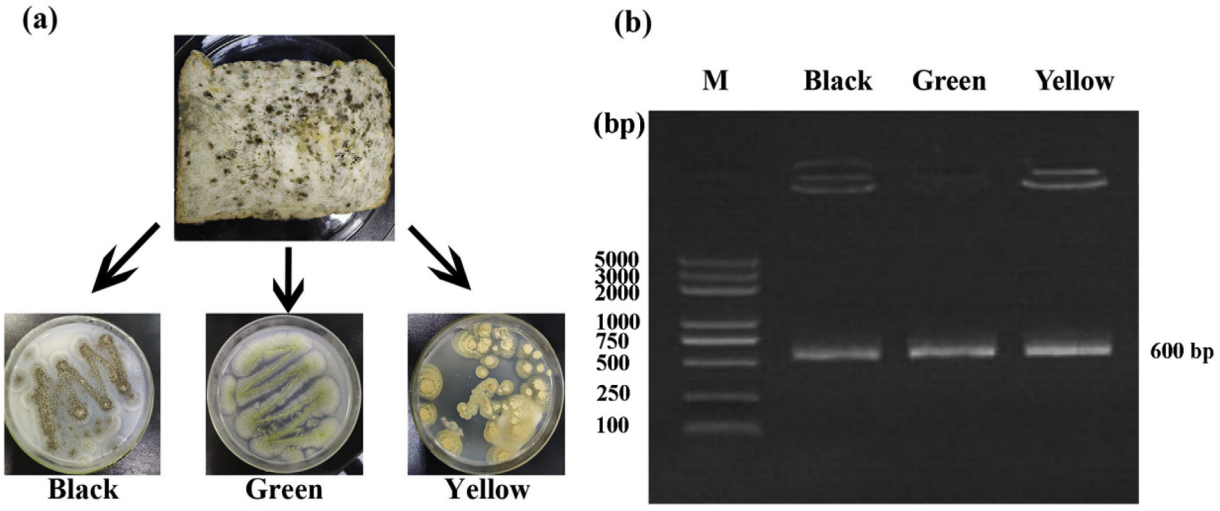

Fig. 1. (a) Three fungi isolated from moldy white bread; (b) Agarose gel electrophoresis of the PCR products with 600 bp target sequence. DNA marker (lane 1), amplified DNA of Black strains (lane 2), amplified DNA of Green strains (lane 3), amplified DNA of Yellow strains (lane 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

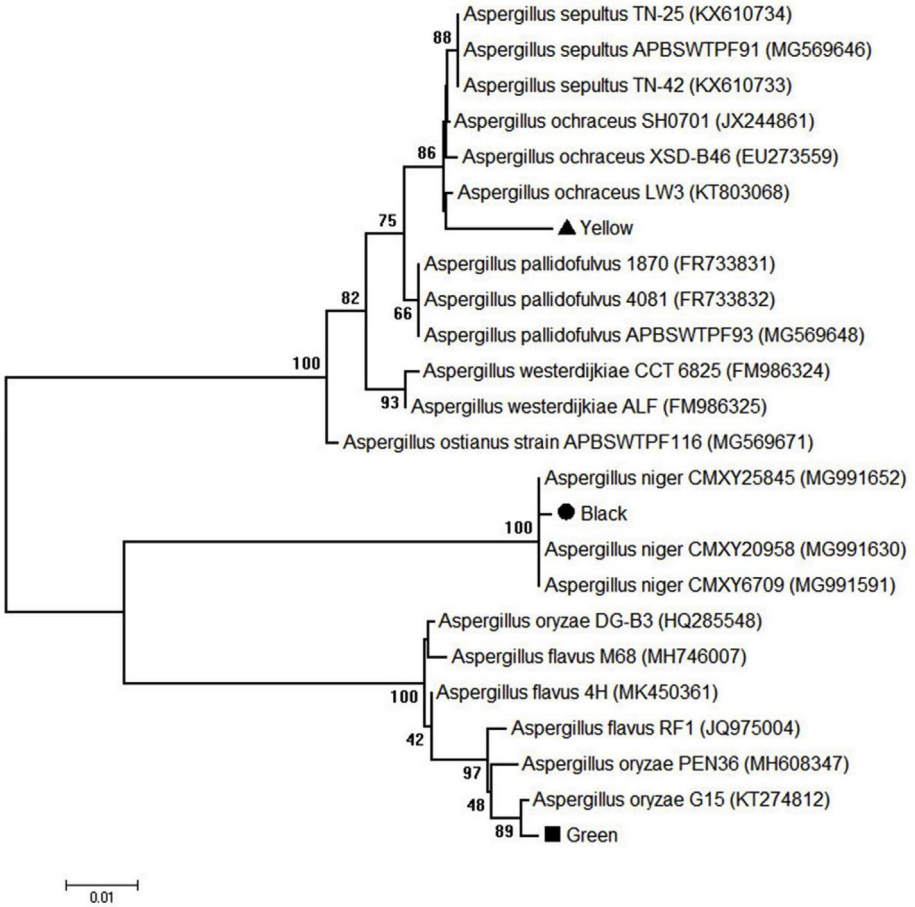

Fig. 2. Phylogenetic relationship of fungal strains. The tree was generated by the Neighbor-joining method and the bar shows 1% sequence divergence (below).

