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Biocontrol of Mycotoxigenic Fungi in Feedstuff Using Spices and *Ganoderma* Mushroom

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ABSTRACT

Food and feed contamination with mycotoxin is a global issue that represents major health risks to both animals and humans. The antifungal properties of spices and *Ganoderma* mushroom, as food preservatives, against seven toxigenic fungi isolated from Egyptian rabbit, poultry, and cow feed were investigated in this study. The growth diameter of fungal colonies was measured separately on plates of Potato dextrose agar medium with different spices at a concentration of 60 g/L. Clove, cinnamon, and turmeric completely inhibited all of the fungi tested, while the rest of the spices had a moderate to variable inhibitory effect, and several spices (coriander, fennel, anise, and caraway) even promoted the fungal growth. The five best effective spices with 16 concentrations (0.05 to 100 g/L) were used to find minimum inhibition concentration (MIC) and minimum fungicidal concentration (MFC) for each fungal isolate separately. Clove and cinnamon were the most effective spices against all tested fungal isolates, with MIC of 0.05-1 g/L and MFC of 3-10 g/L. Hence, clove and cinnamon are recommended, as the best antifungal spices that can easily inhibit fungal growth at a minimal concentration. Mycelial plugs of *Ganoderma mbrekobenum* exhibited a high inhibition activity against the growth of *Monascus ruber*, *Aspergillus ochraceus*, and *Penicillium* sp. The antifungal activities of aqueous and organic extracts of *Ganoderma* mushroom were investigated, and the methanol-chloroform extract was shown to have the maximum activity, making it a good antifungal agent.

Keywords: Antifungal activity; Food spices; *Ganoderma mbrekobenum*; Growth inhibition; Mycotoxins.

INTRODUCTION

Food and animal feedstuff are exposed to infection by many fungi, including those produce low-molecular-weight compounds as secondary metabolites, with confirmed toxic properties referred to mycotoxins. The poor and prolonged storage period gives an opportunity for these fungi to produce their toxins in large quantities causing serious harm to human and animal health (Bhat *et al.*, 2010). Mycotoxins cause adverse health effects and fatal diseases for animals leading to economic losses in the form of decreased productivity. These effects can be mutagenic, carcinogenic, oestrogenic, neurotoxic, and immunosuppressive, interfere with hormonal functions, or exhibit other toxic effects on organs such as the liver and kidney (Turner *et al.*, 2009). Hence, preventive strategies are required to decrease feedstuff fungal contaminations and mycotoxins production according to the ALARA (as low as reasonably achievable) principle.

Many chemical and physical methods have been used to preserve foods, but these methods are restricted in many developed countries due to their negative effects (Köhl *et al.*, 2011). Therefore, the world has recently turned to using natural and safer food preservatives (Sharma *et al.*, 2009). Using spices was the most appropriate natural method, as they have been traditionally used as flavorings for the palatability of food and drinks with no recorded harm to human health. In addition, spices possess a medicinal value containing various bioactive compounds with antioxidant and antimicrobial properties. It is used as a treatment for minor medical conditions such as stomach disorders, sleep problems, poor circulation, ulcers, colds, muscle pain, gout, back pain, dyspepsia, and motion sickness as well as anticancer effects (Rathore *et al.*, 2013). Many spices are known to exert antioxidant activity preventing lipid oxidation in living organisms as well as foods **(**El-Mougy and Abdel-Kader, 2007 and Rathore *et al.*, 2013). Moreover, spices proved antimicrobial ability to prevent contamination with mycotoxin-producing fungi, and limits their growth and ability to produce mycotoxins. Gould (1996) suggested using spices as alternatives for food conservation based on the synergistic effect of antimicrobial compounds from animal, plant and/or microbial origin, to create an inhospitable environment for microbial survival in foods. Incorporation of spice extract or its essential oil into edible packaging exerts antimicrobial activity against the food pathogens thus preventing food spoilage and enhances the shelf-life and also increases the nutritional value of the final product (Ravi *et al.*, 2020). Antimicrobial activity of spices depends on several factors like the kind of spice, composition, and concentration of spice, its occurrence level, substrate composition, processing conditions, and storage (Ceylan and Fung, 2004).

In addition to the amazing benefits of mushrooms for humans, the bioactive compounds extracted from many mushroom types have proven their ability as an anti-cancer, anti-microbial and anti-viral. Several compounds extracted from mushroom revealed antifungal and antibacterial activity. Several studies showed that

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Ganoderma mushroom possess antimicrobial activities, antiviral, anticancer, anti-inflammatory, antioxidant activity, hypoglycemic, immunosuppressive, hepato-protective activities, anti-allergic, anti-hypertension and anti-ageing (Lindequist *et al.*, 2005). In this study, we focused on the importance of using raw spices and *Ganoderma* fungus as natural food preservatives that are safe for animals, as well as to minimize the signify-cant economic losses that may be associated with increased health-care costs. Therefore, the goal of this study was to test the antifungal activity of 13 different food spices, as well as *Ganoderma* mycelium and its fruiting body, against seven toxigenic fungal strains isolated from various types of animal feedstuff.

MATERIALS AND METHODS

Source of toxigenic fungal isolates

Thirty-seven isolates belonging to phylum Asco-mycota were isolated from various samples of Egyptian animal feedstuff of poultry, rabbits, and cattle. Fungal isolation was carried out using the serial dilutions technique on Potato dextrose agar (PDA) plates were incubated in two sets at 25 °C for 5-7 days. Pure fungal cultures were maintained on PDA slopes at 4 °C and stored in 10% glycerol at - 80°C. Seven species were selected based on high production of mycotoxins using UV light detector for fungal cultures on coconut med-ium according to Heenan *et al.*, (1998). These isolates were identified as *Aspergillus flavus* 1, *Aspergillus flavus* 2, *Aspergillus niger*, *Aspergillus ochraceus*, *Asp-ergillus terreus*, *Monascus ruber*, and *Penicillium* sp. based on their morphological (macro and microscopic) characters using the standard keys of Onions *et al.*, (1981), Pitt and Hocking (1997), and Barnett and Hunter (1998).

