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Original Research Article

MOULD CONTAMINATION OF SOME MEAT PRODUCTS WITH REFERENCE TO DECONTAMINATION TRIALS OF *Aspergillus flavus* USING ESSENTIAL OILS

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Abstract: Over the years, researchers, food specialists, and all authorities concerned with food safety around the world could develop many ways to preserve and improve the shelf life of food. Recently, volatile oils have been gaining attention for their antifungal and anti-mycotoxin properties. The objectives of the present study were first to investigate mold contamination of four chilled meat products (luncheon, beef burger, sausage, and minced meat) retailed in Zagazig city, Sharkia Governorate, Egypt. Second, identification of the prevalent mould genera was followed. Third, the inhibitory effects of some essential oils against *Aspergillus flavus* were tested. The results of the present study showed that the mean values of the total mould count were 2.47 ± 0.04, 2.85 ± 0.11, 3.08 ± 0.12 and 2.24 ± 0.02 (log_{10} CFU/g) in luncheon, beef burger, sausage, and minced meat, respectively. The most contaminated samples were that belonging to sausage, followed by beef burger, luncheon, and minced meat. Five mould genera were isolated and identified in the current study, namely, *Aspergillus, Penicillum, Cladosporium, Alternaria,* and *Mucor* Furthermore, *Aspergillus* species was further identified into *A. flavus*, *A. niger*, *A. fumigatus*, *A. ochraceus,* and *A. terreus*. Essential oils of cinnamon, lemongrass, and thyme at 1% and 2% had a clear inhibitory effect against *A. flavus*-artificially contaminated beef burger. The effects of thyme oil 1%, cinnamon oil 1%, and lemongrass oil 1% at the 9th day on the overall acceptability score were 7.33 ± 0.28, 7.13 ± 0.27, and 7.09 ± 0.18, respectively. *A. flavus* counts were 3.79 ± 0.05, 3.18 ± 0.07, and 3.61 ± 0.05, respectively after these treatments. While, at the same day, thyme oil 2% effect > lemongrass oil 2% > cinnamon oil 2% for both overall acceptability score and total *A. flavus* count. These findings suggest that thyme, cinnamon, and lemongrass EOs have significant antifungal activity, which is a promising solution for mould decontamination and consequently extends the shelf life of meat products.

Key words: Mould; *Aspergillus spp*.; *A. flavus*; volatile oils; beef burger

Introduction

Meat and its products have occupied a major portion of our daily meals which positively reflected on its demand all over the world. It is highly perishable food, which is of high nutritional value as it contains animal protein of high quality, several vitamins, and minerals. Meat spoilage might occur via its contamination with bacteria, yeast, and mould (1).

Mould is considered one of the causative agents of meat deterioration; it is responsible for the world's economic losses from food's production by 5-10% (2). Mould presence in food might be attributed to some factors including animals feeding with contaminated rations or due to the contamination during processing and storage of meat (3). There are some toxigenic fungi that can produce secondary metabolites with highly toxic features recognized as mycotoxins leading to carcinogenic, mutagenic, teratogenic, neurotoxic, nephrotoxic, immunosuppressive, and estrogenic effects, even at low concentration (4). Therefore, there was an urgent need to explore novel antifungal with natural features to overcome its negative

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impact on the health of both animals and humans. In addition to, improving the sensory properties and extend the shelf life of meat and meat products (5).

Essential oils (EOs) are volatile oily liquids obtained from different plant parts by several methods. EOs have efficacy on a wide range of Gram-positive, Gram-negative pathogens, mould and yeast, and so they are used as food flavors, antiviral, antibacterial, antimycotic, antitoxigenic, and antiparasitic (6,7). In need, it is necessary to develop a new application for the treatment of *A. flavus* in meat products, especially by application of natural oils that don't affect human health, instead of the chemical additives which pose a threat to human health. As consumer demand increases for minimally processed, preservative-free, more stable, and safe foods, the development of natural preservatives with high antibacterial activities for improving the quality and extending the shelf life of food products is desirable. In this regard, research endeavors focused on the application of natural antimicrobial agents to meat products have emerged as a novel method of preserving food freshness and quality (8.9). Despite the fact that the essential oils investigated have antimicrobial activity, their efficacy in inhibiting mould growth in meat products has not been comparatively assessed. Thus, the objective of this study was to evaluate the mycological status of meat and meat products and to assess the antifungal activities of some EOs on the quality and shelf life of formulated beef burger.