antifungal activity against fruit spoilage fungi such as *Penicillium notatum, Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus microsporus* (Arasu et al., 2019). Bedoya-Serna et al. confirmed the excellent *in vitro* and *in vivo* antifungal effects of oregano (*Origanum vulgare*) on three fungi *Cladosporium* sp., *Fusarium* sp., and *Penicillium* spp. isolated from Minas Padrão cheese (Bedoya-Serna et al., 2018). Matusinsky et al. reported the inhibitory activities of five plant EOs from *Pimpinella anisum*, *Thymus vulgaris*, *Pelargonium odoratissimum*, *Rosmarinus officinalis*, and *Foeniculum vulgare* against five important pathogenic fungal species that cause serious diseases in cereals (Matusinsky et al., 2015). Besides, the appropriate additions of EOs from edible spices generate little effect on sensory properties of food products, while the flavors can be enhanced to a certain extent (Goni et al., 2019). Nevertheless, few studies focused on the antifungal activity of EOs from edible spices

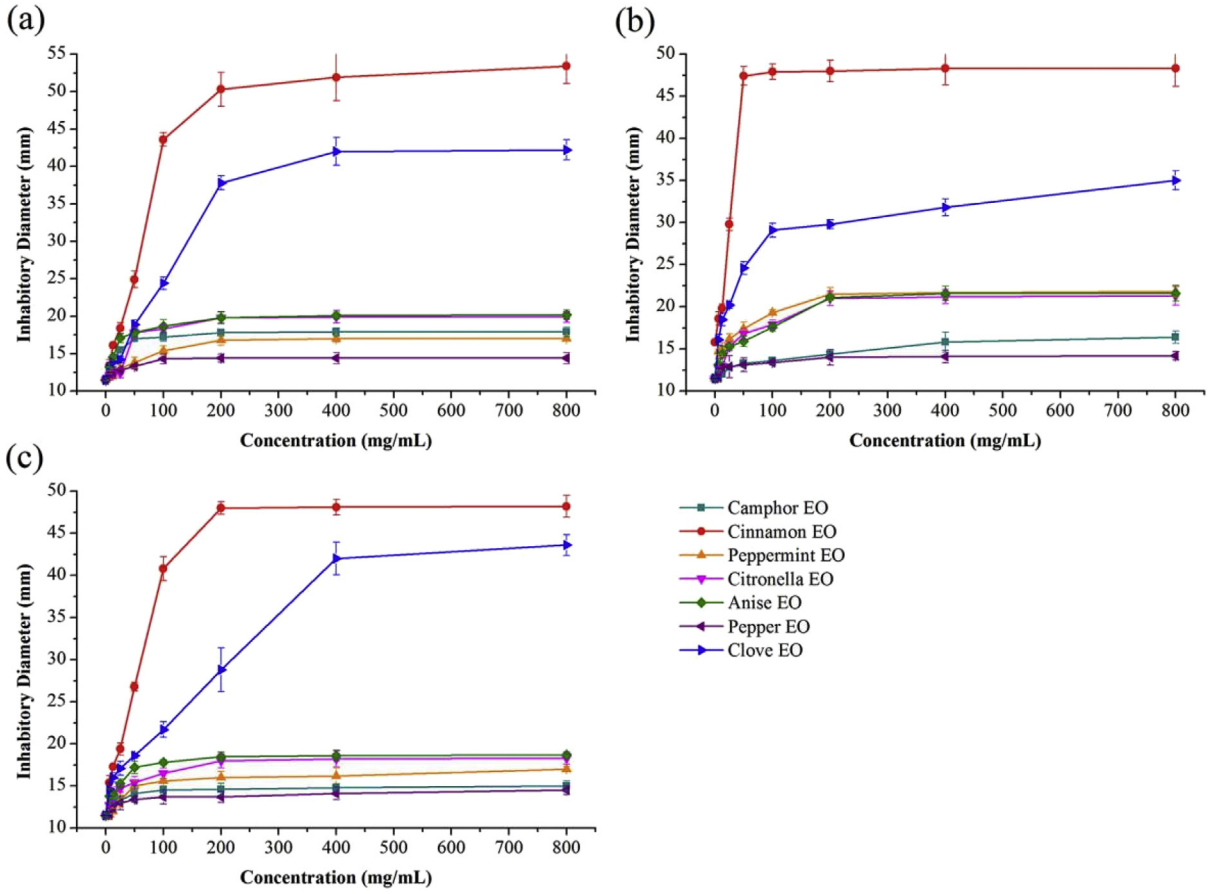

Fig. 3. The inhibition zone (mm) of seven EOs against tested fungi. (a) *A. niger*; (b) *A. oryzae*; (c) *A. ochraceus*. All the data are expressed as means ± SD of three independent experiments. The 0.1% Tween 80 used for dissolving the EOs was taken as a control for antifungal assay.

Table 1
Minimum inhibitory concentration (MIC) of EOs against tested fungi.

Fungal strains	MIC (mg/mL) of essential oils						
	Camphor	Cinnamon	Peppermint	Citronella	Anise	Pepper	Clove
A. niger	2	0.0625	2	2	1	1	0.25
A. oryzae	2	0.125	2	1	1	1	0.25
A. ochraceus	2	0.125	1	2	0.5	1	0.25

against the moldy wheat bread.