Antifungal activity of spices

Thirteen kinds of food spices were collected, for the current study, from different markets located in Damietta Governorate, Egypt (Table1). The spices effect was tested separately at concentration of 60 g/L on the growth of the seven fungal strains according to the method of Zohri *et al.* (2014) with some modify-cations. The spices were ground by an electric blender (Moulnex®, Type 719, France) into a very fine powder and added separately to flasks of PDA medium at concentration of 60 g/L then autoclaved for 20 min at 121°C. After distribution and solidification of media with spices into Petri dishes (9 cm), plates were inoculated with one disc (diameter, 0.4 cm) of fresh PDA-fungal cultures. The plates were incubated at 25°C for four days. The diameter of the fungal colony was measured in comparison to the control colony (growing on PDA with no spices). The inhibition of fungal growth (%) was calculated according to the following formula:

$$\text{Inhibition } \% = \frac{Ccd - TcB}{Ccd} \text{X} 100$$

Where, *Ccd* is the diameter of fungal colony in control plate and *TcB*, is fungal colony diameter in spice-treated plate.

Table (1): Spice plants and the parts that have been investigated

No.	Spices tested		Used part
	Common name	Scientific name	
1	Clove	*Syzygium aromaticum*	Dried flower bud
2	Cinnamon	*Cinnamomum cassia*	Cinnamon bark
3	Cumin	*Cuminum cyminum*	Seeds
4	Thyme	*Thymus vulgaris*	Seeds
5	Ginger	*Zingiber officinale*	Rhizome
6	Black pepper	*Piper nigrum*	Seeds
7	Fennel	*Foeniculum vulgare*	Seeds
8	Cardamon	*Elettaria cardamomum*	Seeds
9	Turmeric	*Curcuma longa*	Rhizome
10	Coriander	*Coriandrum sativum*	Seeds
11	Anise	*Pimpinella anisum*	Seeds
12	Caraway	*Carum carvi*	Seeds
13	Rosemary	*Rosmarinus officinalis*	Dried leaves

Assay for minimum inhibition concentration

Minimum inhibitory concentration (MIC) is defined as the minimum concentration of the material that inhibits the growth of the particular microorganisms. The method is based on growing fungi at varying concentrations of spices in media. Selected five spices (clove, cinnamon, cumin, turmeric and black pepper) were tested separately for their minimum inhibition concentration (MIC) using a series of concentrations (0.05, 0.1, 0.5, 1, 3, 5, 7, 10, 13, 15, 20, 30, 40, 50, 60, and 100 g/L) in PDA media. The diameter of the fungal colony was measured after four days of incubation at 25°C in comparison to the control colony. Fungal growth inhibition percentage (%), minimum inhibition concentration (MIC), at which the spices extract showed the earliest inhibition of the fungal growth and minimum fungicidal concentration (MFC) were determined.

Antifungal activity of *Ganoderma* mycelia

The mycelial culture of *Ganoderma* sp. EGDA, first identified by El-Fallal *et al.* (2015) and later clarified to *Ganoderma mbrekobenum* (Otto *et al.*, 2016), was tested for its ability to suppress the growth of mycotoxin-producing fungus using the agar disc diffusion method. *G. mbrekobenum* fruit bodies were collected from lemon trees grown in Damietta district, Egypt. Inoculation with three days old culture of spore suspension, of investigated fungal strains, was done in the centre of PDA plates, followed by discs insertion (diameter, 1 cm) of 7 days old *Ganoderma* culture. Plates were incubated at 28 °C for 3-5 days. The diameter of clear inhibition zone around the *Ganoderma* disc was measured in millimeters.

Antifungal activity of *Ganoderma* mushroom

Preparation of crude extract

Wild mushroom of *G. mbrekobenum* dried at room temperature and then grinded into fine powder. A sample (2.5 g) of ground mushroom was extracted by 50 ml of Methanol:Chloroform (2:1) mixture by heating and stirring continuously at 60°C for 45 min,

then filtered through Whatman filter paper No.4. The residue was re-extracted twice. All extracts were combined and completely evaporated using a rotary evaporator at 40°C. Distilled water was used for aqueous extraction in the same way as before. The dried organic extracts that contain the bioactive compounds were kept at 4°C until use.

Preparation of different fractions extract

A series of organic solvents were used for several fractions' extraction of *Ganoderma* mushroom in order of increasing polarity as shown in Fig. (1). A sample (2.5 g) of mushroom powder were extracted using 75 ml each of petroleum ether, chloroform, acetone, ethyl acetate, and methanol, successively on a rotary shaker at 150 rpm for one day under dark condition at room temperature. The extracts were filtered through double Whatman filter paper No.1, and the extracts were evaporated to dryness at 40°C using a rotary vacuum evaporator, then stored in refrigerator at 4°C until use

Antifungal assay

Both organic and aqueous extracts were dissolved in a definite volume of Dimethyl Sulfoxide (DMSO) to a concentration of 20 mg/L. Agar well diffusion method was used to test the antifungal activity of all extracts in which 100 µl of each extract was inserted in a well (diameter, 1 cm) made on PDA plates previously inoculated with the tested fungi, DMSO was used as a negative control and then plates were incubated at 28°C. The diameter of the clear zone of inhibition around the wells was measured in millimeter after for 3-5 days.

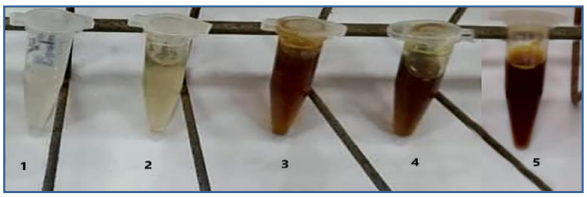

Figure (1): Different organic extracts, based on polarity, of *G. mbrekobenum* mushroom. Solvent used: 1, petroleum ether; 2, chloroform; 3, acetone; 4, ethyl acetate and 5, methanol.

Statistical analysis

All experiments were carried out in three replicates. The mean values of the measured data ±SD were represented and a p value at ≤ 0.01 was considered significant.