Materials and methods

Samples collection

A total of hundred samples of chilled luncheon, beef burger, sausage, and minced meat (25 of each) were randomly collected from supermarkets and butchers at Zagazig city, Sharkia Governorate, Egypt.

Preparation of samples

Under aseptic conditions, 25 g of each sample were homogenized with 225 mL of sterile buffered peptone water 0.1% (BPW, (OXOID, CM0509, UK)). Such homogenate represents the dilution of 10^{-1} and then tenfold serial dilution were prepared to cover the expected range of contamination (10).

Total mould count (TMC)

The total mould count (TMC) was determined by culturing on malt extract agar (MEA) and Czapeck-Dox agar with 5% NaCl (OXOID, BASINGSTOKE, UK) and incubation at 25 °C for 5-7 days. The TMC was obtained by counting of the cultured moulds agar plates (10). The TMC was converted into logarithms 10 of colony forming units per g of samples ($\log_{10}$ CFU/g).

Identification of mould isolates

The identification of the colonies was carried out by observation of the macroscopical and microscopical characteristics of the mould colonies (2).

Macroscopical examination

Both the surface and backside of the colonies were examined. The growing fungi were examined daily using a magnifying hand lens for surface growth consistency, folding pattern (rugae), colony margin distinctness, the rate and growth pattern, and pigment existence either on the surface or the colony reverse or diffusing into the surrounding medium.

Microscopical examination

A loopful of 7 days old mould colony was transferred to a clean glass slide, stained with lactophenol cotton blue stain (LPCB, S016, Himedia, India), and examined microscopically under low power and oil immersion lenses lens (Optika DM-15 binocular digital microscope with built-in Digital Camera, Italy) to typify the morphological structures of the mould isolates (11).

Trials to improve meat quality and decrease mould count

Twenty EOs of cinnamon (*Cinnamomum zeylandicum*), clove (*Syzygiumaromaticum*), lemongrass (*Cymbopogon citratus*), garlic (*Allium sativum*), tea tree (*Melaleuca alternifolia*), thyme (*Thymus vulgaris*), black seed (*Nigella sativa*), marjoram (*Origanum majorana*), cumin (*Cuminum cyminum*), rosemary (*Rosmarinus officinalis*), yaqtinseed (*Lagenaria siceraria*), turnip (*Brassica rapa subsp. rapa*), ginger (*Zingiber officiale*), green tea (*Camellia sinensis*), wheat germ (*Triticum vulgare*), arugula (*Eruca Sativa*), argan (*Argania spinosa*), sesame (*Sesamum indicum L.*), moringa *oleifera* seed oil (Moringaceae) and moringa leaf extract (*Moringaoleifera*) were obtain-

ed from the squeezing and extraction of natural oils unit and from the Egyptian scientific society of moringa, the National Research Centre, Dokki, Giza. These oils were used to determine the most effective pure oil (100% conc.) on *A. flavus* by agar well diffusion method (12). Subsequently, the best effective oils in the preliminary screening were selected for further investigation at different concentrations of 50%, 25%, 10%, 5%, 2%, 1%, and 0.5% (v/v) as previously described (12). The different concentrations of EOs were prepared as Oil-in-water emulsion (tested oil, Tween 80 (Sigma-Aldrich Ltd), and water) before utilization for evaluation of their antimicrobial properties as previously described by Ghabraie et al. (13). The experiment was performed in triplicate. It is considered positive when the mean of inhibition zone diameters was ≥ 10 mm (14).

Preparation of A. flavus spore suspension

Aspergillus flavus isolates were subcultured on MEA plates supplemented with chloramphenicol (50 mg/L) and incubated at 37°C before each trial. After 72 h, Conidia were harvested by flooding the plates with sterile phosphate buffered saline (PBS) containing 0.01% (v/v) Tween 20, centrifuged at 3500 rpm for 30 min and quantified by counting with a hemocytometer (15).

