INHIBITORY ACTIVITIES OF *Myristica fragrans* ESSENTIAL OIL ON AFLATOXIGENIC STRAINS

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ABSTRACT

Aflatoxin B1 is a highly toxic and carcinogenic metabolite produced by aflatoxigenic strains that commonly contaminate food and agricultural commodities. This study evaluates the inhibitory effects of *Myristica fragrans* Houtt (nutmeg) essential oil extracted by hydrodistillation on the mycelial growth, sporulation, and aflatoxin B1 production of *Aspergillus flavus* IMI 242684 *and Aspergillus parasiticus* IMI 283883 by fumigation and contact application. An analysis of *M. fragrans* essential oil using the chromatography-mass spectrometry showed that its major components are safrole (42.50%), 4-terpineol (23.81%) and methyl eugenol (11.14%). At a concentration of 1000 ppm of essential oil, the mycelial growths of both *Aspergillus* strains were completely inhibited by vapor treatment but only reduced by about 70% by contact treatment. However, the sporulation and aflatoxin B1 production were completely inhibited by both contact and vapor treatments. Vapor treatment induced a higher level of inhibition than contact treatment. In conclusion, nutmeg essential oil is a potential biochemical agent that can help prevent contamination of stored foods and feeds.

Keywords: Aflatoxigenic fungi, *Aspergillus flavus*, *Aspergillus parasiticus*, nutmeg essential oil

INTRODUCTION

Mycotoxins is a group of structurally diverse secondary metabolites produced by various fungal species. Aflatoxins is a group of mycotoxins mainly produced by filamentous fungi such as *Aspergillus flavus* and *A. parasiticus* (Pandey *et al.* 2016). These aflatoxins belong to four major groups, namely: B1, B2, G1, and G2. Aflatoxin B1 (AFB1) is the most toxic and prevalent and is classified as a Group 1a carcinogen by the International Agency for Research on Cancer (IARC 2002). Several strategies have been applied to prevent and control the growth of aflatoxin-producing fungi in grains, crops, and human foods. The exploitation of naturally occurring antimicrobials in essential oils has also received attention (Kedia *et al.* 2014; Pandey *et al.* 2016; Thanaboripat *et al.* 2016).

Myristica fragrans Houtt belongs to the family Myristicaceae. The nutmeg of *M. fragrans* Houtt is the seed kernel inside the fruit, while mace is the lacy covering (aril) on the kernel. Members of this family are widely used as spices and for numerous traditional medicines (Dorman *et al.* 2000). In addition, *M. fragrans* Houtt exhibits antifungal and antibacterial activities (Chatterjee 1990; Dorman & Deans 2000; Singh *et al.* 2005; Kamble & Patil 2008; Valente *et al.* 2011; Suthagar *et al.* 2012; Ashish *et al.* 2013). However, little is known of the antifungal activity of *M. fragrans* (nutmeg) essential oil against aflatoxigenic strains. The objectives of this study, therefore were to analyze the chemical composition of *M. fragrans* (nutmeg) essential oil using the gas chromatography-mass spectrometry (GC-MS), to evaluate the effect of this essential oil on mycelial growth, sporulation, and aflatoxin production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883, and to compare the effects of contact and vapor treatment on these aflatoxins.

MATERIALS AND METHODS

Plant Material and Extraction Procedure

The seed kernel inside the fruits of *Myristica fragrans* Houtt were collected from a local market

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in Bangkok, Thailand. The seeds were washed twice with distilled water and subsequently air dried in the dark at room temperature, before homogenizing. *M. fragrans* seeds of 100 g were placed in 1 L round-bottom distillation flask and added with 300 mL double distilled water. The essential oil was obtained by hydrodistillation for 3 hours using a Clevenger-type apparatus (Clevenger 1928). The oily layer on top of the aqueous distillate was separated and dried over anhydrous sodium sulfate and was stored in a tightly closed, dark vial at 4 ºC.