RESULTS

Fungal isolates

Forty-three isolates, belonging to Ascomycota (37 isolates) and Zygomycota (6 isolates) phyla, were obtained from different feedstuff samples (poultry, rabbits and cattle). Rabbit feed recorded the highest significant ($p \leq 0.01$) source in fungal species richness (32%), followed by poultry (20%) and cattle feed (18%). The most prevalent fungi in the feed samples were *Aspergillus* and *Penicillium* species, which were detected in 100% and 75% of the samples, respectively. Seven species were selected based on their highest production of mycotoxins on coconut medium. They were morphologically identified as *Aspergillus flavus* 1, *Aspergillus flavus* 2, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus terreus*, *Monascus ruber* and *Penicillium sp*.

Antifungal activity of Spices

The measured inhibition percentage of fungal growth using the thirteen spices at a concentration of 60 g/L showed that cloves, cinnamon and cumin were lethal (with a 100% inhibition) to all tested mycotoxigenic fungal strains, as presented in Fig. (2). Turmeric and black pepper showed a stronger inhibitory effect on all tested strains than the rest of the spices. Cardamom, rosemary, ginger and thyme showed a moderate inhibitory effect on fungi, with the highest activity against *A. flavus* 1 (66.97, 61.47, 52.29 and 37.61 %, respectively) and *A. flavus* 2 (75.00, 64.29, 53.57 & 36.61 %, respectively).

On the other hand, thyme had a noticeable reducing effect on the spore production for all the tested fungal strains as it inhibited the spore maturation and their distinctive pigments for all the tested fungi strains as shown in Fig. (3). Meanwhile, anise had a moderate effect on *A. flavus* 1 and *A. flavus* 2, and was weak on *A. terreus* and *Penicillium* sp., but induced growth increases in *A. ochraceus*, *A. niger*, and *M. ruber*. Tested fungi were least affected by caraway, coriander, and fennel. Compared to the control, caraway was slightly effective on *A. flavus* 1, *A. flavus* 2, *A. terreus*, and *Penicillium sp*. However, it had no impact on *A. ochraceus*, and nourished the fungal growth of *A. niger* (Fig. 4). Furthermore, fennel had a negligible effect on *A. flavus* 1 and 2. In parallel, it had no effect on *Penicill-ium* sp., but boosted the development of *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus terreus*, and *M. ruber*. While coriander was ineffective against all the fungi tested, it assisted *A. flavus* 1, *A. flavus* 2, *A. terreus*, and *Penicillium* sp. grow better.

MIC and MFC of the tested species

Five spices out of the thirteen were selected based on the highest inhibitory effect against the growth of the seven fungal strains. The percentage of fungal growth inhibition increased obviously with the increase of the spices' concentration. Clove, cinnamon and cumin approved their ability to totally (100%) eliminate the growth of all tested fungi, while turmeric and black pepper reached maximum inhibition activity of 80% only for all fungi.

For MFC and MIC (Table 2), clove recorded MFC (5 g/L) for all fungi with 100% inhibition of growth except for *A. niger* and *Penicillium* sp. (3 g/L). Meanwhile, MIC was 0.05 g/L for all tested fungi except for *M. ruber* (0.1 g/L). However, in the case of cinnamon, MIC recorded 0.05 g/L for *A. flavus* 1, *A. flavus* 2, *A. terreus* and *M. ruber*, 0.1 g/L for *A. niger* and *Penicillium* sp. and 1 g/L for *A. ochraceus*. MFC was at concentration of 7 g/L for *A. niger*, *A. terreus* and *M. ruber* and 10 g/l for *A. flavus* 1, *A. flavus* 2, *A. ochreceus* and *Penicillium* sp. with 100% inhibition of growth. Cumin was the third spice in the fungicidal activity and its MIC was 0.05 g/L for *A. terreus* and *M. ruber*, 0.1 g/L for *A. flavus* 2, 0.5 g/L for *A. flavus* 1, 1 g/L for *A. niger*, and 3 g/L for *A. ochraceus* and *Penic-*