Formulation of the Burger

For the experimental trails, a total of 12 Kg (4 kg/ trial of three replicates) of fresh beef burger was aseptically prepared. For burger formulation, fresh beef minced meat and fat were purchased from a local meat supplier. Beef burger contains 70% minced meat, 15% fat, 3.5% starch, 10% cold water and 1.5% salt was manufactured according to Alsaiqali et al. (16). All the ingredients were well homogenized and each group was manually shaped by a burger- maker to obtain round burger weighed 100g with 10 cm diameter and 1cm thickness.

The experimental design

All groups except the negative control were inoculated with one mL of *A. flavus* spore suspension broth that was adjusted to 0.5 McFarland standard (1.5×10^8 spore/mL) (17) by pipetting over each meat burger in a sterile plastic Ziploc bag and gently massaged for the inoculum uniformity dispersal. The inoculated burger was left at 25°C for 1 h to allow the absorption of the inoculum. After that, cinnamon oil, lemongrass oil, and thyme oil at concentrations of 1% and 2% of each oil was individually added to the other six groups. Then, it was left for another 1h at 25°C. Finally, each group was packaged individually in a sterile plastic Ziploc bag, labeled, and chilled at 3 ± 1°C. The last group is control positive group contains one mL of *A. flavus* spore suspension without oil addition. The control as well as the treated groups were examined at zero, 3rd, 6th, 9th, and 12th days of the chilling storage to evaluate the effect of the above-mentioned oils against *A. flavus*. *A. flavus* count were recorded, calculated, and expressed as $\log_{10}$ CFU/g.

Sensory evaluation

The sensory assessment (color, odour, consistency, juiciness, appearance, purchase probability, and overall acceptability) of the beef burger was carried out according to Bastos et al. (18).

Statistical analysis

Total mould count values were presented as means ± standard error (SE). The colony count was converted to $\log_{10}$CFU/g value. Data were subjected to the statistical package for social sciences (SPSS-16.; Chicago, IL, USA) software and one-way Analysis of Variance (ANOVA) at 95% level of confidence. Significant differences among the means were determined by Tukey's Kramer HD test considering $P < 0.05$ as significant.

Results

As presented in Table 1, the most contaminated sample was sausage (80%), followed by beef burger (72%), then luncheon (64%) and minced meat (40%).

As depicted in Table 2, five mould genera were identified, and the most prevalent genera were *Cladosporium, Aspergillus* followed by *Penicillium*. *Aspergillus* species were isolated and identified at 50% and 60% from sausage and minced meat, respectively. Moreover, *Penicillium* and *Cladosporium* species were isolated from luncheon, beef burger, and sausage only and failed to be isolated from minced meat samples. *Alternaria* species was isolated only from luncheon samples. While *Mucor* species was isolated from sausage (12.50%) and minced meat samples (40%).

Table 1: Mean values of total mould count (log_{10}CFU/g) in meat and meat product samples (No. = 25 of each).

	Positive samples		Minimum	Maximum	Mean ± SE
	No.	%			
Luncheon	16	64	2.30	2.78	2.47±0.04 [b]
Beef burger	18	72	2.30	3.60	2.85±0.11 [a]
Sausage	20	80	2.00	3.78	3.08±0.12 [a]
Minced meat	10	40	2.00	2.30	2.24±0.02 [b]

Means within the same column carrying different superscripts are significantly different at $P < 0.05$.

Table 2: Prevalence of isolated mould species in meat and meat product samples

	Luncheon		Beef burger		Sausage		Minced meat		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Aspergillus spp.	4	16.67	4	16.67	16	50	6	60	30	33.33
Penicillium spp.	4	16.67	4	16.67	4	12.50	-	-	12	13.33
Cladosporium spp.	14	58.33	16	66.66	8	25	-	-	38	42.22
Alternaria spp.	2	8.33	-	-	-	-	-	-	2	2.22
Mucor spp.	-	-	-	-	4	12.50	4	40	8	8.9
Total	24	100	24	100	32	100	10	100	90	100

Percentages were calculated according to the total number of the isolates from each sample.