GC-MS Analysis of *M. fragrans* Houtt Essential Oil

The chemical composition of the essential oil was analyzed using the gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis was performed on an Agilent 6890 gas chromatograph in electron impact mode (70 eV) equipped with an HP 5973 mass selective detector and fitted with a fused silica capillary column (HP-5MS; 30.0 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The GC column oven temperature was increased from 100 ºC to 188 ºC at a rate of 3 ºC/min and then to 280 ºC at a rate of 20 ºC/min, with a final hold time of 3 minutes. The injector and detector temperatures were maintained at 280 ºC. Chromatograms were screened in scan mode, from m/z 50 to 500, at a rate of 3.25 scan/s, with the ionization source temperature set at 200 ºC. Diluted samples (20%, in dichloromethane) of 0.2 µL were injected in split mode (ratio of 1:50). The peaks were identified using standard reference by comparison with mass spectra available on MS database (National Institute of Standards and Technology and Wiley 8 libraries). The relative percentages of the essential oil constituents were expressed by peak area normalization.

Preparation of Conidial Suspensions

The aflatoxigenic strains *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 were obtained from the International Mycological Institute, Egham, Surrey, UK. These strains were cultured on potato dextrose agar (PDA) for 7−10 days at 28±1ºC. The conidia were harvested aseptically by adding 10 mL of sterile 0.05% Tween 80 solution to the culture and gently scraping the mycelial surface with a sterile inoculating loop to free the spores. The conidia concentration was determined using a hemocytometer and adjusted to 10^6 mL^{-1} (Nguefack *et al.* 2004).

Effect of *M. fragrans* Essential Oil on Mycelial Growth of the Two *Aspergillus* Strains

The antifungal effect of *M. fragrans* essential oil on both *Aspergillus* strains was determined based on mycelial growth inhibition that happened after application of the contact and vapor treatments described previously by Soliman and Badeaa (2002) and Soylu *et al.* (2010) with some modifications. The effect of contact treatment with *M. fragrans* essential oil was assessed by adding appropriate amounts of essential oil prepared in 0.05% Tween 80 to sterilized molten PDA (20 mL). The final concentrations of essential oil were 0, 100, 200, 300, 600, and 1000 ppm. A sterile Whatman No. 1 filter paper disc with a diameter of 6 mm was placed at the center of each plate and inoculated with 10 µL of the spore suspension (10^6 spores mL^{-1}). The plate was immediately sealed with parafilm and incubated for 5 days at 30±1ºC in darkness. The effect of vapor treatment was determined in a similar manner. Various amounts of *M. fragrans* essential oil were applied to the paper disc, which was placed at the center of the lid of each plate.

The efficacy of *M. fragrans* essential oil on the strains was evaluated by calculating the average of two perpendicular diameters of each colony daily. Both treatments were performed in triplicate. Relative growth inhibition after treatment compared with the control (RGI, %) was calculated as a percentage using the following equation: RGI (%) = [(dc−dt) / dc]×100, where dc is the diameter of the fungal colony in the control Petri dish and dt is the diameter of the fungal colony in the essential oil-treated Petri dish. The fungitoxicity (fungistatic/fungicidal activity) of *M. fragrans* essential oil was determined using a modified method of Thompson (1989). The inhibited fungal strains of the essential oil-treated sets were reinoculated with fresh PDA, and revival of their growth was recorded. After growth evaluation, sporulation and AFB1 production were analyzed in all samples.

Effect of *M. fragrans* Essential Oil on the Sporulation of the Two *Aspergillus* Strains

Spore production of the two *Aspergillus* strains was determined using the method of Tzortzakis and Economakis (2007). The spores from the colonies of both strains were collected by adding 5 mL of sterile water containing 0.1% Tween 80 to each Petri dish and gently scraping the mycelial surface three times with a sterile L-shaped spreader to free the spores. Spore production was estimated using a hemocytometer and a light microscope. The percentage inhibition of spore production was calculated using the following equation: Inhibition of sporulation (%) = [(Nc−Ns) / Nc]×100, where Nc is the number of spores in the control sample and Ns is the number of spores in the treated sample.