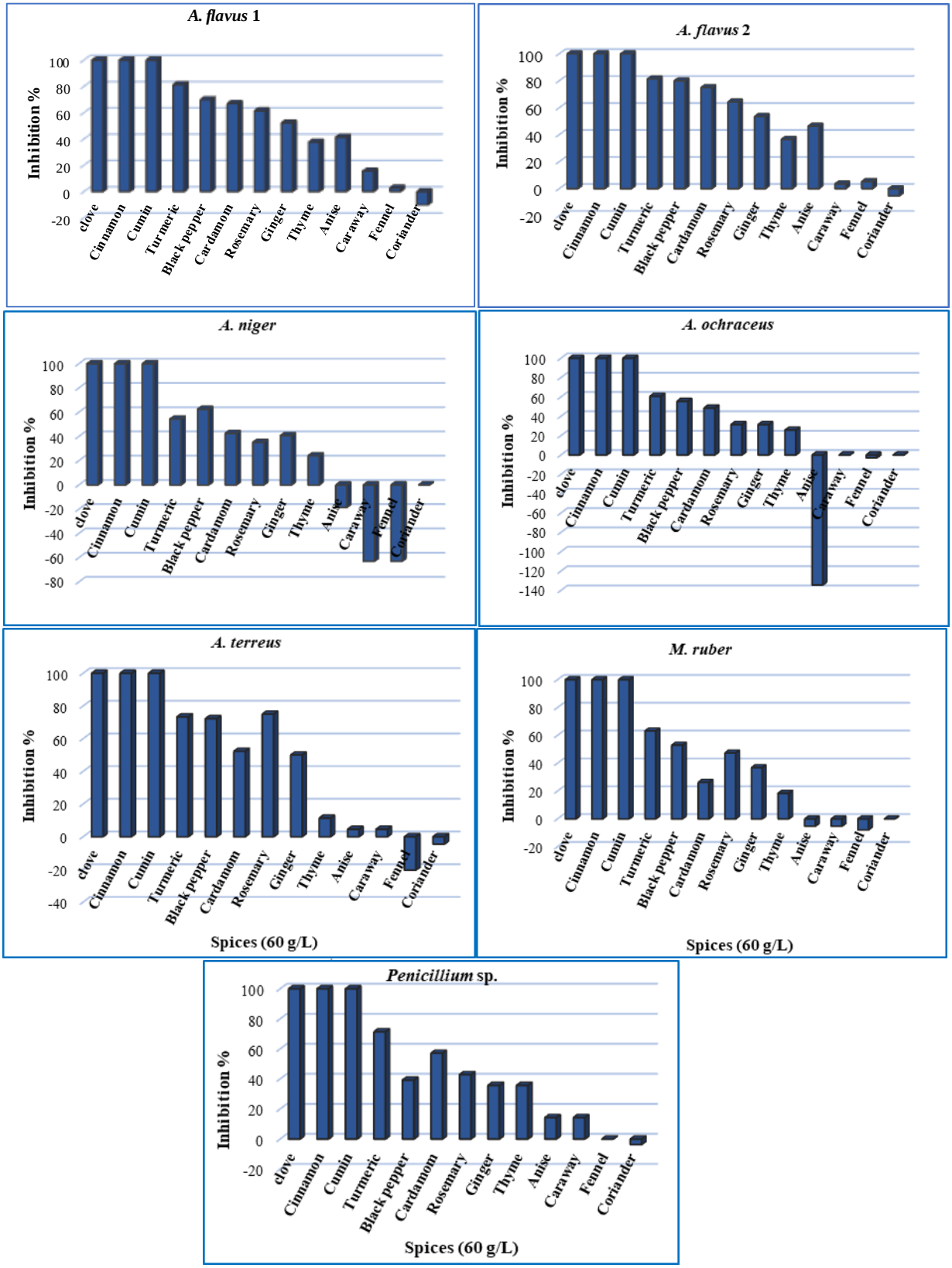

Figure. (2):. Antifungal activity was evaluated, for different spices at a concentration of 60 g/L on PDA medium, after four days of incubation. All tests were carried out in three replicates and the mean values of results were represented. Antifungal activity was tested on five *Aspergillus* species, *Monascus ruber* and *Penicillium* sp.

illium sp. While MFC was at concentration of 15 g/L for *A. flavus* 1, 30 g/L for *M. ruber*, 40 g/l for *A. terreus*, 50 g/L for *A. niger* and *Penicillium* sp., 60 g/L for *A. flavus* 2 and *A. ochraceus* with 100% inhibition of growth**.** However, turmeric was completely non-lethal. Its inhibitory effect was very close and increased slowly starting from 10 g/L with increasing concentrations. MIC was 0.05 g/L for all tested fungal isolates except *A. terreus* was 0.1 g/L. Likewise; black pepper was not completely fatal. It did lead to steady growth despite the increase in concentrations, starting from a concentration of 13 g/L, with a sudden and severe decrease at 100 g/L for *A. flavus* 1 (88.68%) for the fourth day. MIC was 0.05 g/L for all tested fungal isolates except *A. flavus* 1 and *Penicillium* sp. was 0.1 g/L as presented in Table 3.

Antifungal activity of *Ganoderma* mycelia

The highest inhibition zone was observed for *M. ruber* followed by *A. ochraceus* and finally *Penicillium* sp. (25 ±0.5, 16 ±0.0 and 15 ± 0.4 mm, respectively). However, no effect was observed on the rest of the tested fungi (Figures 6 and 7).

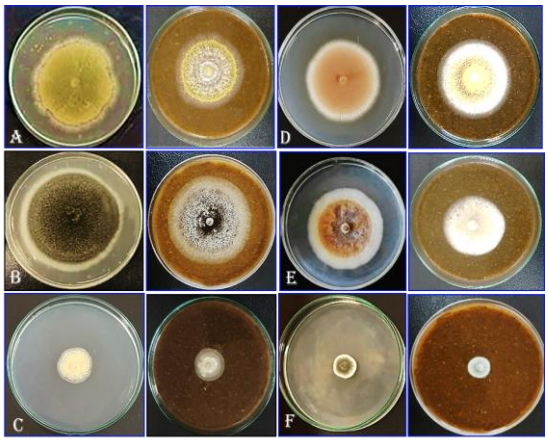

Figure (3): Antifungal effect of thyme (60 g/L in PDA medium) on fungal growth after four days (control colony on right and colony with tested spice on left); (A) *A. flavus*2, (B) *A. niger*, (C) *A. ochraceus*, (D) *A. terreus*, (E) *M. rubber* and (F) *Penicillium* sp.

Antifungal activity of *Ganoderma* mushroom

As shown in Fig. 8, different mushroom extracts had varying levels of inhibition against the majority of the fungal species examined, except for *A. flvus* 1,

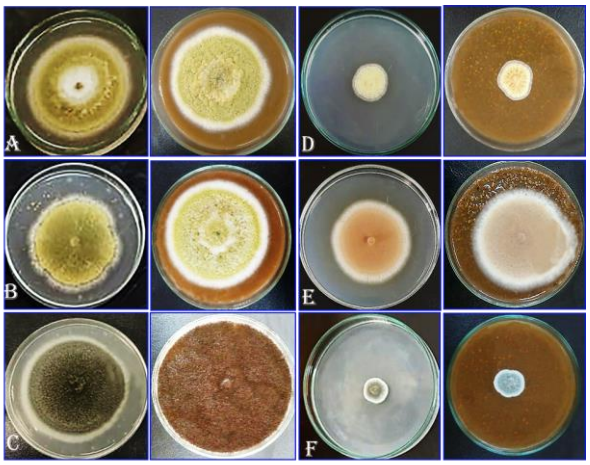

Figure (4): Antifungal effect of caraway (60 g/L in PDA medium) on fungal growth after four days of incubation (control colony on right and colony with tested spice on left); (A) *A. flavus* 1, (B) *A. flavus* 2, (C) *A. niger*, (D) *A. ochraceus*, (E) *A. terreus* and (F) *Penicillium sp*.