Therefore, in this study, three kinds of fungi naturally grown on whole wheat bread were isolated and identified. The antifungal activity of EOs from seven common edible spices was tested against the three fungi and compared through agar disc diffusion and gradient plate method. Then after, cinnamon and clove EOs were selected to further study the effect on the mycelial growth and spore germination of these three fungi. Moreover, the efficacy of cinnamon and clove EOs as antifungal preservatives in fresh whole wheat bread was also estimated to access the possibility of practical application of selected EOs as novel and safe preservatives.

2. Material and methods

2.1. Materials

Whole wheat bread was purchased from the local market (Hefei City, Anhui province, China). Seven different EOs such as anise (*Pimpinella anisum*), Peppermint (*Mentha haplocalyx*), clove (*Syzygium aromaticum)*, cinnamon (*Cinnamomum zeylanicum*), Pepper (*Zanthoxylum bungeanum*), Citronella (*Cymbopogon nardus*), and Camphor (*Cinnamomum camphora*) were extracted by hydro distillation in a Clevenger-type apparatus and their active compounds were analyzed using GC-MS according to the previously reported method (Tu et al., 2018). The obtained EOs were preserved at 4 °C until next use.

2.2. Isolation of fungal strains

The three fungal strains used in this study were isolated and identified from moldy whole wheat bread in natural condition. Plate scribe method was used for isolation and purification. The isolated fungal colonies selected from naturally contaminated bread were dissolved in sterile saline to make a fungal suspension, which were spread on Petri dishes containing potato dextrose agar (PDA) medium (Hangzhou Microbial Reagent Co., Ltd., China) and incubated at 28 °C for 3–5 days until the complete growth of fungi. The grown colonies were re-cultured to obtain the pure cultures and transferred to PDA slant medium and stored at 4 °C for further studies.