Effect of *M. fragrans* Essential Oil on AFB1 Production of the Two *Aspergillus* Strains

The anti-aflatoxigenic effect of *M. fragrans* essential oil was also examined. After sporulation was determined, cultures on PDA medium were extracted with 10 mL of 70% methanol, shaken for 5 minutes, and filtered using Whatman No. 4 filter paper. The AFB1 content of the extracts was analyzed using a DOA-Aflatoxin ELISA Test Kit from Department of Agriculture (DOA), Ministry of Agriculture and Cooperatives, Thailand, as described previously by Chinaphuti *et al.* (2002). Fifty µL of AFB1 standards was added into the antibody coated wells in 96 well plates and 50 µL of diluted sample was added into the other wells. Each well was then added with 50 µL of AFB1-horseradish peroxidase conjugate, slightly shaken and incubated at room temperature for 30 minutes. The contents of the well were then discarded into the appropriate waste container and the plates were washed 3-5 times with 0.5% Tween 20 in 0.01 M phosphate buffer saline. One hundred µL of tetramethylbenzidine substrate was added into the well, incubated for 10 minutes at room temperature and added with 100 µL of stopping solution (0.3 M phosphoric acid). The solution was read at 450 nm using the automated MicroELISA reader. The concentration of AFB1 of samples was calculated from the slope between % maximum binding and standard AFB1 concentrations. The percentage inhibition of AFB1 production was evaluated using the following equation: Inhibition of AFB1 production (%) = (AFB1 concentration in the control sample−AFB1 concentration in the treated sample)×100 / AFB1 concentration in the control sample.

Statistical Analysis

The chemical composition of *M. fragrans* essential oil was qualitatively and quantitatively analyzed by GC-MS. The data were first tested for normality and then subjected to an analysis of variance (ANOVA) based on the three factors: (1) contact and vapor treatment, (2) concentration of essential oil at 0, 100, 200, 300, 600, and 1000 ppm, and (3) growth of fungal strain of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883. Factor interactions were also investigated in terms of inhibition of mycelial growth, sporulation, and AFB1 production. Significant differences between the mean values were determined using a multiple comparisons test (Tukey's post-hoc test).

RESULTS AND DISCUSSION

The hydrodistillation of *M. fragrans* essential oil achieved a yield of 2.12–2.22% based on dry weight, which is relatively lower than that reported by Muchtaridi *et al.* (2010) which is 6.85% Analysis of the chemical components, identified seven components that accounted for 99.96% of the total oil composition (Table 1). Safrole (42.50%) was the major component, followed by 4-terpineol (23.81%) and methyl eugenol (11.14%). Muchtaridi *et al.* (2010) however, reported sabinene (21.38%), 4-terpineol (13.92%), and myristicin (13.57%) as major components in *M. fragrans* oil. Variability in the proportion of each compound and the composition of essential oil depends on several parameters including genetic variability, geographical location, environmental and agronomic conditions, and the extraction method (Runyora *et al.* 2010).

Very few data are available regarding the antimicrobial activity of *M. fragrans* essential oil particularly, regarding its effects on sporulation and AFB1 production. In another study, the application of 0.1% of *M. fragrans* essential oil

Table 1 Chemical compounds found in the *M. fragrans* essential oil

No.	Compound	Retention time (min)	Area (%)
1	trans-sabinene hydrate	4.313	7.70
2	cis-sabinene hydrate	4.888	5.88
3	4-terpineol	6.718	23.81
4	alpha terpineol	7.091	2.59
5	safrole	9.920	42.50
6	methyl eugenol	13.688	11.14
7	elemicin	19.129	6.34

inhibited the growth of *A. flavus* and *A. ochraceus* by 43 and 65%, respectively (Valente *et al.* 2015). At a concentration of the 0.3%, the growth of *A. flavus* and *A. ochraceus* was inhbited by 84 and 79%, respectively.

This study results indicated that the three-factor interaction of the treatment type, the concentration of essential oil, and the fungal strain significantly ($p = 0.000$) affected the mycelial growth of both *Aspergillus* strains (Table 2).