2 and *A. terreus*. *M. ruber*, *A. ochreceus*, *Penicillium* sp., and *A. niger*, where no activity was recorded. The highest antifungal activity was recorder for mushroom extract with methanol chloroform mixture (20 ±0.6, 19 ±0.6, 16 ±0.0 and 26 ±0.3 mm) against *M. ruber*, *A. ochre-ceus*, *Penicillium* sp. and *A. niger*. The extract induced the production of pink pigment in *A. ochraceus* and inhibited the maturation of *A. niger* spores (white colored). However, some extracts such as petroleum ether, acetone, and ethyl acetate were non effective on *Penicillium* sp. Meanwhile, acetone has no effect on *A. niger*.

DISCUSSION

The incidence of mycotoxigenic fungal infections particularly ascomycetes are becoming more wide-spread over the world. The resistance of some fungal species to antifungal drugs, high treatment costs and toxic effects of current drugs all have encouraged researchers to look for alternative natural compounds. The spices are recently used to preserve feed and combat fungal growth and mycotoxins production due to their antimicrobial and antioxidant properties and no negative impact on animal and human health (Liu *et al.*, 2017 and Purkait *et al.*, 2020). Many investi-gations reported the inhibitory effect of spices on fungi with variable responses to different types

Table (2): MFC, MIC and its opposite growth inhibition % recorded for spices against the tested fungi.

Plant Spice used	Tested Fungi													
	***A. flavus*1**		***A. flavus* 2**		***A. niger***		***A. ochraceus***		***A. terreus***		***M. ruber***		***Penicillium* sp.**	
	ǂ**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Clove	5.00	0.05	5.00	0.05	3.00	0.05	5.00	0.05	5.0	0.05	5.0	0.10	3.00	0.05
Cinnamon	10.0	0.05	10.0	0.05	7.00	0.10	10.0	1.00	7.0	0.05	7.0	0.05	10.0	0.10
Cumin	15.0	0.50	60.0	0.10	50.0	1.00	60.0	3.00	40.0	0.05	30.0	0.05	50.0	3.00
Turmeric	nd	0.05	nd	0.05	nd	0.05	nd	0.05	nd	0.1	nd	0.05	nd	0.05
Black pepper	nd	0.1	nd	0.05	nd	0.05	nd	0.05	nd	0.05	nd	0.05	nd	0.10

ǂ1, Minimal fungicidal concentration, MFC (g/L); 2, Minimal inhibitory concentration of the tested materials, MIC (g/L); nd, not detected.

of spices (Bokhari, 2007 and Bokhari & Aly, 2009). In the current study, thirteen food spices were tested for their effectiveness against seven mycotoxin-producing fungal species isolated from different feedstuff. Most of the spices showed promising antifungal effects against all the tested fungi, these effects may be attributed to their content of essential oils that can penetrate fungal cells causing alterations in its structure and function and diverse groups of phytochemicals with mechanisms of action such as strong antioxidant phenolic and γ-terpinene compo-

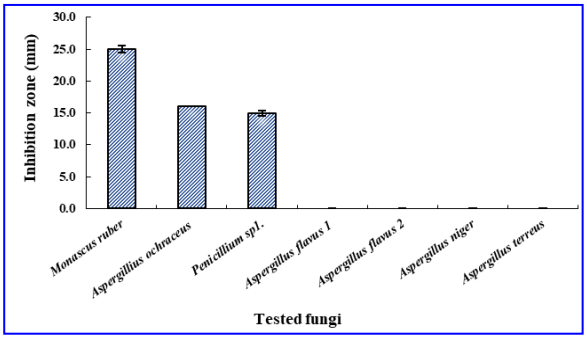

Figure (6): Antifungal activity of *Ganoderma mbrekobenum* mycelia.

unds (Bassolé & Juliani, 2009). In addition, clove proved the highest inhibitory activity against all tested fungi at a concentration of 5 g/L. In accordance, Zohri *et al.* (2014) reported that clove was the most effective against *A. flavus*, *A. ochraceus* and *P. citrinum*, and lethal to them at a concentration of 5 g/L. Several other studies have also reported antifungal (inhibitory and fungicidal) action of clove (Reddy *et al.*, 2007 and Achar *et al.*, 2020*).* Cinnamon came secondly in its antifungal activity with complete inhibition of all tested fungi at a concentration of 10 g/L. likewise; Kumar *et al.* (2020) reported that cinnamon and clove exhibited great antifungal effic-iency with wide zones of inhibition. Bokhari(2007) and Zohri *et al.* (2014) recorded a complete growth inhibition of *A. flavus* and *A. ochraceus* by cinnamon at the same concentration (10 g/L). Cumin came in the third position of antifungal activity with 100% inhibition efficiency at a concentration of 15 g/L. These results agree with the previous studies of Bokhari (2007) and Zohri *et al.* (2014). Eugenol is the major aromatic oil of clove (70-90%), also found in lower concentrations in cinnamon and other aromatic spices and possess powerful antioxidant and antimicrobial benefits. It was identified as the antifungal component, responsible for the growth inhibition of Aspergilli (Reddy *et al.*, 2007) and showed fungistatic and fungicidal activities on solid and broth medium, respectively (Chee & Lee, 2007). Li *et al.* (2014) and Liu *et al.* (2017) suggested the possible mechanism of antifungal activity of clove and cinnamon. They reported that eugenol and cinnamon oil can destroy cell walls and membranes causing the cytoplasmic leakage, inhibiting the ergosterol synthesis then inhibit the normal synthesis of DNA and proteins.

Furthermore, the antifungal activity of the rest of the spices decreased gradually from turmeric to thyme (Fig. 2). However turmeric and black pepper didn't prove any MFC for any of the tested fungi, they prove a MIC at 0.05 and 0.1 g/L, respectively (Table 3), and their inhibition activity reached 80%. Oza *et al.* (2021) reported the antifungal effect of raw turmeric powder against *Aspergillus* sp. and *Fusarium* sp. The antif-ungal activity of turmeric was attributed to the essential turmeric oil and a polyphenolic compound called curcumin (Apisariyakul *et al.*, 1995), while piperine is the major bioactive component of pepper the proved a significant antifungal activity in previous study of Yohannes *et al.*, (2018). Thyme caused a clear inhi-bition in the reproductively of all tested fungal strains leading to a decrease in the spore production. Borugă *et al.* (2014) also reported this antifungal activity.