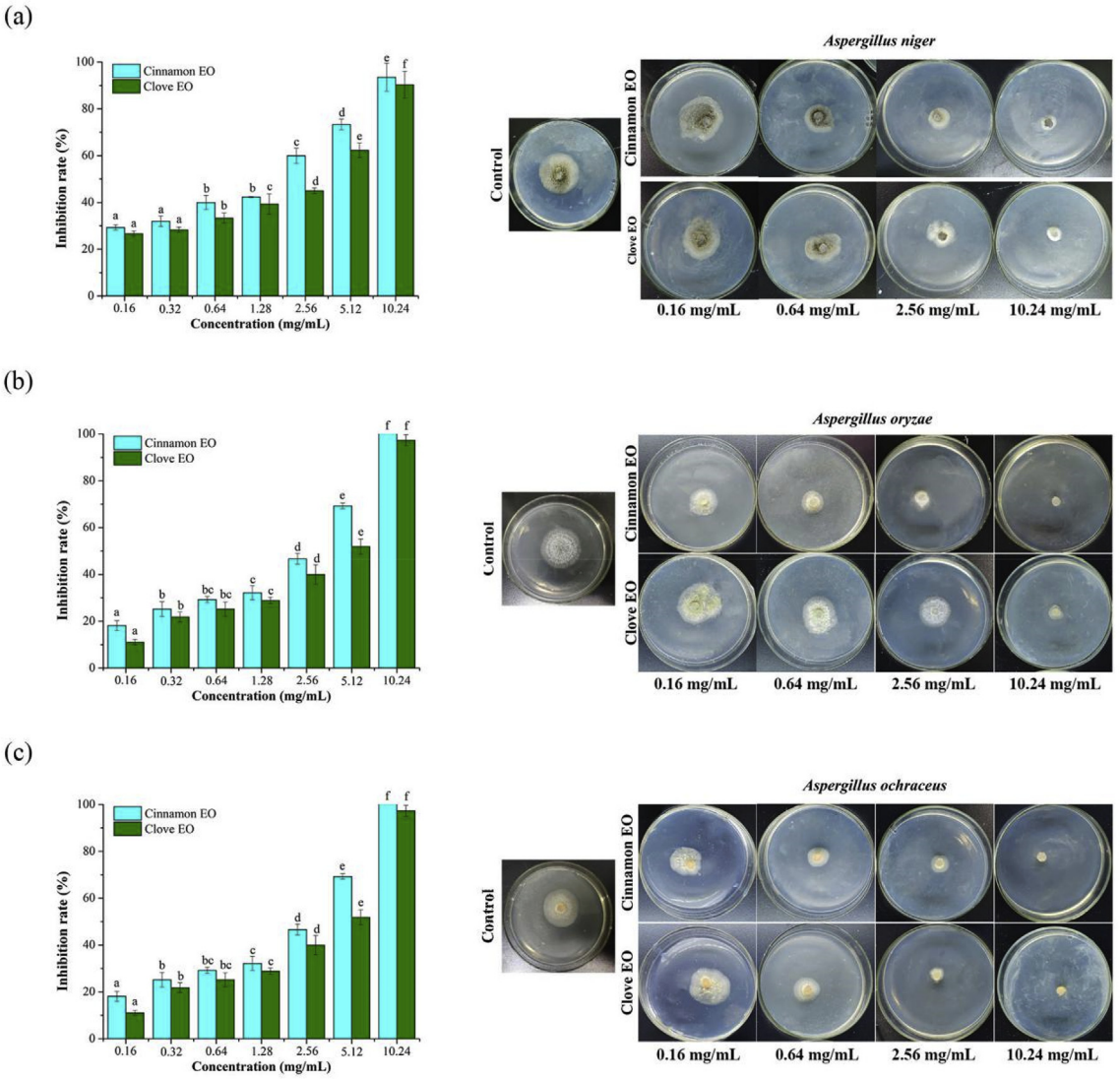

Fig. 4. Inhibitory effect of cinnamon and clove EOs on the mycelial growth against tested fungi. (a) *A. niger*; (b) *A. oryzae*; (c) *A. ochraceus*. All the data are expressed as means ± SD of three independent experiments. Statistical analysis was performed using one-way ANOVA at $p < 0.05$ designated by superscripts a, b, c, d, e, and f.

2.3. Identification of fungal strains

To identify the fungal strains, the genomic DNA was extracted and amplified using primer sequence ITS1: 5′-TCCGTAGGTGAACCTG CGG-3′, ITS4: 5′-TCCTCCGCTTATTGATATGC and the PCR product was confirmed by agarose gel electrophoresis (Raja et al., 2017; Toju et al., 2012). The nucleic acid sequences of the regions ITS A, 5.8 S gene and ITS B of rDNA of different fungi strains were aligned with Clustral W. The neighbor joining tree in Mega 4.0 was constructed to establish the phylogenetic relationships among the fungal strains.

2.4. Antifungal activity

The *in vitro* antifungal activity of seven EOs against *A. niger*, *A.oryzae*, and *A. ochraceus* was determined by the agar diffusion method (Perumal et al., 2016). Three fungal strains were cultured at 28 °C for 3–5 days, and the fungal spores on the plates were dissolved in sterile saline and diluted to approximate proportion of 10^6 CFU/mL. The EOs were dissolved in 0.1% Tween 80 to obtain different concentrations (800, 400, 200, 100, 50, 25, 12.5, and 6.25 mg/mL) and the diluted EOs were filtered by a 0.45 μM microporous filter. Then, 100 μL of each fungal suspension was spread onto PDA plate medium and the sterile filter paper (6.0 mm diameter, 1.0 mm thick) was impregnated with 10 μL of each EO and placed on the surface of seeded Petri plates. The filter paper loaded with solvent was used as a control. The plates were placed in an incubator at 28 °C for 3–5 days, and the diameter of the inhibition zone was measured and recorded as an indication of antifungal activity. The agar diffusion assay for each EOs against three test fungi was performed in triplicates.

2.5. Determination of minimal inhibitory concentration

The lowest concentration of EOs without visual growth of fungi after 24 h was considered as minimum inhibitory concentration (MIC). MIC of EOs against three tested fungi was determined by gradient plate method. PDA medium was mixed with EO solution to obtain the concentration of 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, and 0.0313 mg/mL in each dish. PDA medium without EO was used as control group and each treatment was performed in triplicates. Then after, 100 μL of the prepared fungi suspension (10^6 CFU/mL) was spread on the surface of the Petri plates. The plates were incubated at 28 °C for 3–5 days to observe the fungal growth.

