MANAGEMENT OF AFLATOXIGENIC STRAINS VIA VAPOR AND CONTACT TREATMENT WITH ESSENTIAL OIL OBTAINED FROM *Myristica fragrans*

ORATAI SUKCHAROEN[1*], PRAMOTE SIRIROTE[2] AND DUSANEE THANABORIPAT[2]

[1]Department of Biotechnology, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand
[2]Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand
**Corresponding author, e-mail: osukcharoen@hotmail.com*

Received …….. / Accepted ……..

ABSTRACT

This study evaluated the inhibitory effects of essential oil extracted from *Myristica fragrans* Houtt (nutmeg) by hydrodistillation on mycelial growth, sporulation, and aflatoxin B1 production of *Aspergillus flavus* IMI 242684 *and Aspergillus parasiticus* IMI 283883 by fumigant and contact application. Gas chromatography-mass spectrometry analysis of *M. fragrans* essential oil showed that safrole (42.50%) was the major component, followed by 4-terpineol (23.81%) and methyl eugenol (11.14%). At an essential oil concentration of 1000 ppm, the mycelial growth of both *Aspergillus* strains was significant completely inhibited by vapor treatment but only reduced by about 70% by contact treatment. By contrast, sporulation and aflatoxin B1 production were completely inhibited by both contact and vapor treatment. Vapor treatment induced a higher level of inhibition than contact treatment. In conclusion, nutmeg essential oil is a potential chemical agent and can help to prevent infestation of stored foods and feeds.

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

INTRODUCTION

Mycotoxins are a group of structurally diverse secondary metabolites produced by various fungal species. Aflatoxins are a group of mycotoxins mainly produced by filamentous fungi such as *Aspergillus flavus* and *A. parasiticus* (Pandey *et al.* 2016). There are four major aflatoxins: 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).

* Corresponding author: osukcharoen@hotmail.com

Myristica fragrans Houtt (nutmeg) belongs to the family Myristicaceae 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 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 *et al.* 2008; Valente *et al.* 2011; Suthagar *et al.* 2012; Ashish *et al.* 2013). To our knowledge, there is no report of the antifungal activity of *M. fragrans* essential oil against aflatoxigenic strains. The objectives of this study were to analyze the chemical composition of *M. fragrans* essential oil via 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.

MATERIALS AND METHODS

Plant material and extraction procedure

Myristica fragrans Houtt was collected from local market, Bangkok, Thailand. *M. fragrans* seeds were washed twice with distilled water, subsequently air dried in darkness at room temperature, before homogenizing. *M. fragrans* (100 g) were placed in 1 L round-bottom distillation flask and 300 mL double distilled water was added. The essential oil was obtained by hydrodistillation for 3 h using a Clevenger-type apparatus according to 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 4ºC for further need.

GC-MS analysis of *M. fragrans* Houtt essential oil

The chemical composition of the essential oil was analyzed via GC-MS. This analysis was performed on an Agilent 6890 gas chromatograph in electron impact mode (70 eV) coupled to 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 100ºC to 188ºC at a rate of 3ºC/min and then to 280ºC at a rate of 20ºC/min, with a final hold time of 3 min. The injector and detector temperatures were maintained at 280ºC. 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 200ºC. Diluted samples (20%, in dichloromethane) of 0.2 μL were injected in split mode (ratio of 1:50). Peaks were identified using standard and 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). The strains were cultured on potato dextrose agar (PDA) for 7–10 days at 28±1ºC. 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 spores. The conidia concentration was determined using a hemocytometer and adjusted to 10^6 mL^{-1} (Nguefack *et al.* 2004).

Assessment of the 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 following contact and vapor treatment as described previously with some modifications (Soliman and Badeaa. 2002; Soylu *et al.* 2010). 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 in the center of the lid of each plate.

The efficacy of *M. fragrans* essential oil was evaluated by calculating the average of two perpendicular diameters of each colony daily. All 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 discs of the essential oil- treated sets were reinoculated into fresh PDA, and

revival of their growth was recorded. After growth evaluation, sporulation and AFB1 production were analyzed in all samples.