On the other hand, caraway, anise, fennel and coriander caused some stimulation and flourish of the fungal growth. This stimulatory effect may be ascribed to the presence of myrcene in both spices (Khalil *et al.*, 2018) since the addition of myrcene significantly promoted the spore germination of *P. digitatum* (Che *et al.*, 2020). This may agree with the observation of El-Mougy & Abdel-Kader (2007) for a lower antifungal activity of fennel powder than cinnamon, and Thyagaraja & Hosono (1996) for a weaker antifungal effect of coriander than cumin and pepper. While it may contradict the study of Hassan *et al.* (2020), who recorded a significant antifungal activity of caraway and its essential oil (carvone) against *A. niger*. To the best of our knowledge, this is the first study done on *M. rubber*, which is known for its high production of citrinin toxin, and the results showed that cloves, cinnamon and cumin were able to inhibit its growth completely.

The antimicrobial activity of extracts and comp-ounds from *Ganoderma* indicate that this genus is worthy of further study according to Reddy (2018). Both mycelium and mushroom of different mushrooms contain several compounds which possess antifungal and antibacterial activities such as terpenoids, steroids,

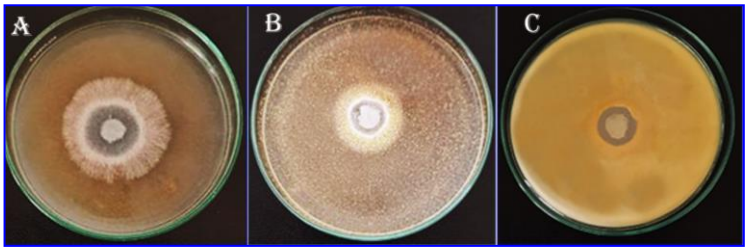

Fig. (7). Antifungal activity of *Ganoderma mbrekobenum* mycelia on: (A) *M. rubber*, (B) *A. ochraceus* and (C) *Penicillium* sp.

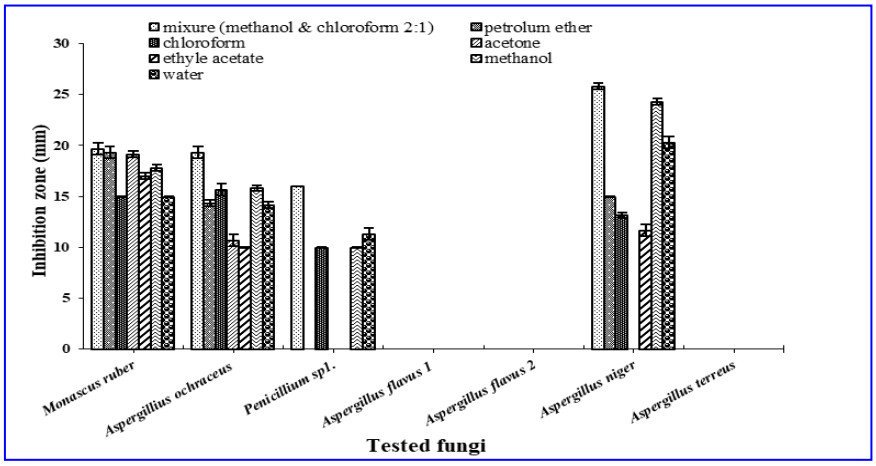

Fig. 8. Antifungal activity of different extracts of *G. mbrekobenum* fruiting body.

polyphenol, polyketides, polyglucan, flavonoids, alkaloids, polysaccharides and dietary fibers which exert several pharmacological activities (Chowdhury *et al.*, 2015). The antifungal activity of *G. mbrekobenum* was evaluated using two different methods: mycelial agar disc, mushroom extractions. The mycelial discs showed a potential high antifungal activity against *M. ruber*, *A. ochraceus* and *Penicillium sp*. This effect may be attributed to both intercellular components and extracellular metabolites as enzymes, toxins, antioxidants.

On the other hand, mushroom extract using methanol chloroform mixture proved the strongest antifungal activity compared to other extracts using organic solvents and water. The highest effect was against *A. niger*, followed by *M. ruber*, then *A. ochraceus*, and finally *Penicillium* sp. No inhibition zone was recorded against *A. flavus* 1 and 2, and *A. terreus*. The various antifungal activities of different solvent extracts may be attributed to different extracted bioactive compounds as aliphatic esters, aromatic esters, fatty acids, alkaloids, saturated aromatic compounds and isocyanide. Likewise, Ameri *et al.* (2011) reported that aqueous extracts of *G. lucidum*, *G. Praelongum* and *G. resinaceum* didn't have antimicrobial activity.

The low activity of water extract might be due to poor solubility of bioactive compounds in water compared to organic solvent. Sridhar *et al.*, (2011) reported that methanol and aqueous extracts of *G. lucidum* mushroom possessed strong antibacterial and antifungal activity. However, *G. lucidum* extracts showed slightly higher activity against *Penicillium* and lower activity against *A. niger*. The antifungal activity of aqueous extract against *A. flavus* was showed no antifungal effect as obtained in current study. In addition, Jonathan & Awotona (2010) proved that water extracts of *G. lucidum*, *G. applanatum* and *G. austral* mushrooms exerted less antimicrobial activity than methanolic and ethanolic extracts against fungal pathogens. Furthermore, Heleno *et al.* (2013) revealed that *G. lucidum* possessed a high activity against different fungal species; *A. ochraceus*, *A. fumigatus*, *A. niger*, *A. versicolor*, *P. verrucosum*, *P. funiculosum*, *P. ochrochloron* and *Trichoderma viride*.