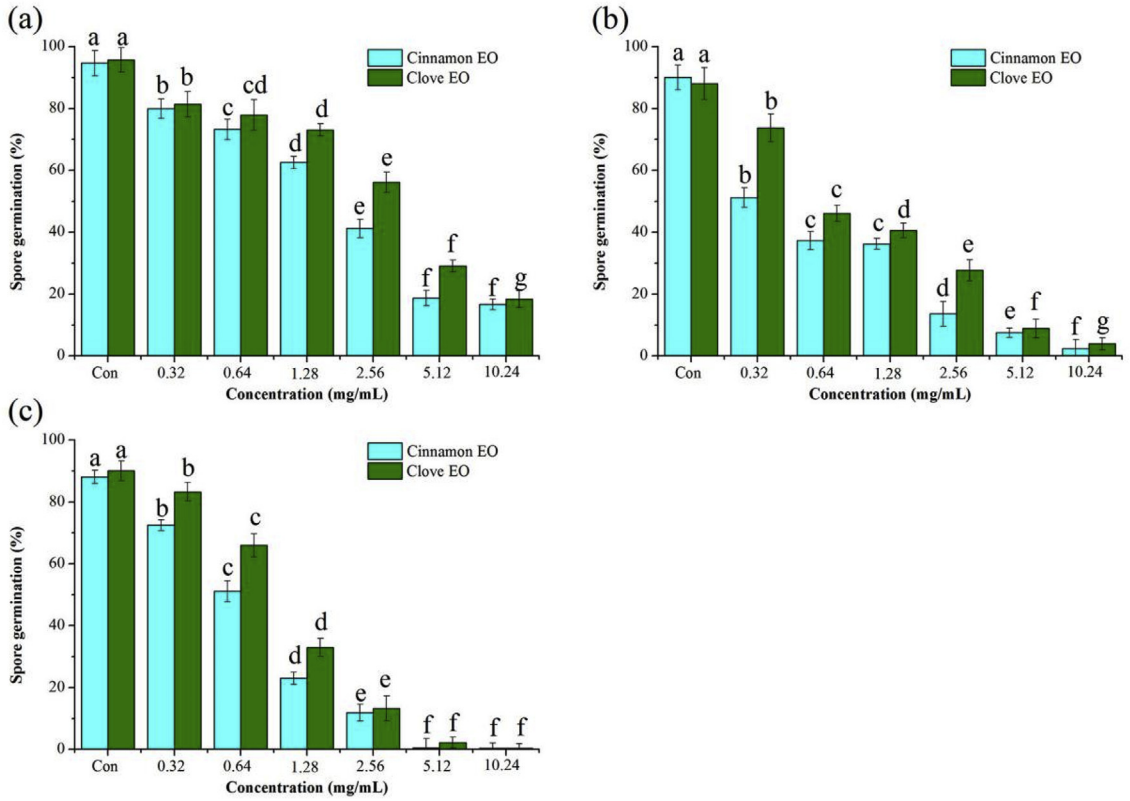

Fig. 5. Inhibitory effect of cinnamon and clove EOs on the spore germination against tested fungi. (a) *A. niger*; (b) *A. oryzae*; (c)*A. ochraceus*. All the data are expressed as means ± SD of three independent experiments. Statistical analysis was performed using one-way ANOVA at $p < 0.05$ designated by superscripts a, b, c, d, e, and f.

2.6. Antifungal effect of EO on mycelial growth

The effect of cinnamon and clove EOs on mycelial growth of three tested fungi was evaluated according to previously reported method with few modifications (Bomfim et al., 2015). The EOs were dispersed as an emulsion in 0.1% Tween 80 and added to PDA medium immediately (medium temperature of 40–45 °C). The mixed PDA medium was transferred to Petri dish to obtain the EO concentrations of 0.16, 0.32, 0.64, 1.28, 2.56, 5.12, and 10.24 mg/mL. PDA medium mixed with sterile 0.1% Tween 80 was used as control. Then after, 5 mm diameter plugs of mycelium were cut from the edge of the actively growing colony of tested fungi and they were inverted and placed on the surface of each PDA plate. The treated plates were incubated at 28 °C for 3–5 days. The growth of the other groups was observed when the mycelial growth diameter in control reached to 30 ± 2 mm. The diameter (mm) of the fungal mycelium growth was measured from the center to the edge of the colony, and each group was treated three times. Antifungal effect of EOs on mycelial growth was presented as percent inhibition of mycelial growth (%) and calculated according to the following formula (Farzaneh et al., 2015):

$$\text{Inhibition } \% = \frac{C - T}{C} \times 100$$

where C is the mean (mm) of the colony diameter for the control group, and T is the average of the colony diameter for the treated group (mm).

2.7. Antifungal effect of EO on spore germination

Antifungal effects of cinnamon and clove EOs on spore germination were observed under light microscope (XS-212-201) (Bajpai et al., 2008). Briefly, fungal spore suspensions were prepared by sterile distilled water and the spore concentration was adjusted to 1×10^6 spores/mL. 1 mL of 0.1% Tween 80 was used to dissolve cinnamon and clove EOs to obtain series of concentration such as 0, 0.32, 0.64, 1.28, 2.56, 5.12, and 10.24 mg/mL. Then after, 100 μL of the spore suspension was inoculated into each test tube containing the above EO solution (1 mL). 10 μL of the solution was added to the concave slide and placed in an incubator for 20 h at 28 °C to observe the spore germination. Spores were considered germinated when the length of hypha was longer than the length of the diameter of the spore under the light microscope. About 200 spores were counted and the percentage of spore germination was calculated from the evaluated spores. Addition of 0.1% Tween 80 to the fungi suspension was used as control and each treatment was performed in triplicates (Rguez et al., 2018).

2.8. In vivo antifungal efficacy of EO

The filter paper discs (2 × 4 cm) containing cinnamon and clove EOs solution were fixed to beneath surface of each plates (180 mm). Fresh whole wheat bread was purchased from local market and placed into above plates. The plates were sealed and incubated at 28 °C for 10 days to observe the mold growth on bread. The bread was considered contaminated when mold spots were detected on the surface. The group without EO treatment was set as control and each treatment was performed in triplicates.

2.9. Statistical analysis

Data were analyzed by one-way ANOVA/Manova using the software IBM SPSS Statistics 22.0 (SPSS, USA). Mean values were compared using the Duncan and LSD tests at significance level of $P \leq 0.05$.