Assessment of the effect of *M. fragrans* essential oil on sporulation of the two *Aspergillus* strains

Spore production by the two *Aspergillus* strains was determined using the method of Tzortzakis & Economakis (2007). Spores from colonies of both *Aspergillus* 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 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.

Assessment of the effect of *M. fragrans* essential oil on AFB1 production by the two *Aspergillus* strains

The anti-aflatoxigenic effect of *M. fragrans* essential oil was studied. After sporulation was determined, cultures on PDA medium were extracted with 10 mL of 70% methanol, shaken for 5 min, 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, described previously by Chinaphuti *et al.* (2002). By adding 50 µl of AFB1 standards into the antibody coated wells in 96 well plates and 50 µl of diluted sample into the other wells followed by adding 50 µl of AFB1-horseradish peroxidase conjugate to each well of plates were slightly shake before incubated at room temperature for 30 min. The contents of the well were then discarded into the appropriate waste container and washed the plate 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 min at room temperature before adding 100 µl of stopping solution (0.3M 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

All analyses were performed in triplicate. Data were recorded as means ± standard deviations. Data were analyzed for normality and then subjected to an analysis of variance (ANOVA) based on three factors: (1) contact and vapor treatment, (2) concentration of essential oil (0, 100, 200, 300, 600, and 1000 ppm), and (3) fungal strain (*A. flavus* IMI 242684 and *A.parasiticus* IMI 283883). The effects of factor interactions on mycelial growth, sporulation, and AFB1 production were also investigated. Factor interactions were also investigated in terms of inhibition of mycelial growth, sporulation, and AFB1 production. Significant differences between mean values were determined using a multiple comparison test (Tukey's post hoc test), where $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Hydrodistillation of *M. fragrans* essential oil achieved a yield of 2.12–2.22% based on dry weight, which contrasts with the yield of 6.85% reported by Muchtaridi *et al.* (2010). The chemical composition of *M. fragrans* essential oil was qualitatively and quantitatively analyzed by GC-MS. The identified chemical components, retention times and percentage compositions are presented in Table 1. Seven components accounted for 99.96% of the total oil composition. Safrole (42.50%) was the major component, followed by 4-terpineol (23.81%) and methyl eugenol (11.14%). On the other hand, Muchtaridi *et al.* (2010) reported sabinene

Table 1 Chemical composition of *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

(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; in particular, there are no data regarding its effects on sporulation and AFB1 production. Valente *et al.* (2015) reported that treatment with 0.1% of *M. fragrans* essential oil inhibited the growth of *A. flavus* and *Aspergillus ochraceus* by 43 and 65%, respectively. At a concentration of the 0.3%, growth of *A. flavus* and *A. ochraceus* was inhibited by 84 and 79%, respectively.

The results from statistical analyses 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 in Table 2.

At 1000 ppm, vapor treatment with essential oil completely inhibited mycelial growth of both *Aspergillus* strains, while contact treatment only induced ~70% inhibition. Growth increased following the transfer of discs showing mycelial growth to PDA lacking essential oil. 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 more by vapor treatment than by contact treatment. Other studies also reported 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*. Our results indicate that the antifungal effect of nutmeg essential oil is related to its main components safrole and 4-terpineol, which are phenyl-propenes 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). Devi *et al.* (2010) reported the mechanism underlying the antifungal action of phenylpropenes, whose lipophilicity enables them to the permeability of cell membranes and also to inhibit specific cellular processes or enzymes. However, 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).

