INHIBITORY EFFECT OF PLANT ESSENTIAL OILS ON GROWTH AND AFLATOXIN PRODUCTION BY *Aspergillus flavus* IMI 242684 AND *Aspergillus parasiticus* IMI 283883

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ABSTRACT

Aflatoxin B1 (AFB1) is highly toxic and carcinogenic metabolite produced by aflatoxigenic strains and commonly contaminated in food and agricultural commodities. Prevention of the growth of aflatoxigenic fungi is the best way to avoid the contamination of aflatoxin in food and feeds. Various treatments have been studied for controlling aflatoxin producing fungi and aflatoxin production. The use of plant essential oils is one of the alternative choices as it is safety and environmental friendly. The aims of this research were to determine the efficacy of plant essential oils for the control of two aflatoxigetic strains, i.e. *Aspergillus flavus* IMI 242684 and *Aspergillus parasiticus* IMI 283883. Eighty eight plants were preliminary screened and essential oils were extracted by hydrodistillation using a Clevenger-type apparatus for 3 h and identified by gas chromatography-mass spectrometry. The results showed that only 56 plants contained essential oils. The essential oils from 56 plants were further tested for mycelial growth inhibition using disc diffusion method. It was found that only 19 plants exhibited strong inhibitory activities with the diameter of inhibition zone more than 30 mm. Nineteen plants were further tested for minimum inhibitory concentration (MIC), minimum fungicidal inhibition (MFC) on mycelial growth by broth microdilution method. The essential oils with MIC less than 0.5 mg/ml were selected for further investigation. The results showed that 12 essential oils exhibited MIC less than 0.5 mg/ml. These 12 essential oils were then examined for the inhibitory effects on mycelial growth, sporulation, and aflatoxin B1 production of two aflatoxigenic strains by comparison between contact and vapor treatments.

This study reports for the first time that five aromatic essential oils from *Brassica juncea* L., *Cinnamomum porrecrum* (Roxb.) Kosterm., *Michelia alba* DC., *Limnophila geoffrayi* Bonati and *Kaempferia galangal* L. have considerable effects on the growth, sporulation, and aflatoxin B1 inhibition of *Aspergillus* strains. The inhibitory effects are varied according to the type and concentration of essential oils. All of the essential oils in vapor treatment gave better inhibition than contact treatment. Four essential oils from *Cinnamomum porrecrum* (Roxb.) Kosterm,, *Cinnamomum zeylanicum* Blume, *Myristica fragrans* Houtt. and *Kaempferia galangal* L. showed remarkably high inhibition against both *Aspergillus* strains in both treatments with IC50 values ranging from 107.7-383.3 ppm against mycelial growth, 128.4-284.7 ppm against sporulation, and 96.0-200.0 ppm against aflatoxin B1 production. This findings indicated that essential oils from these four aromatic plants can be used as the biocontrol agent for aflatoxigenic strains in food and agricultural commodities especially *via* fumigation and could further be developed as natural fungicides.

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CHAPTER 1

INTRODUCTION

1.1 Statement and significant of the problem

Thailand, one of the largest exporters of various agricultural products, in situated the tropical area with high temperature and humidity, unseasonal rains during harvest, and flash floods that can lead to infestation aflatoxigenic fungi, and thereby contamination of aflatoxins. Among all aflatoxins produces, aflatoxin B1 is classified as a group I carcinogen by the International Agent for Research on Cancer (2002). Aflatoxins were reported to reduce the efficiency of immunization in children and led to enhance risk of infections (Hendrickse, 1997). Aflatoxin B1 accumulated in the liver can be activated by cytochrome P450 enzymes and converted to AFB1-8,9-epoxide, which is highly reactive with DNA forming adducts at the N7 position of guanine that is responsible for its carcinogenic effects (Denissenko *et al.*, 1999). Aflatoxin has been found to be related to hepatocellular carcinoma (HCC), especially in the presence of hepatitis B virus (HBV) and hepatitis C virus (HCV) (Kar, 2014). Epidemiological data indicated that the areas in the world with the high levels of aflatoxin are correlated with the high incidence of human hepatic cancer (IARC, 1985). A number of outbreaks of aflatoxicosis have been recorded to be responsible for the death of human and animals in Asia and Africa, thus strengthening the severity of the problem of food contamination with aflatoxin (Reddy and Raghavender, 2007; Mittal and El-Serag, 2013). Aflatoxin management at a global level requires a thorough control of aflatoxin contamination from agricultural farming to agricultural commodities, food and feeds which has impacts on human and animal health and also economic losses (Mishra and Das, 2003).