At 1000 ppm, the vapor treatment with essential oil completely inhibited the mycelial growth of both *Aspergillus* strains, while the contact treatment only induced ~70% inhibition. However, the inhibited mycelial discs exhibited growth revival after their transfer to the fresh PDA medium. These results indicate that vapor treatment with essential oil at 1000 ppm had fungistatic activity against both *Aspergillus* strains. For all concentrations of essential oil, the mycelial growth of both *Aspergillus* strains was inhibited significantly better by vapor treatment than by contact treatment. Other studies also documented the antifungal activity of nutmeg essential oil. Valente *et al.* (2015) reported that myristin is the major antifungal agent in nutmeg (*M. fragrans*) against *A. flavus* and *A. ochraceus*. This study results indicate that the antifungal effect of nutmeg essential oil is related to its main components safrole and 4-terpineol, which are phenylpropenes with very potent antifungal properties (Simic *et al.* 2004). Compounds present at lower concentrations in this essential oil, such as elemicin and methyl eugenol, also have efficient antimicrobial activities (Kubo *et al.* 1993; Sudhakar *et al.* 2009). The mechanism underlying the antifungal action of phenylpropenes is its lipophilicity that enables the permeability of cell membranes and also inhibit specific cellular processes or enzymes (Devi *et al.* 2010). Moreover, fungal cell death is reported to be mediated either by the formation of plasma membrane lesions or the alteration of membrane permeability (Pinto *et al.* 2009; Khan *et al.* 2010).

Effect of *M. fragrans* Essential Oil on the Sporulation of the Two *Aspergillus* Strains

Statistical analyses indicated that the three-factor interaction significantly ($p = 0.044$) affected sporulation. Sporulation of both *Aspergillus* strains was significantly ($p < 0.05$) inhibited by increasing the concentration of essential oil (Table 3). Vapor treatment with essential oil at 600 ppm completely inhibited the sporulation of both *Aspergillus* strains. Sporulation inhibition was significantly ($p < 0.05$) better by vapor treatment than by contact treatment when compared at same concentration levels of 200, 300 and 600 ppm. In similar studies of Paranagama *et al.* (2003) and Sonker *et al.* (2014), lemongrass essential oil reduces the spore formation of the *Aspergillus* species. Another study on *Cymbopogon citratus* L. essential oil show that its effect on sporulation reflect the effects of volatile compounds emitted by this oil on the surface of developing mycelia and/or the perception or transduction of signals involved in the switch from vegetative to reproductive development (Mahanta *et al.* 2007).

Table 2 Effects of contact and vapor treatments at different concentrations of *M. fragrans* essential oil on the mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883

Inhibition (%)		Concentration of essential oil (ppm)					
		Control	100	200	300	600	1000
A. flavus	Contact	0^{l}	3.6 ± 1.0^{kl}	8.8 ± 2.4^{ij}	25.4 ± 0.0^{g}	36.7 ± 1.0^{e}	72.6 ± 1.4^{b}
	Vapor	0^{l}	19.9 ± 2.1^{h}	29.3 ± 1.0^{fg}	48.1 ± 2.1^{d}	55.9 ± 0.9^{c}	100^{a}
A. parasiticus	Contact	0^{l}	5.1 ± 1.0^{jk}	10.3 ± 2.4^{i}	26.9 ± 0.5^{fg}	37.2 ± 1.0^{e}	71.9 ± 0.9^{b}
	Vapor	0^{l}	17.6 ± 0.9^{h}	30.9 ± 1.0^{f}	38.2 ± 0.5^{e}	47.5 ± 1.9^{d}	100^{a}

Note: Values are means (n=3) ± standard deviation;
Mean values with the same superscripts do not significantly differ according to ANOVA and Tukey's multiple comparisons test ($p < 0.05$)

Table 3 Effects of contact and vapor treatments at different concentrations of *M. fragrans* essential oil on the sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883