Table 3: Prevalence of *Aspergillus* species in meat and meat product samples

	Luncheon		Beef burger		Sausage		Minced meat		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
A. flavus	4	100	4	100	2	12.50	-	-	10	33.33
A. niger	-	-	-	-	6	37.50	6	100	12	40.00
A. fumigatus	-	-	-	-	2	12.50	-	-	2	6.67
A. ochraceus	-	-	-	-	4	25.00	-	-	4	13.33
A. terreus	-	-	-	-	2	12.50	-	-	2	6.67
Total	4	100	4	100	16	100	6	100	30	100

Percentages were calculated according to the total number of isolated *Aspergillus* species from each sample.

A. flavus was found in luncheon and beef burger with a prevalence of 100% and *A. niger* at 100% in minced meat samples. Sausage samples were contaminated with all identified *Aspergillus* species (Table 3).

In Table 4, the most effective EOs on *A. flavus* were garlic oil, lemongrass oil, cinnamon oil, thyme oil, cumin oil, and rosemary oil with a mean of inhibition zone diameters of 100 ± 0.0 mm followed by marjoram oil with 98.33 ± 1.67 mm and clove oil with 81.67 ± 9.28 mm.

Table 4: Efficacy of volatile oils' pure concentration (100%) against *A. flavus* by using agar well diffusion method

Applied treatments	Inhibition zone diameter* (mm) mean ± SE
(+ve) Itraconazole 10 µg /disc	34.33 ± 0.33^{c}
(-ve) tween 80	0.00 ± 0.00^{f}
Garlic oil	100.00 ± 0.00^{a}
Lemongrass oil	100.00 ± 0.00^{a}
Cinammon oil	100.00 ± 0.00^{a}
Thyme oil	100.00 ± 0.00^{a}
Cumin oil	100.00 ± 0.00^{a}
Rosemary oil	100.00 ± 0.00^{a}
Marjoram oil	98.33 ± 1.67^{a}
Clove oil	81.67 ± 9.28^{b}
Tea tree oil	17.33 ± 1.45^{d}
Green tea oil	6.67 ± 6.67^{e}
Yaqtin seed oil	6.33 ± 6.33^{e}
Ginger oil	3.33 ± 3.33^{e}
Turnip oil	2.33 ± 2.33^{e}
Black seed oil	0.00 ± 0.00^{f}
Wheat germ oil	0.00 ± 0.00^{f}
Arugula oil	0.00 ± 0.00^{f}
Argan oil	0.00 ± 0.00^{f}
Sesame oil	0.00 ± 0.00^{f}
Moringa seed oil	0.00 ± 0.00^{f}
Moringa leaf extract	0.00 ± 0.00^{f}

*Values carrying different small letters (a, b, c, d, e, and f) within the same column indicates statistical significance at $P < 0.05$

Table 5: Antifungal activity of different concentrations of volatile oils against *A. flavus* by using agar well diffusion method