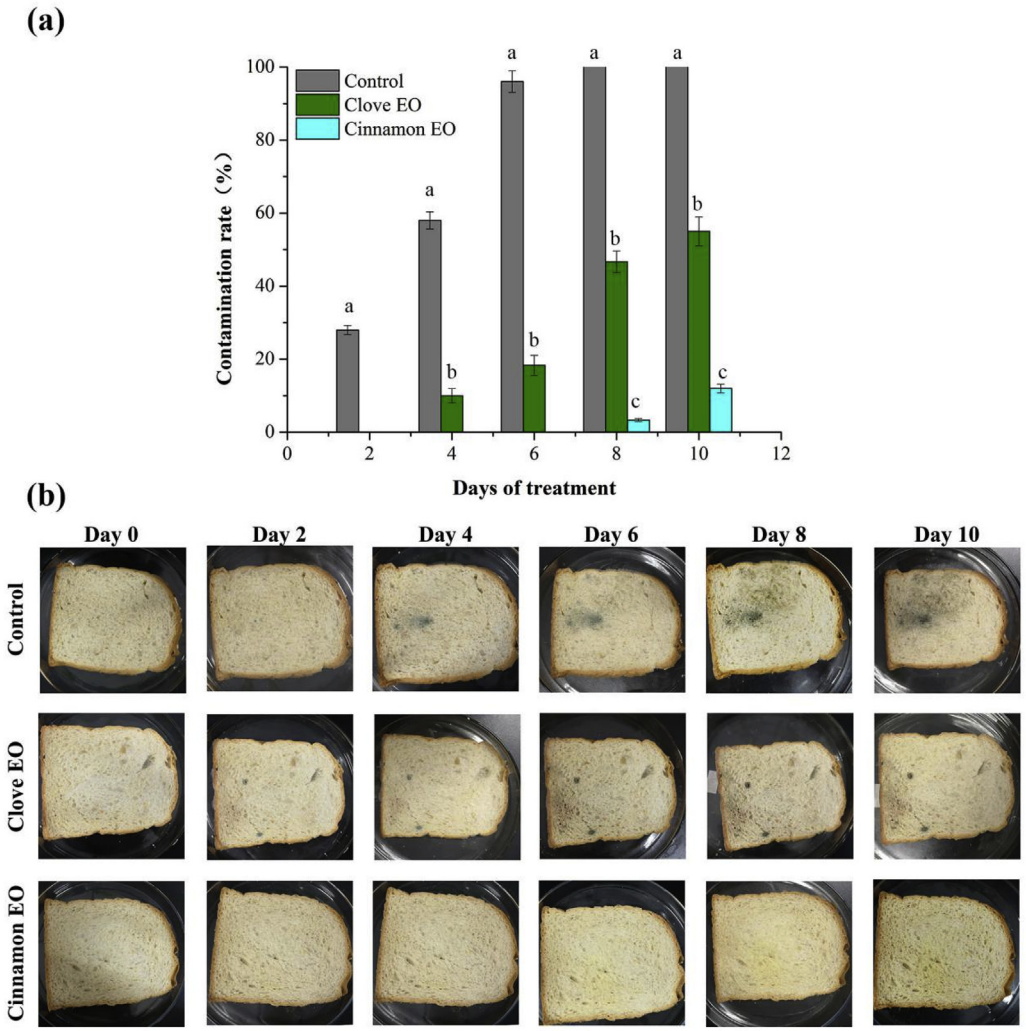

Fig. 6. Effect of cinnamon and clove EOs on naturally grown molds on bread. (a) Contamination rate of whole wheat bread; (b) Representative figures of bread samples with or without EOs treatment during 10 days incubation. All the data are expressed as means ± SD of three independent experiments. Statistical analysis was performed using one-way ANOVA at $p < 0.05$ designated by superscripts a, b, c.

3. Results

3.1. Identification of fungal strains

The fungal cultures picked from the moldy whole wheat bread were subjected to isolation, characterization (phenotypic and genotypic), and purification steps. As shown in Fig. 1a, the obtained three single colonies were temporarily named as black, green, and yellow strains. For the genotypic characterization, the resulting PCR products were run on agarose gel electrophoresis prior to sequencing.

The results of the sequencing alignment with the NCBI sequence showed that the black, green and yellow strains were identified as *Aspergillus niger*, *A. oryzae*, and *A. ochraceus*, respectively. As shown in Fig. 2, phylogenetic analysis was performed using the regions ITS A, 5.8 S gene and ITS B of rDNA sequences for the comparison of all the isolated fungal strains, using Neighbor joining tree in Mega 4.0.

3.2. Antifungal activity of seven EOs against three fungi

The inhibitory effects of seven EOs against three tested fungi were shown in Fig. 3. It can be clearly seen that antifungal effect of EOs increased with their increasing concentration until it reached the maximum diameter of inhibition zone (DIZ). For the same test strain, when the concentration of EOs reached to 800 mg/mL, cinnamon EO presented the strongest antifungal activity against three fungi with the maximum DIZ between 45 and 55 mm, followed by clove EO. Citronella EO was also found to have significant inhibitory effects on three fungi. For anise EO, it showed remarkable inhibition on *A. oryzae* with the maximum DIZ of 21.6 mm, while presented moderate inhibitory effect against *A. niger* and *A. ochraceus*. The maximum DIZ of the camphor EO against *A. niger* was 17.79 mm, while the feeble inhibitory effects against *A. oryzae* and *A. ochraceus* were noticed. On the other hand, pepper EO only revealed slight inhibitory effect on the three fungal strains.