Table 2 Effect of contact and vapor treatment with different concentrations of *M. fragrans* essential oil on 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 ± 1.1^{kl}	8.8 ± 2.4^{ij}	25.4 ± 0.0^{g}	36.8 ± 1.1^{e}	72.7 ± 0.0^{b}
Vapor	0^{l}	19.9 ± 2.1^{h}	29.3 ± 1.2^{fg}	48.1 ± 2.2^{d}	55.8 ± 3.4^{c}	100^{a}
A. parasiticus						
Contact	0^{l}	5.2 ± 1.1^{jk}	10.3 ± 2.4^{i}	26.9 ± 0.5^{fg}	37.2 ± 1.1^{e}	71.9 ± 0.9^{b}
Vapor	0^{l}	17.6 ± 0.9^{h}	30.9 ± 1.1^{f}	38.3 ± 0.5^{e}	47.5 ± 2.0^{d}	100^{a}

Values are means (n=3) ± standard deviation.

Mean values followed by the same letter are not significantly different according to an ANOVA and Tukey's multiple comparisons test ($p < 0.05$).

Effect of *M. fragrans* essential oil on sporulation of the two *Aspergillus* strains

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

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 by 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 inhibited significantly ($p < 0.05$) more by vapor treatment than by contact treatment when compare on the same concentration of 300 and 600 ppm.

The effects of essential oils on fungal growth, sporulation, and AFB1 production have been investigated (Rasooli & Abyaneh. 2004). Similar results were reported by Thanaboribat *et al.* (2004), who reported that essential oils of citronella inhibit growth, AFB1 production, and sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 102566 in maize grain. Kedia *et al.* (2014) also showed that essential oil obtained from *Cuminum cyminum* L. seeds inhibit both *A. flavus* LHP(C)-D6 growth and aflatoxin production. Vilela *et al.* (2009) reported that vapor treatment with essential oil obtained

Table 3 Effect of contact and vapor treatment with different concentrations of *M. fragrans* essential oil on 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}	4.17 ± 3.6^{gh}	12.5 ± 6.3^{fgh}	17.9 ± 1.4^{fg}	35.8 ± 5.2^{de}	100^{a}
Vapor	0^{j}	25.0 ± 6.3^{ef}	56.3 ± 10.0^{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}	51.3 ± 8.6^{cd}	62.8 ± 5.3^{c}	100^{a}	100^{a}

Values are means (n=3) ± standard deviation.
Mean values followed by the same letter are not significantly different according to an ANOVA and Tukey's multiple comparison tests ($p < 0.05$).

Table 4 Effect of contact and vapor treatment with different concentrations of *M. fragrans* essential oil on 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}	6.5 ± 3.5^{hi}	25.2 ± 8.0^{ef}	36.5 ± 3.8^{de}	53.3 ± 5.0^{c}	100^{a}
Vapor	0^{i}	12.8 ± 8.1^{gh}	41.6 ± 3.8^{cd}	74.4 ± 2.3^{b}	100^{a}	100^{a}
A. parasiticus						
Contact	0^{i}	15.1 ± 3.3^{fgh}	24.8 ± 8.3^{efg}	29.6 ± 4.6^{de}	35.5 ± 5.4^{de}	100^{a}
Vapor	0^{i}	15.1 ± 3.3^{fgh}	30.7 ± 3.1^{de}	67.5 ± 2.5^{b}	74.4 ± 4.9^{a}	100^{a}

Values are means (n=3) ± standard deviation.
Mean values followed by the same letter are not significantly different according to an ANOVA and Tukey's multiple comparison tests ($p < 0.05$).

from leaves of *Eucalyptus globules* completely inhibits the growth of *A. flavus* Link and *A. parasiticus* Speare in lower concentration when compared with contact treatment. It appears to have a similar effect on aflatoxin production. The mechanism of inhibition of aflatoxin B1 production was not clear. Some studies have shown that the direct correlation between fungal growth and aflatoxin B1 production (Kumar *et al.* 2008; Rezaei-Kahkha 2014). On the other hand, the inhibition of AFB1 production by essential oils may be related to inhibition of aflatoxin biosynthesis involving lipid peroxidation and oxygenation (Bluma *et al.* 2008).