Inhibition (%)		Concentration (ppm)					
		Control	100	200	300	600	1000
A. flavus	Contact	0^{j}	4.1 ± 3.6^{gh}	12.5 ± 6.2^{fgh}	17.9 ± 1.4^{fg}	35.8 ± 5.2^{de}	100^{a}
	Vapor	0^{j}	25.0 ± 6.2^{ef}	56.2 ± 10.6^{c}	82.9 ± 10.1^{b}	100^{a}	100^{a}
A. parasiticus	Contact	0^{j}	14.7 ± 3.4^{fgh}	18.2 ± 6.4^{fg}	22.1 ± 4.0^{ef}	36.8 ± 10.8^{de}	100^{a}
	Vapor	0^{j}	26.7 ± 5.1^{ef}	51.9 ± 7.7^{cd}	62.8 ± 5.3^{c}	100^{a}	100^{a}

Note: Values are means (n=3) ± standard deviation;
Mean values with the same superscripts do not significantly differ according to ANOVA and Tukey's multiple comparisons test ($p < 0.05$)

Statistical analyses indicated that the three-factor interaction significantly ($p = 0.01$) affected AFB1 production. Most concentrations of essential oil significantly ($p < 0.05$) inhibited AFB1 production of the two *Aspergillus* strains compared with the control (Table 4). However, contact treatment with essential oil at a concentration of 100 ppm did not significantly affect AFB1 production by *A. flavus* IMI 242684. AFB1 production was better inhibited significantly ($p < 0.05$) by vapor treatment than by contact treatment when compared at the same concentration levels of 300 and 600 ppm.

Other investigations on the effects of essential oils on fungal growth, sporulation, and AFB1 production have shown similar results (Rasooli & Abyaneh 2004). Essential oils from citronella grass inhibit the growth, AFB1 production, and sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 102566 in maize grain (Thanaboripat *et al.* 2004). The essential oil obtained from *Cuminum cyminum* L. seeds inhibited both *A. flavus* LHP(C)-D6 growth and aflatoxin production (Kedia *et al.* 2014). The vapor treatment at lower concentration of essential oil obtained from the leaves of *Eucalyptus globules* completely inhibited the growth of *A. flavus* Link and *A. parasiticus* Speare when compared with contact treatment (Vilela *et al.* 2009). It appeared to have a similar effect on aflatoxin production but the inhibition of AFB1 production was not clear. Some study results have shown that direct correlation exists between fungal growth and AFB1 production (Kumar *et al.* 2008; Rezaei-Kahkha *et al.* 2014). This inhibition of AFB1 production by essential oils may be related to inhibition of aflatoxin biosynthesis involving lipid peroxidation and oxygenation (Bluma *et al.* 2008).

Table 4 Effects of contact and vapor treatments at different concentrations of *M. fragrans* essential oil on the aflatoxin production by *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883

Inhibition (%)		Concentration (ppm)					
		Control	100	200	300	600	1000
A. flavus	Contact	0^{i}	6.4 ± 3.4^{gh}	25.2 ± 8.0^{ef}	36.4 ± 3.7^{de}	53.2 ± 5.0^{c}	100^{a}
	Vapor	0^{i}	12.8 ± 8.0^{fg}	41.5 ± 3.7^{cd}	74.4 ± 2.3^{b}	100^{a}	100^{a}
A. parasiticus	Contact	0^{i}	15.0 ± 3.2^{fg}	24.7 ± 8.3^{ef}	29.6 ± 4.5^{de}	35.5 ± 5.3^{de}	100^{a}
	Vapor	0^{i}	15.0 ± 3.2^{fg}	30.7 ± 3.0^{de}	67.4 ± 2.4^{b}	74.3 ± 4.8^{b}	100^{a}

Note: Values are means (n=3) ± standard deviation;
Mean values with the same superscripts do not significantly differ according to ANOVA and Tukey's multiple comparisons test ($p < 0.05$)

CONCLUSION

The inhibitory effect of the essential oil from *Myristica fragrans* Houtt (nutmeg) has demonstrated its potential efficacy as a natural control for mycelial growth, spore production, and aflatoxin production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883. The antifungal activity of nutmeg essential oil vapor can be applied in the treatment and prevention of various fungal infections. However, an in-depth study is needed to fully understand the dynamics of essential oil application in order to explore the role of these eco-friendly agents to protect foods and feeds from contamination.