Applied treatments	Concentrations of oil						
	0.5%	1%	2%	5%	10%	25%	50%
Control (+ve) Itraconazole 10 µg/disc	34.33 ±0.33^{a}	34.33 ±0.33^{a}	34.33 ±0.33^{a}	34.33 ±0.33^{b}	34.33 ±0.33^{b}	34.33 ± 0.33^{d}	34.33 ±0.33^{c}
Control (-ve) Tween 80	0.00 ±0.00^{f}	0.00 ±0.00^{f}	0.00 ±0.00^{e}	0.00 ±0.00^{h}	0.00 ±0.00^{g}	0.00 ±0.00^{h}	0.00 ±0.00^{f}
Garlic oil	7.00 ±1.15^{cd}	8.00 ±1.15^{cd}	10.00 ±1.15^{bc}	14.00 ±1.15^{d}	20.00 ±5.77^{d}	100.00 ±0.00^{a}	100.00 ±0.00^{a}
Lemongrass oil	7.00 ±0.58^{cd}	10.00 ±0.58^{c}	13.00 ±3.06^{b}	18.67 ±2.96^{c}	27.00 ±7.02^{c}	44.00 ±4.58^{c}	100.00 ±0.00^{a}
Cinnamon oil	13.00 ±1.15^{b}	15.00 ±1.15^{b}	39.00 ±3.79^{a}	48.00 ±1.53^{a}	60.33 ±4.67^{a}	70.00 ±2.89^{b}	98.33 ±1.67^{a}
Thyme oil	9.33 ±1.86^{c}	10.67 ±2.19^{c}	11.67 ±0.67^{b}	13.00 ±1.15^{de}	15.00 ±1.15^{e}	23.00 ±0.58^{e}	51.00 ±1.15^{b}
Clove oil	6.33 ±0.88^{de}	7.00 ±0.58^{d}	8.00 ±0.58^{c}	10.00 ±2.89^{ef}	10.00 ±1.15^{f}	18.33 ±4.41^{f}	26.00 ±1.15^{d}
Cumin oil	4.00 ±2.00^{e}	4.00 ±2.00^{e}	7.00 ±0.58^{c}	11.00 ±2.31^{e}	14.67 ±5.36^{e}	15.00 ±1.15^{fg}	25.00 ±1.15^{d}
Marjoram oil	4.00 ±2.00^{e}	4.00 ±2.00^{e}	4.67 ±2.33^{cd}	7.67 ±0.33^{fg}	9.00 ±0.58^{f}	11.33 ±0.33^{g}	14.67 ±1.45^{e}
Rosemary oil	4.00 ±2.00^{e}	4.33 ±2.19^{e}	4.33 ±2.19^{d}	4.33 ±2.19^{g}	7.00 ±4.04^{f}	10.67 ±3.18^{g}	12.67 ±3.18^{e}

It is considered positive when the mean of inhibition zone diameters for each surveyed oil was ≥ 10 mm. Values carrying different small letters (a, b, c, d, e, f, g) within the same column indicates statistical significance at $P < 0.05$.

Discussion

The cinnamon, lemongrass, and thyme oils at concentrations of 1% and 2% were the most effective concentrations having anti-*A. flavus* activity with the most acceptable sensory properties (Table 5). All the treated groups were exhibit a bioactivity till the last day of storage than the control group in terms of acceptability and antifungal effects (Table 6). *A. flavus* was inhibited by oils in a concentration-dependent manner. However, the overall acceptability score was not in complete harmony with the oil concentration all the time, but it is still better than the untreated group. The higher the concentration, the greater the effects on the *A. flavus* count and the lower the overall acceptability score. For examples, the effects of thyme oil 1%, cinnamon oil 1% and lemongrass oil 1% at the 9th day on the overall acceptability score were 7.33 ± 0.28, 7.13 ± 0.27, and 7.09 ± 0.18, respectively. *A. flavus* counts were 3.79 ± 0.05, 3.18 ± 0.07, and 3.61 ± 0.05, respectively after these treatments. While, at the same day, thyme oil 2% effect > lemongrass oil 2% > cinnamon oil 2% for both overall acceptability score and total *A. flavus* count.

Contamination of food with mould is a serious issue around the world. Not only causing economic losses, but also initiating hazards to animals and human. Mould detection in meat is considered one of the suggestive parameters of the hygiene status of the products, which describe the environment, the condition, and all circumstances around the manufacturing process.

In the current study, the total mould count in luncheon samples was nearly similar to that were previously reported (2.27 $\log_{10}$ CFU/g and 2.74 ± 1.3 $\log_{10}$ CFU/g) by Ebraheem and Ghadam (19), and Hamad et al. (20). Higher counts of 4.74 $\log_{10}$ CFU/g, 3.49 ± 2.48 $\log_{10}$ CFU/g, and 3.54 ± 3.5 $\log_{10}$ CFU/g were previously recorded (21-23). Meanwhile, lower count of 2.2 $\log_{10}$ CFU/g was reported by Ouf et al. (24). The TMC in beef burger (2.85 $\log_{10}$ CFU/g) was consistent with 2.87 ± 1.79 $\log_{10}$ CFU/g obtained by Hamad et al (20) and higher than 2.84 $\log_{10}$ CFU/g that was declared by Maktabi et al. (25).