CONCLUSION

In conclusion, this study demonstrates the potential efficacy of *M. fragrans* essential oil as a natural compound that effectively controls mycelial growth, spore production, and aflatoxin production by *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883. The antifungal activity of nutmeg essential oil vapor could be widely applicable in the treatment and prevention of various fungal infections. However, an in-depth study is needed to fully understand the mechanism-of-action of essential oil and the mode of application in order to exploit these eco-friendly agents to protect foods and feeds from infestation.

ACKNOWLEDGEMENTS

The authors thank Miss Sujitra Sukonthamut for her advice on statistical analysis and the Faculty of Science, King Mongkut's Institute of Technology Ladkrabang for financial support.

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 105:203-214.

Chatterjee D. 1990. Inhibition of fungal growth and infection in maize grains by spice oils. Lett Appl Microbiol 11:148-151.

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 Journal : Nat Sci 36:179 – 186.

Clevenger JF. 1928. Apparatus for dertermination of volatile oil. J Amer Pharm Assoc 17: 346-349.

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 130:107-115.

Dorman HJ, Fiqueiredo AC, Barroso JG, Deans SG. 2000. In-vitro evaluation of antioxidant activity of essential oils and their components. Flavour Fragr J 15:12-16.

Dorman HJ, Deans SG. 2000. Antimicrobial agents from plants : antibacterial activity of plant volatile oils. J Appl Microbiol 8:308-316.

IARC (International Agency for Research on Cancer). 2002. Aflatoxins. In : Monograph on the evaluation of carcinogenic risks to humans. Vol. 82. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC, Lyon, France, 171-300.

Kamble VA, Patil SD. 2008. Spice-derived essential oils : Effective antifungal and possible therapeutic agents. J Herb Spices Med Plants 14:129-143.

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 3:168-169.

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 161:816-823.

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

Kubo I, Muroi H, Himejima M. 1993. Combination effects of antifungal nagilactones against *Candida albicans* and two other fungi with phenylpropanoids. J Nat Prod 56:220-226.

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 22:525-530.

Muchtaridi M, 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: 4771-4781.

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: 329-334.

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: Current trends in plant disease diagnostics and management practices. Edited by Kumar P, Gupta VK, Tiwari AK, Kamle M.: Springer International Publishing. 405-427.

Paranagama PA, Abeysekera KHT, 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-Siva L, Cavaleiro C, Salgueiro L. 2009. Antifungal acitivity of the clove essential oil from *Syzygium aromaticum (Eugenia caryophyllus)* on *Candida, Aspergillus* and dermatophyte species. J Med Microbiol 58:1454-1462.

Rasooli, I, Abyaneh MR. 2004. Inhibitory effects of Thyme oils on growth and aflatoxin production by *Aspergillus parasiticus*. Food Control 15:479-483.

Rezaei-Kahkha MR, Amanloo S, Kaykhaii M. 2014. Antiaflatoxigenic activity of *Carum copticum* essential oil. Environ Chem Lett 12:231-234.

Runyora D, Ngassapan 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 119:311-316.

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 18:713–717.

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 70:141-148.

Soliman KM, Badeaa RI. 2002. Effect oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem Toxicol 40:1669-1675.

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–M634.

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 143:183–189.

Sudhakar P, Latha P, Sreenivasula Y, Reddy BV, Hemlatha TM, Balakrishna M, Reddy KR. 2009. Inhibition of *Aspergillus flavus* colonization and aflatoxin (AFB1) in peanut by methyl eugenol. Indian J Exp Biol 47:63-67.

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):572-578.

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.

Thanaboripat D, Monkontanawut 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.

Thomson DP. 1989. Fungitoxic activity of essential oil components on food storage fungi. Mycologia 81: 151-153.

Tzortzakis NG, Economakis CD. 2007. Antifungal activity of lemongrass (*Cymbopogon citratus* L.) essential oil against key postharvest pathogens. Innov Food Sci Emerg Technol 8:253-258.

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 4:51-57.

Vilela GR, Almeida GS, D' Arce MABR, Moreaes MHD, Brito JO, Silva MFGF, Silva SC, Piedade SMS, Calori-Domingues MA, 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 45:108–111.