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REFERENCES

Ashish DG, Vipin KB, Vikash B, Nishi M. 2013. Chemistry, antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans* Houtt.). J Genet Eng Biotechnol 11(1):25-31.

Bluma R, Amaiden MR, Daghero J, Etcheverry M. 2008. Control of Aspergillus section Flavi growth and aflatoxin accumulation by plant essential oils. J Appl Microbiol 105:203-14.

Chatterjee D. 1990. Inhibition of fungal growth and infection in maize grains by spice oils. Lett Appl Microbiol 11:148-51.

Chinaphuti A, Trikarunasawat C, Wongurai A, Kositcharoenkul S. 2002. Production of in-house ELISA test kit for detection of aflatoxin in agricultural commodities and their validations. Kasetsart J (Nat Sci) 36:179-86.

Clevenger JF. 1928. Apparatus for dertermination of volatile oil. J Amer Pharm Assoc 17:346-9.

Devi KP, Nisha SA, Sakthivel R, Pandian SK. 2010. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrance. J Ethnopharmacol 130:107-15.

Dorman HJD, Figueiredo AC, Barroso JG, Deans SG. 2000. In-vitro evaluation of antioxidant activity of essential oils and their components. Flavour Fragr J 15:12-6.

Dorman HJD, Deans SG. 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J Appl Microbiol 88:308-16.

International Agency for Research on Cancer [IARC]. 2002. Aflatoxins. In: Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Lyon (FR): IARC Press. p. 171-300. [IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, *Vol. 82*.]

Kamble VA, Patil SD. 2008. Spice-derived essential oils: Effective antifungal and possible therapeutic agents. J Herb Spices Med Plants 14:129-43.

Kedia A, Prakash B, Mishra PK, Dubey NK. 2014. Antifungal and antiaflatoxigenic properties of *Cuminum cyminum* (L.) seed essential oil and its efficacy as a preservative in stored commodities. Int J Food Microbiol 3:168-9.

Khan A, Ahmad A, Akhter F, Yausuf S, Xess I, Khan LA, Manzoor N. 2010. *Ocimum sanctum* essential oil and its active principles exert their antifungal activity by disrupting ergosterol biosynthesis and membrane integrity. Res Microbiol 161:816-23.

Kubo I, Muroi H, Himejima M. 1993. Combination effects of antifungal nagilactones against *Candida albicans* and two other fungi with phenylpropanoids. J Nat Prod 56:220-6.

Kumar V, Basu MS, Rajendran TP. 2008. Mycotoxin research and mycoflora in some commercially important agricultural commodities. Crop Prot 27:891-905.

Mahanta JJ, Chutia M, Bordoloi M, Pathak MG, Adhikary RK, Sarma TC. 2007. *Cymbopogon citratus* L. essential oil as a potential antifungal agent against key weed moulds of *Pleurotus* spp. Spawns. Flavour Frag J 22:525-30.

Muchtaridi, Subarnas A, Apriyantono A, Mustarichie R. 2010. Identification of compounds in the essential oil of nutmeg seeds (*Myristica fragrans* Houtt.) that inhibit locomotor activity in mice. Int J Mol Sci 11:4771-81.

Nguefack J, Leth V, Zollo PA, Mathur SB. 2004. Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. Int J Food Microbiol 94:329-34.

Pandey AK, Sain SK, Singh P, Palni UT, Tripathi NN. 2016. Mycotoxin menace in stored agricultural commodities and their management by plant volatiles: An overview. In: Kumar P, Gupta VK, Tiwari AK, Kamle M, editors. Current Trends in Plant Disease Diagnostics and Management Practices. Cham (ZG): Springer International Publishing. p. 405-27.

Paranagama PA, Abeysekera KH, Abeywickrama K, Nugaliyadde L. 2003. Fungicidal and anti-aflatoxigenic effects of the essential oil of *Cymbopogon citratus* (DC.) Stapf. (lemongrass) against *Aspergillus flavus* Link. isolated from stored rice. Lett Appl Microbiol 37(1):86–90.

Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. 2009. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida, Aspergillus* and dermatophyte species. J Med Microbiol 58:1454-62.

Rasooli, I, Abyaneh MR. 2004. Inhibitory effects of Thyme oils on growth and aflatoxin production by *Aspergillus parasiticus*. Food Control 15:479-83.

Rezaei-Kahkha MR, Amanloo S, Kaykhaii M. 2014. Antiaflatoxigenic activity of *Carum copticum* essential oil. Environ Chem Lett 12:231-4.

Runyora D, Ngassapa O, Vagionas K, Aligiannis N, Graikou K, Chinou I. 2010. Chemical composition and antimicrobial activity of the essential oils of four *Ocimum* species growing in Tanzania. Food Chem 119:311-6.

Simic A, Sokovic MD, Ristic M, Grujic-Jovanovic S, Vukojevic J, Marin PD. 2004. The chemical composition of some Lauraceae essential oils and their antifungal activities. Phytother Res 18:713–7.

Singh G, Marimuthu P, de Heluani CS, Catalan C. 2005. Antimicrobial and antioxidant potentials of essential oil and acetone extract of *Myristica fragrans* Houtt. J Food Sci 70:141-8.

Soliman KM, Badeaa RI. 2002. Effect oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem Toxicol 40:1669-75.

Sonker N, Pandey AK, Singh P, Tripathi NN. 2014. Assessment of *Cymbopogon citratus* (DC.) Stapf essential oil as herbal preservatives based on antifungal, antiaflatoxin and antiochratoxin activities and *in vivo* efficacy during storage. J Food Sci 79:M628–34.

Soylu EM, Kurt S, Soylu S. 2010. *In vitro* and *in vivo* antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. Int J Food Microbiol 143:183–9.

Sudhakar P, Latha P, Sreenivasula Y, Reddy BVB, Hemalatha TM, Balakrishna M, Reddy KR. 2009. Inhibition of *Aspergillus flavus* colonization and aflatoxin (AFB1) in peanut by methyl eugenol. Indian J Exp Biol 47:63-7.

Suthagar PP, Roziahanim M, Shanmugapriya P. 2012. Determination of antibacterial activity of essential oil of *Myristica fragrans* Houtt. using tetrazolium microplate assay and its cytotoxic activity against vero cell line. Int J of Pharmacol 8(6):572-8.

Thanaboripat D, Mongkontanawut N, Suvathi Y, Ruangrattanametee V. 2004. Inhibition of aflatoxin production and growth of *Aspergillus flavus* by citronella oil. KMITL Sci J 49(1):1-8.

Thanaboripat D, Sarutipaisan C, Puangtong C, Chatpongsatorn P, Suvatti Y, Sukonthamut S, Charoensettasilp S. 2016. Effects of four essential oils on the growth of aflatoxin producing fungi. KMITL Sci Tech J 16:64-71.

Thompson DP. 1989. Fungitoxic activity of essential oil components on food storage fungi. Mycologia 81:151-3.

Tzortzakis NG, Economakis CD. 2007. Antifungal activity of lemongrass (*Cymbopogon citratus* L.) essential oil against key postharvest pathogens. Innov Food Sci Emerg Technol 8:253-8.

Valente VMM, Jham GN, Dhingra OD, Ghiviriga I. 2011. Composition and antifungal activity of the Brazilian *Myristica fragrans* Houtt essential oil. J Food Saf 31(2):197-202.

Valente VMM, Jham GN, Jardim CM, Dhingra OD, Ghiviriga I. 2015. Major antifungals in nutmeg essential oil against *Aspergillus flavus* and *A. Ochraceus*. J Food Res 4:51-7.

Vilela GR, Almeida GS, D' Arce MABR, Moraes MHD, Brito JO, Silva MFGF, … Gloria EM. 2009. Activity of essential oil and its major compound, 1,8-cineole, from *Eucalyptus globulus* Labill., against the storage fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. J Stored Prod Res 45:108-11.