Table 6: Efficacy of cinnamon oil, lemongrass oil and thyme oil at 1%, and 2% on the overall acceptability and total *A. flavus* count in beef burger artificially contaminated with *A. flavus* during storage at 3±1 °C

Applied treatments per day		Overall acceptability	*A. Flavus* count
Control	zero	8.97 ± 0.03^{aA}	3.92 ± 0.07^{aA}
	3rd	7.29 ± 0.03^{abA}	3.97 ± 0.06^{aA}
	6th	5.45 ± 0.04^{bcA}	4.11 ± 0.02^{aA}
	9th	4.60 ± 0.09^{cB}	4.12 ± 0.03^{aA}
	12th	3.60 ± 0.24^{cB}	4.15 ± 0.03^{aA}
Cinnamon oil 1%	zero	8.80 ± 0.07^{aA}	3.50 ± 0.02^{aBC}
	3rd	8.00 ± 0.30^{aA}	3.37 ± 0.04^{aBC}
	6th	7.32 ± 0.18^{aA}	3.12 ± 0.07^{aC}
	9th	7.13 ± 0.27^{aA}	3.18 ± 0.07^{aC}
	12th	6.44 ± 0.20^{aA}	3.41 ± 0.04^{aB}
Cinnamon oil 2%	zero	8.51 ± 0.07^{aA}	3.37 ± 0.04^{aBC}
	3rd	7.47 ± 0.33^{abA}	3.18 ± 0.07^{aC}
	6th	6.73 ± 0.26^{abA}	3.18 ± 0.07^{aC}
	9th	6.53 ± 0.28^{abA}	3.12 ± 0.07^{aC}
	12th	5.93 ± 0.20^{bA}	3.30 ± 0.00^{aB}
Lemongrass oil 1%	zero	8.80 ± 0.10^{aA}	3.64 ± 0.02^{aAB}
	3rd	7.84 ± 0.29^{abA}	3.58 ± 0.07^{aB}
	6th	7.29 ± 0.18^{abA}	3.50 ± 0.13^{aBC}
	9th	7.09 ± 0.18^{abA}	3.61 ± 0.05^{aB}
	12th	6.29 ± 0.17^{bA}	3.66 ± 0.02^{aB}
Lemon grass oil 2%	zero	8.71 ± 0.12^{aA}	3.28 ± 0.08^{aC}
	3rd	7.56 ± 0.19^{abA}	3.24 ± 0.11^{aBC}
	6th	6.83 ± 0.20^{abA}	3.19 ± 0.12^{aC}
	9th	6.76 ± 0.26^{abA}	3.37 ± 0.04^{aBC}
	12th	6.15 ± 0.17^{bA}	3.43 ± 0.06^{aB}
Thyme oil 1%	zero	8.68 ± 0.09^{aA}	3.87 ± 0.01^{aAB}
	3rd	8.01 ± 0.36^{aA}	3.78 ± 0.02^{aAB}
	6th	7.44 ± 0.21^{aA}	3.77 ± 0.04^{aAB}
	9th	7.33 ± 0.28^{aA}	3.79 ± 0.05^{aB}
	12th	6.41 ± 0.24^{aA}	3.82 ± 0.06^{aAB}
Thyme oil 2%	zero	8.64 ± 0.12^{aA}	3.53 ± 0.03^{aBC}
	3rd	7.79 ± 0.24^{abA}	3.42 ± 0.07^{Abc}
	6th	7.04 ± 0.17^{abA}	3.47 ± 0.05^{aBC}
	9th	6.89 ± 0.29^{abA}	3.54 ± 0.07^{aBC}
	12th	5.95 ± 0.14^{bA}	3.64 ± 0.02^{aB}

Values are mean ± SE. Values carrying different small letters (a, b, c) within each treatment indicates statistical significance at $P < 0.05$ (Tests the effect of preservation time). Values carrying different large letters (A, B, C) within the same time indicates statistical significance at $P < 0.05$ (Tests the effect of essential oil)