3.3. The minimum inhibition concentrations (MICs) of seven EOs

The results of obtained MIC of seven EOs against three kinds of whole wheat bread spoilage fungi were shown in Table 1. According to MIC analysis, among all the EOs, remarkable antifungal activities were observed for clove and cinnamon EOs with the MIC of 0.25 mg/mL and 0.0625–0.125 mg/mL, respectively. Peppermint, citronella, anise, and pepper EOs also presented considerable antifungal activity against three bread spoilage fungi, while camphor EO showed the largest MIC range (2 mg/mL) in the concentration dependent range. These MIC results were consistent with the agar diffusion test stating that cinnamon and clove EOs could be regarded as an effective natural fungicidal agent in food industry to prevent the fungal contamination.

3.4. Inhibition of mycelial growth

According to the above results, the effects of clove and cinnamon EOs on the mycelial growth of spoilage fungi were determined. As seen in Fig. 4, compared with the control group, cinnamon and clove EOs possessed apparent inhibitory effects on the mycelial growth of the three fungi, and the cinnamon EO exhibited stronger inhibitory effect on the mycelial growth than clove EO at the same concentration, which is consistent with the MIC results. The increasing concentration of EOs promoted the inhibition of mycelial growth of all tested fungi strains. At the concentration of 10.24 mg/mL, the mycelial growth of three fungi was completely inhibited. The mycelial growth inhibition rate of cinnamon EO on *A. niger*, *A. oryzae* and *A. ochraceus* reached to 93.51%, 100% and 100%, respectively and the mycelial growth inhibition rate of clove EO on *A. niger*, *A. oryzae* and *A. ochraceus* reached to 90.33%, 97.31%, and 100%, respectively. The sensitivity of the three fungi to EOs was different with the following order: *A. ochraceus* > *A. oryzae* > *A. niger*.

3.5. Inhibition of spore germination

The effects of clove and cinnamon EOs on the spore germination rate of three whole wheat bread fungi were presented in Fig. 5. The clove and cinnamon EOs could significantly inhibit the spore germination of three kinds of bread spoilage fungi in a dose dependent manner. When the EOs concentrations were between 0.32 and 10.24 mg/mL, the spore germination rate of three kinds of tested fungi ranged from 11.11% to 100%, while the spore germination rate of cinnamon EO ranged from 18.15% to 100%. Clove and cinnamon EO with the concentration of 10.24 mg/mL could completely suppress the spore germination of *A. ochraceus* and the inhibition rates against *A. oryzae* were 97.31% and 100%, on the other hand, the inhibition rate against *A. niger*were 90.33% and 93.51%.

3.6. In vivo antifungal efficacy of EO

The efficacy of cinnamon and clove EOs on naturally contaminated whole wheat bread was illustrated in Fig. 6. As seen in Fig. 6 (a) and (b), treatment with cinnamon and clove EOs could effectively reduce the incidence and development of natural mold spoilage of wheat bread sample at 28 °C during ten days storage period. During the storage period, compared with the control group, cinnamon and clove EOs could effectively control the fungal contamination of the bread and prolong the storage time. Cinnamon EO could completely inhibit the natural mold on the surface of bread at least after six days of storage, while clove EO could extend the shelf-life of bread up to two days. However, the inhibitory effect of EOs against the mold spoilage of bread was weakened with the increasing contamination rate as the storage time was extended, which may be the consequence of rapid dispersing of the vapor phase in EOs.

4. Discussion

Seven plant EOs from edible spices displayed varying degrees of inhibitory actions on the three tested fungi, *Aspergillus* spp. These findings were consistent with the previous studies (Chaemsanit et al., 2018; Jia et al., 2019; Zorzi et al., 2018). Ju et al. reported that cinnamon and clove EOs exhibited strong antifungal activity against two fungi (*Penicillium* spp. and *Aspergillus* spp.) from mold baked food with DIZ significantly larger than 15 mm (Ju et al., 2017). It has been well demonstrated that the differences in fungicidal activity of plant EOs can be related to the active components contained therein, such as phenols, aldehydes, and ketones (Oussalah et al., 2007). In our previous study, GC-MS analysis revealed that the active contents varied in different EOs. For example, eugenol (84.036%) and cinnamaldehyde (86.216) represented the main components in clove EO and cinnamon EO, respectively. It is assumed that the majority of phenols and aldehydes in clove EO and cinnamon EO may lead to secondary membrane damage resulting to higher anti-fungal activity, respectively (Tu et al., 2018). Nevertheless, synergistic effect of some minor components may also contribute to fungicidal activity of plant EOs. Xie et al. investigated structure-activity relationships of cinnamaldehyde from cinnamon bark EO, eugenol from clove bud EO and their derivatives against plant pathogenic fungi, *Rhizoctonia solani* and *Fusarium oxysporum*and found that a conjugated double bond structure and the length of CH chain outside the benzene ring may be responsible for the antifungal activityof EOs (Xie et al., 2017). Therefore, the differences in chemical composition of different EOs may contribute to the antifungal diversity. High content of phenols in clove EO and aldehydes in cinnamon EO may be responsible for severe lesions of the membrane leading to secondary membrane damage which can result in the higher fungicidal activity.