Over the last few decades, various synthetic fungicides are commonly used for protection of food commodities from fungal deterioration as well as aflatoxin contaminations. However, undesirable side effects in the food chain, i.e., environmental persistence, residual toxicity, fungal and pest resistance, and mammalian toxicity are increased (Isman, 2006). Thus, plant-based essential oils are gaining interest as a source of natural antioxidants, insecticidal, and antimicrobial properties (Kumar *et al.*, 2016) which are safe to the environment as well as to human (Prakash *et al.*, 2012). In general, most of the essential oils are preferred by Environment Protection Agency in view of their favorable safety profiles and proven as generally recognized as safe (GRAS) by the United State Food and Drug Administration (FDA). Several strategies including the application of essential oils have been examined for the prevention and control of aflatoxin-producing fungi and aflatoxin contamination (Umesha *et al.,* 2017, Rasooli and Abyaneh, 2004; Thanaboripat *et al.,* 2007, 2016). Furthermore, the application of essential oils on microbial inhibition in food and feed protection by vapor phase have been reported (Lopez *et al.*, 2005; Tullio *et al.*, 2007; Laghchimi *et al.*, 2014).

1.2 Research objectives

The objective of this study was to determine the inhibitory effects of fifty-six essential oils from nineteen families of plants against the aflatoxigenic strains, *Aspergillus flavus* IMI 242684 and *Aspergillus parasiticus* IMI 283883. The chemical composition of the plant essential oils was also investigated by gas chromatography-mass spectrometry (GC-MS) and their effects on mycelial growth, sporulation and aflatoxin B1 production of strains *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 were investigated and compared between the contact and the vapor treatments. Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) were determined by the broth microdilution method.

1.3 Scopes of sesearch study

1. Preparation of fifty-six plant essential oils by hydrodistillation
2. Determine the chemical composition of essential oils by gas chromatography-mass spectrometry (GC-MS).
3. Preliminary screening of essential oils against *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using the disc diffusion method

4. Determination of the minimum Inhibitory concentration and minimum fungicidal concentration using the broth microdilution method
5. Inhibitory effect of essential oils on mycelial growth, sporulation, and aflatoxin B1 production of the aflatoxigenic strains, *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 by comparison between the contact and the vapor treatments.

1.4 Benefits of the study

To be able to select the potential essential oils for the use in controlling aflatoxigenic strains and aflatoxin B1 contamination as an alternative choice to synthetic fungicides for the application on the food chain.

CHAPER 2

THEORY AND LITERATURE REVIEWS

2.1 Chemical properties of aflatoxin

Aflatoxins belonging to a group of mycotoxins which contaminate a quarter of the world's food crops especially in tropical and subtropical regions. The contamination can take place at any point along the food chain from the field, harvest, handling, shipment and storage (Magan *et al*., 2003). Four types of the main aflatoxins generally found in nature are aflatoxins B1, B2, G1, and G2 (Figure 1), which are named based on the illuminated fluorescent properties under ultraviolet light. Aflatoxins B1 and B2 show strong blue fluorescence under UV light, whereas G1 and G2 show greenish yellow fluorescence. The structures of aflatoxins B and G are similar and the potency of B1 is the most toxic followed by G1, B2, and G2 (Bennett and Klick, 2003). The International Agency for Research on Cancer (IARC) has classified both aflatoxins B and G as Group 1 (Carcinogenic to humans), whereas AFM1 is classified as Group 2A (Probably carcinogenic to human). (IARC, 2002).