CONCLUSION

This study presents powder of spices and mushroom mycelia and fruit body as available, inexpensive, and easy-to-use natural products to combat the growth of certain mycotoxin-producing fungal strains isolated from various animal feed. They considered a promising antifungal alternative for feed safety with the superior inhibitory effect attributed to their essential oils, which may provide a wide and useful application for several aspects including food preservation and disease prevention. Clove and cinnamon are recommended as the best anti-fungal spices that can be used, as they can inhibit the fungal growth significantly at a minimal concentration of 0.05 g/L. In addition, they are abundant with low prices and affordable for many people. While using coriander, fennel or caraway is not recommended, as they do not affect the growth of those mycotoxin-producing strains. Furthermore, both mycelial plugs and mush-room extracts of *G. mbrekobenum* can be recommended as antifungal activity against *M. rubber*, *A. ochraceus* and *Penicillium* sp.

REFERENCES

ACHAR, P.N., P. QUYEN, E.C. ADUKWU, A. SHARMA, H.Z. MSIMANGA, H. NAGARAJA AND M.Y. SREENIVASA. 2020. Investigation of the antifungal and anti-aflatoxigenic potential of plant-based essential oils against *Aspergillus flavus* in peanuts. Journal of Fungi, 6(383): 1-19.

AMERI, A., J.G. VAIDYA AND S.S. DEOKULE. 2011. In vitro evaluation of anti-staphylococcal activity of *Ganoderma lucidum*, *Ganoderma praelongum* and *Ganoderma resinaceum* from Pune, India.

African Journal of Microbiology Research, 5(3): 328-333.

APISARIYAKUL, A., N. VANITTANAKOM AND D. BUDDHASUKH. 1995. Antifungal activity of turmeric oil extracted from *Curcuma longa* (Zingiberaceae). Journal of Ethnopharmacology, 49(3): 163-169.

BARNETT, H.L. AND B.B. HUNTER. 1998. Illustrated genera of imperfect fungi. Illustrated genera of imperfect fungi., 4th ed. American Phytopathological Society.

BASSOLÉ, I.H.N. AND H.R. JULIANI. 2012. Essential oils in combination and their antimicrobial properties. Molecules, 17(4): 3989-4006.

BOKHARI, F. AND M.M. ALY. 2009. Trials towards reduction of fungal growth and aflatoxin G1 production in Arabic coffee using different additives. African Journal of Food Science, 3(3): 068-076.

BOKHARI, F.M. 2007. Spices mycobiota and mycotoxins available in Saudi Arabia and their abilities to inhibit growth of some toxigenic fungi. Mycobiology, 35(2): 47-53.

BORUGĂ, O., C. JIANU, C. MIŞCĂ, I. GOLEŢ, A.T. GRUIA AND F.G. HORHAT. 2014. Thymus vulgaris essential oil: chemical composition and antimicrobial activity. Journal of Medicine and Life, 7(3): 56-60.

CEYLAN, E., AND D.Y. FUNG. 2004. Antimicrobial activity of spices 1. Journal of Rapid Methods and Automation in Microbiology, 12(1): 1-55.

CHE, J., Y. CHEN, Y. WU, L. LI AND N. TAO. 2020. Metabolomics analysis reveals that myrcene stimulates the spore germination of *Penicillium digitatum* via the upregulation of central carbon and energy metabolism. Postharvest Biology and Technology, 170: 111329.

CHEE, H.Y. AND M.H. LEE. 2007. Antifungal activity of clove essential oil and its volatile vapour against dermatophytic fungi. Mycobiology, 35(4): 241-243.

CHOWDHURY, M.M.H., K. KUBRA AND S.R. AHMED. 2015. Screening of antimicrobial, antioxidant properties and bioactive compounds of some edible mushrooms cultivated in Bangladesh. Annals of clinical microbiology and antimicrobials, 14(1): 1-6.

EL-FALLAL, A.A., A.K.A. EL-SAYED AND S.R. El-ESSEILY. 2015. First record of two *Ganoderma* species from North East Nile Delta-Egypt. Mycosphere, 6(3): 248-259.

EL-MOUGY, N.S. AND M.M. ABDEL-KADER. 2007. Antifungal effect of powdered spices and their extracts on growth and activity of some fungi in relation to damping-off disease control. Journal of Plant Protection Research, 47(3): 267-278.

GOULD, G.W. 1996. Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. Journal of Food Protection, 59(13): 82-86.

HASSAN, H.A., M.M. GENAIDY, M.S. KAMEL AND S.F. ABDELWAHAB. 2020. Synergistic antifungal activity of mixtures of clove, cumin and caraway essential oils and their major active components. Journal of Herbal Medicine, 24: 1-5.

HEENAN, C.N., K.J. SHAW AND J.I. PITT. 1998. Ochratoxin A production by *Aspergillus carbonarius* and *A. niger* isolates and detection using coconut cream agar. Journal of food mycology, 1(2): 67-72.

HELENO, S.A., I.C. FERREIRA, A.P. ESTEVES, A. ĆIRIĆ, J. GLAMOČLIJA, A. MARTINS AND M.J.R. QUEIROZ. 2013. Antimicrobial and demelanizing activity of *Ganoderma lucidum* extract, p-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. Food and chemical toxicology, 58: 95-100.

JONATHAN, S.G. AND F.E. AWOTONA. 2010. Studies on antimicrobial potentials of three *Ganoderma* species. African Journal of Biomedical Research, 13(2): 131-139.

KHALIL, N., M. ASHOUR, S. FIKRY, A.N. SINGAB AND O. SALAMA. 2018. Chemical composition and antimicrobial activity of the essential oils of selected Apiaceous fruits. Future Journal of Pharmaceutical Sciences, 4(1): 88-92.

KÖHL, J., J. POSTMA, P. NICOT, M. RUOCCO AND B. BLUM. 2011. Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. Biological Control, 57(1): 1-12.

KUMAR, A., P.P. SINGH, V. GUPTA AND B. PRAKASH. 2020. Assessing the antifungal and aflatoxin B1 inhibitory efficacy of nanoencapsulated antifungal formulation based on combination of *Ocimum* spp. essential oils. International Journal of Food Microbiology, 330, 108766, 1-13.