In sausage samples, the TMC (3.08 $\log_{10}$ CFU/g) was similar to that found in other studies (3.04 ± 2.15 $\log_{10}$ CFU/g and 3.12 ±1.23 $\log_{10}$ CFU/g) (26, 20). Higher TMCs in sausage (3.28$\log_{10}$ CFU/g and 4.6 ± 4.1 $\log_{10}$ CFU/g) were reported by Abdel Gawaad and El Leboudi (21) and by Hassan et al. (23), respectively. Meanwhile, lower counts of 2.46 ± 1.96 $\log_{10}$ CFU/g was recorded by Algammal et al. (27). In minced meat samples, TMC (2.24 $\log_{10}$ CFU/g) was coincided with 2.24 ± 1.72 $\log_{10}$ CFU/g and 2.4 ± 2.0 $\log_{10}$ CFU/g reported by Algammal et al. (27) and Saad et al.(28), respectively and lower than 3.2 ± 2.8 $\log_{10}$ CFU/g reported by Hassan et al. (23).

The mould species isolated from luncheon, beef burger, sausage, and minced meat were in agreement with those reported in previous studies (19, 22, 23, 26-30). The contamination of meat products with several mould genera could be due to several steps occur during meat manufacture including grinding, mincing, cooking, cooling, and packaging. Through all these steps, mould can find it's easily way to the meat products. So, existence of mould in meat products indicates the unsanitary conditions where these products are processed. In addition, food additives like; spices and flavorings which are raw, unsterilized and of inferior quality. Also, lacking personnel hygiene of workers dealing with meat is sharing a percent. Filling and casing sausage in contaminated intestine and insufficient heat treatment of luncheon also gives a chance for mould to persist.

The effect of different EOs concentrations varied from oil to oil. There is a need to select the most appropriate oil and concentration which exert efficacy against *A. flavus* without causing adverse organoleptic changes in formulated beef burger. Therefore, the results of this study suggested the potency of cinnamon, lemongrass, and thyme oil on *A. flavus* in corporation with chilling.

Previous investigation found that treatment of minced meat by cinnamon oil extended its shelf life and inhibited the mould growth compared with the control group, which showed deterioration signs at 6th day (28). Meanwhile, Tzortzakis and Economakis (31) recorded that 25 ppm of lemongrass oil could retard fungal spore production up to 70% and 500 ppm could inhibit the fungal sporulation completely. Moreover, Omidbeygi et al. (32) analyzed some volatile oils including thyme oil to monitor its antifungal effect. The results revealed that, thyme oil completely inhibit *A. flavus* growth *in vitro* with concentration of 350 ppm. Viuda-Martos et al. (33) approved the inhibitory effect of some volatile oils including thyme oil on *Aspergillus* genus mainly; *A. flavus* and *A. niger*. Also, thyme oil was more active on *A. flavus* than *A. niger*. Moreover, El Bayomi et al. (34) recommended the addition of thyme oil and marjoram oil to improve the wellbeing condition of meat and their products.

EOs has a lipophilic nature, which makes EO able to adhere to cell membrane structures, causing its damage, impermeability, inhibiting respiration, altering ion transport processes and finally cell death (35). The antimicrobial activities of the EOs depend on the concentration and the quality of its bioactive compounds, which in turn depend on the species, the harvest conditions, and the place where they have grown (36), the extraction method and the conditions used (solvent, temperature, and time) (37). The active compound of cinnamon EOs is trans-cinnamal-dehyde, which, has an electronegative property and in turn inhibit the microbial growth by reacting with nucleic acids and proteins (38). While, the major components of thyme were thymol (33.14%), carvacrol (19.59%), linalool (16.00%) and β-cymene (10.30%), which have key role in their antifungal activities against *A. flavus* (32).

Conclusion

The mould contamination of some meat products marketed in Zagazig city, Sharkia Governorate, Egypt demonstrating unsatisfactory hygienic measures during processing and packaging of such meat products. The most prevalent mould genera were *Aspergillus*. Such moulds might have potential health hazards. Thus, strict hygienic measures must be applied during the processing and storage of meat products. In addition, application of natural bioactive agents such as volatile oils in meat is a promising solution for mould decontamination especially thyme, cinnamon, and lemongrass oil which have antifungal activity and could extend the shelf life of the meat products.

The authors announce no conflict of interest.

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