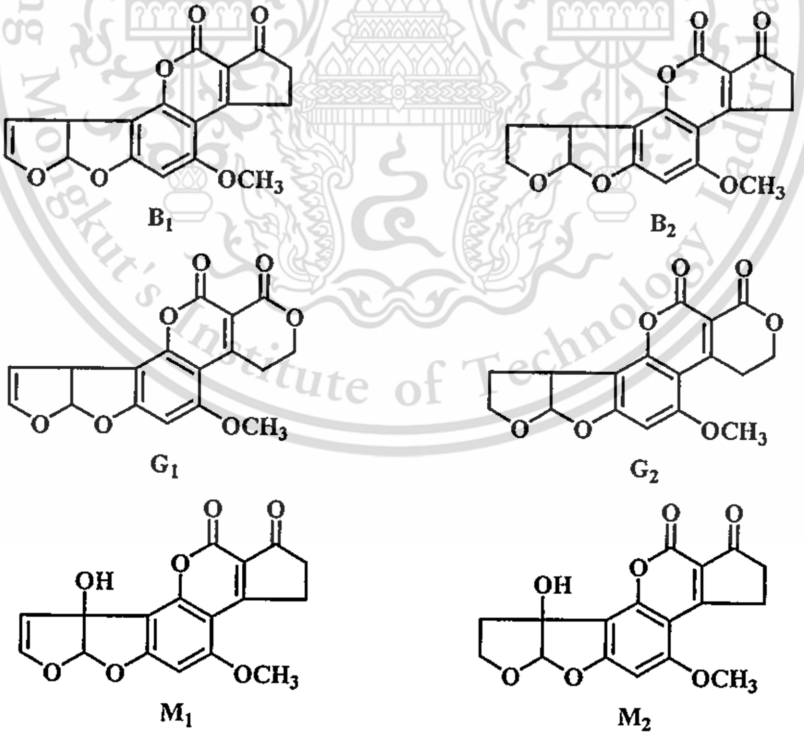

Figure 1 Structure of Aflatoxin (Bennett and Klick, 2003)

2.2 Biology of aflatoxigenic fungi

Aflatoxins are produced by the predominant species of *Aspergillus* such as *A. flavus, A. parasiticus*, which are common in soils, air, and on crop. Aflatoxigenic strains were shown in Table 1. Dominant aflatoxins produced by *A. flavus* are AFB1 and B2, whereas *A. parasiticus* produces two additional G1 and G2 (Payne and Brown, 1998). *A. flavus* and *A. parasiticus* are the most distinct species involved in aflatoxin production prior to harvest or during storage. *A. flavus* is the major contaminant of crops (Cary and Ehrlich, 2006) especially in corn, cottonseed, and tree nuts, while *A. parasiticus* is a main economic problem in peanuts (Bennett and Klick, 2003). *A. nomius* was determined to be the most abundant aflatoxin-producing species from the soil often found in different regions of Thailand. *A. pseudotamarii* is also reported as aflatoxin-producing species from Thailand (Ehrlich *et al.*, 2007). The major aflatoxin producing strains of *A. flavus* can be classified as L or S strains according to the size of sclerotia. The S strains produce numerous small sclerotia that are less than 400 μm while the L strains produce sclerotia greater than 400 μm. The S strains produced aflatoxin more than L strains (Bennett and Klich, 2003).