LI, Y., Y. NIE, L. ZHOU, S. LI, X. TANG, Y. DING AND S. LI. 2014. The possible mechanism of antifungal activity of cinnamon oil against *Rhizopus nigricans*. Journal of Chemical and Pharmaceutical Research, 6(5): 12-20.

LINDEQUIST, U., T.H. NIEDERMEYER AND W.D. JÜLICH. 2005. The pharmacological potential of mushrooms. Evidence-based complementary and alternative medicine, 2(3): 285-299.

LIU, Q., X. MENG, Y. LI, C.N. ZHAO, G.Y. TANG AND H.B. LI. 2017. Antibacterial and antifungal activities of spices. International Journal of Molecular Sciences, 18(6): 1283.

ONIONS, A.H.S., G. SMITH AND D. ALLSOPP. 1981. Introduction to industrial mycology. (Cambridge University Press1992), 406.

OTTO, E.C., R.A. BLANCHETTE, AND B.W. HELD. 2016. *Ganoderma mbrekobenum* E.C. Otto, Blanchette, Held, C.W. Barnes & Obodia, sp. nov. Fungal Planet description sheets: 449-4. Persoonia: 36, 416-417.

OZA, K., B.K. JAIN, AND B. MAITREYA. 2021. Spoilage of fruits and vegetables due to fungal infection and its post-harvest control: A review. International Journal of Botanical Studies, 5: 694-697.

PITT, J.I. AND A.D. HOCKING. 1997. Fungi and Food Spoilage. 2nd Ed.: Blackie Academic and Professional, London.

PURKAIT, S., A. BHATTACHARYA, A. BAG AND R.R. CHATTOPADHYAY. 2020. Synergistic antibacterial, antifungal and antioxidant efficacy of cinnamon and clove essential oils in combination. Archives of Microbiology, 202(6): 1439-1448.

RATHORE, S.S., S.N. SAXENA AND B. SINGH. 2013. Potential health benefits of major seed spices. International Journal of Seed Spices, 3(2): 1-12.

RAVI, K., K. GOYAL, S. PRIYADARSHI AND M.M. NAIDU. 2020. Spice bioactives in edible packaging. Journal of Spices and Aromatic Crops, 29(2): 81-97.

REDDY, C.S., K.R.N. REDDY, M. PRAMEELA, U.N. MANGALA AND K. MURALIDHARAN. 2007. Identification of antifungal component in clove that inhibits *Aspergillus* spp. colonizing rice grains. Journal of Mycology and Plant Pathology, 37(1): 87-94.

REDDY, E.P. 2018. Phytochemical Analysis and Antifungal Activity of *Ganoderma lucidum*. Indian Journal of Public Health Research & Development, 9(12): 130-135.

SHARMA, R.R., D. SINGH AND R. SINGH. 2009. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. Biological Control, 50(3): 205-221.

SRIDHAR, S., E. SIVAPRAKASAM, R. BALA-KUMAR, AND D. KAVITHA. 2011. Evaluation of antibacterial and antifungal activity of *Ganoderma lucidum* (Curtis) P. Karst fruit bodies extracts. World Journal of Science and Technology, 1(6): 08-11.

THYAGARAJA, N. AND A. HOSONO. 1996. Effect of spice extract on fungal inhibition. LWT-Food Science and Technology, 29(3): 286-288.

TURNER, N.W., S. SUBRAHMANYAM AND S.A. PILETSKY. 2009. Analytical methods for determination of mycotoxins: a review. Analytica Chimica Acta, 632(2): 168-180.

YOHANNES, A., K. EYALARASAN, L. EYOB AND A. YEMANE. 2018. Antibacterial and antifungal activities of easily grown Eritrean black pepper. International Journal of Engineering Research and Technology, 7(2): 81-83.

ZOHRI, A.A., S.M. SABER, M.S. YOUSSEF AND M. MARWA ABDEL-KAREEM. 2014. Biocontrol of some toxigenic fungal strains and their toxins production using some spices. Journal of Environmental Studies [JES], 13: 13-20.

المكافحة الحيوية للفطريات المنتجة للسموم الفطرية في العلف الحيواني باستخدام التوابل

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تسبب استهلاك الأغذية والأعلاف الملوثة الي تفشي الامراض الصعبة، واصبحت مشكلة منشره في جميع أنحاء العالم، وتشكل مخاطر صحية خطيرة على الحيوانات والبشر. صممت هذه الدراسة لدراسة التأثير المضاد للفطريات كمواد حافظة غذائية للتحكم في نمو بعض الفطريات المسببة للسموم الفطرية. تم اختبار التأثير التثبيطي لثلاثة عشر نوعا من البهارات ضد سبعة عزلات فطرية من الأعلاف المصرية المختلفة. تم قياس قطر نمو المستعمرات الفطرية على أطباق من وسط أجار دكستروز البطاطس تحتوي توابل بتركيز ٦٠ جم / لتر. أظهر القرنفل والقرفة والكركم تثبيطًا تامًا لجميع الفطريات المختبرة ، ولوحظ تأثير مثبط متوسط ومتغير لبقية التوابل ، بينما عززت بعض التوابل (الكزبرة ، والشمر ، واليانسون ، والكراوية) نمو الفطريات. تم استخدام أفضل خمسة توابل فعالة بتركيز ١٦ (٠,٠٥ إلى ١٠٠ جم / لتر) لإيجاد الحد الأدنى لتركيز التثبيط (MIC) والحد الأدنى لتركيز مبيد الفطريات (MFC) لكل فطر على حدة. كان القرنفل والقرفة أكثر التوابل فاعلية ضد جميع الفطريات المختبرة ، حيث بلغMIC ٠,٠٥-١جم / لتر و MFC ٣-١٠ جم / لتر للفطريات المختبرة. ومن ثم ، يوصى بها كأفضل التوابل المضادة للفطريات التي يمكن أن تمنع نمو الفطريات بسهولة عند الحد الأدنى من التركيز. بينما الكزبرة أو الشمر أو الكراوية لا ينصح بها لأنها لا تؤثر على نمو تلك السلالات المسببة للسموم الفطرية.