Many studies had confirmed the strong antifungal activity of plant EOs with broad inhibition spectrum, which emphasizes on their high potential as novel preservatives to substitute the synthetic fungicides. Besides, the natural anti-fungal agents have high volatility, good biodegradability, low residue generation, low toxicity, and they are safe for non-target organisms. It has been reported that the camphor EO was proved to be the most effective EO for antifungal activity against *Stemphylium solani*, which caused heavily gray leaf spot on tomato among ten tested EOs with the MIC of 3 μL/mL (Zorzi et al., 2018). Chaemsanit et al. indicated that the MIC of peppermint EO against *A. flavus*, *Penicillium* and *A. niger* were 700 μL/mL, 550 and 550 μL/mL, respectively (Chaemsanit et al., 2018). Another study evaluated 16 different EOs against 21 fungi isolated from herbal drugs and found that anise EO could inhibit the growth of most fungi with the MIC range of 0.7 mg/mL to 2.2 mg/mL, while the high activity of anise EO was mostly dependent on the existence of trans-anethole (Stevi, 2014; Huang et al., 2010). Previous research confirmed that the inhibitory effects of plant EOs on many fungi were attributed to the components with low-molecular weight and highly lipophilic characteristics which can easily disrupt cell membrane and lead to cytoplasmic leakage (Chao et al., 2005). Carson et al. showed that in the presence of tea tree EO, severe membrane permeability and interference on respiratory chain activity in *Candida albicans* cells resulted in the death of microorganisms (Carson et al., 2006; Armstrong, 2010). In addition, Soylu et al. observed alternations in plasma membrane, cytoplasm, and nucleus through scanning and transmission electron microscopic analysis of *Phytophthora infestans* treated with plant EOs (Soylu et al., 2006).

The inhibition of mycelial growth, spore germination as well as aflatoxin biosynthesis of plant derived EOs in fungi were reported to be associated with the destruction of the endomembrane system of fungal cells. Gupta et al. revealed that the antifungal activity of cinnamon EO was mainly due to the high content of cinnamaldehyde, which interfered the several biological process such as electron transfer and can react with nitrogen containing compounds (Gupta et al., 2008). It had also been reported that phenolics in plants such as eugenol, the major component in clove EO, are known to play important role in the antimicrobial activity by damaging the morphology and membranes of tested bacterial cells (El-Maati et al., 2016).

In the previous study, cinnamon EO (0.4%) combined with gumarabic (10%) was found to inhibit the mycelial growth and spore germination inhibition against *Colletotrichum musae* and *Colletotrichum gloeosporioides*, while *in vivo*results showed that 0.4% of cinnamon EO combined with 10% of gumarabic was the optimal concentration to prevent the rot caused by *C. musae* and *C. gloeosporioides* inartificially contaminated bananas and papayas (Maqbool et al., 2011). Songsamoe et al. reported that limonene, a component from bergamot EO could affect some enzyme functions involved in spore germination by extending the lag phase during spore germination after UV-C radiation (Songsamoe et al., 2016). After treatment with *Citrus sinensis* EO, the loss of cytoplasm in fungal hyphae, thinner and distorted hyphal wall

and damaged cell wall were observed in *A. niger* (Sharma and Tripathi, 2008). In addition, Grbić et al. found that *Nepeta rtanjensis*EO could significantly suppress the spore germination of *Cladosporium cladosporioides*, *Trichoderma viride* and two mold from *Alternaria*. Among these fungi, *Cladosporium cladosporioides*was so sensitive that 0.1 mg/L of *Nepeta rtanjensis* EO could entirely inhibit the spore germination, in comparison to 0.6mg/L of EO for Alternaria. (Grbić et al., 2011).

There are many EOs extracted from different plant species with an aim to inhibit the *in vivo* fungal development to control the food deterioration. Frankova et al. applied the cinnamon and clove essential EOs to the storage of apple and found that cinnamon (16 mL/L) and clove (4 mL/L) EOs effectively reduced the incidence of *P. expansum* with minimal adverse effect on the sensory properties (Frankova et al., 2016). Another research indicated that vapor with *Michelia alba* EO could completely inhibit the natural mold grown on brown rice for at least 12 weeks if artificially infected with *A. flavus* spores (Songsamoe et al., 2017). Ribes et al. found remarkable fungicidal activity of cinnamon bark EO emulsified with zinc gluconate and trans-ferulic acid in strawberry jams with a reduction of 2log-cycles after seven days of *A. niger* contamination (Ribes et al., 2018).

5. Conclusions

To summarize, three fungi were isolated from the moldy wheat bread and identified as *A. niger*, *A. oryzae*, and *A. ochraceus*. Seven plant EOs from edible spices displayed varying degrees of inhibitory actions on the three tested fungi. For the same test strain, cinnamon EO had the strongest antifungal activity, showing the largest DIZ and the lowest MIC range, followed by clove EO. Furthermore, both cinnamon and clove EOs could inhibit the mycelial growth and spore germination of three kinds of bread spoilage fungi. Finally, the *in vivo* experiment confirmed that cinnamon and clove EOs could effectively control the incidence and development of natural mold spoilage and extend the shelf-life of bread. The obtained results evidently demonstrated that the selected EOs can be developed as natural preservatives to prevent the fungal contamination in food industry.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by the Major Projects of Science and Technology in Anhui Province (17030701024, 17030701025, 17030701028, and 17030701058), the Key Research and Development Projects of Anhui Province (1804h07020147), and the National Natural Science Foundation of China (31301657).

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