Table 1 Aflatoxin producing fungi and types of aflatoxins produced

Species	Type of aflatoxin
Aspergillus section *Flavi*	
A. arachidicola	AFB1, B2, G1, G2
A. bombycis	AFB1, B2, G1, G2
A. flavus	AFB1, B2
A. minisclerotigenes	AFB1, B2, G1, G2
A. nomius	AFB1, B2, G1, G2
A. novoparasiticus	AFB1, B2, G1, G2
A. parasiticus	AFB1, B2, G1, G2

Table 1 Aflatoxin producing fungi and types of aflatoxins produced (contd.)

Species	Type of aflatoxin
A. parvisclerotigenus	AFB1, B2, G1, G2
A. pseudocaelatus	AFB1, B2, G1, G2
A. pseudonomius	AFB1
A. pseudotamarii	AFB1, B2, G1, G2
A. togoensis	AFB1
A. transmontanensis	AFB1, B2, G1, G2
A. mottae	AFB1, B2, G1, G2
A. sergii	AFB1, B2, G1, G2
Aspergillus section *Ochraceorosei*	
A. ochraceoroseus	AFB1, B2
A. rambellii	AFB1, B2
Aspergillus section *Nidulantes*	
A. astellatus (=*Emericella astellata*)	AFB1
A. olivicola (=*Emericella olivicola*)	AFB1
A. venezuelensis (=*Emericella venezuelensis*)	AFB1

Source: Adapted from Baranyi *et al.* (2013)

2.3 Biosynthesis of aflatoxins

Aflatoxins are synthesized through a polyketide pathway from hexanoyl-CoA and 7 malonyl-CoAs to condensed polyketide noranthrone. Noranthrone is then converted to norsolorinic acid (NOR) which is the first stable intermediate (Bennett *et al.*, 1997). The conversion of NOR to averantin (AVN) and AVN to hydroxyaverantin (HAVN) was presented in Table 2. After the conversion of versicolorin B (VERB) to versicolorin A (VERA), there is a branch point in the pathway that leads to aflatoxin formation (Figure 2). O-methyltransferase for the conversion of sterigmatocystin (ST) to O-methylsterigmatocystin (OMST) and DMST to dihydro-O-methylsterigmatocystin (DHOMST) (Bhatnaga *et al.*, 1988). Two cluster-specific regulators are also known; i.e. *aflR,* a gene which encodes a transcription activator of the promoter regions of aflatoxin structural genes (Payne *et al.*, 1993), and *aflS,* a gene involved in the regulation of transcription of *aflR* (Meyer *et al.*, 1998). The gene *aflD* encodes a ketoreductase enzyme involved in the conversion of norsolorinic acid to averantin (AVN). Deletion or disruption of this gene determines losses of aflatoxin pathway (Papa, 1982). *aflaM* encoding a ketoreductase, which is required for the conversion versicolorin A (VERA) to demethyl sterigmatocystin (DMST) and versicolorin B (VERB) to demethyldihydrosterigmatocystin (DMDHST) (Skory *et al.*, 1992) and *aflP* encodes an O-methyltransferase for the conversion of sterigmatocystin (ST) to O-methylsterigmatocystin (OMST) and DMST to dihydro-O-methylsterigmatocystin (DHOMST) (Bhatnaga *et al.*, 1988). Two cluster-specific regulators are also known; i.e. *aflR,* a gene which encodes a transcription activator of the promoter regions of aflatoxin structural genes (Payne *et al.*, 1993), and *aflS,* a gene involved in the regulation of transcription of *aflR* (Meyer *et al.*, 1998).

Table 2 Importance genes in aflatoxin biosynthesis

Gene	Enzyme or product	Pathway
aflA	Fatty acid synthase α	malonyl CoA → condensed polyketide noranthrone
aflB	Fatty acid synthase β	malonyl CoA → condensed polyketide noranthrone
aflC	Polyketide synthase	malonyl CoA → condensed polyketide noranthrone
hypC	Anthrone oxidase	noranthrone → norsolonic acid
aflD	Reductase	norsolonic acid (NOR) → averantin (AVN)
aflE	NOR reductase	norsolonic acid (NOR) → averantin (AVN)
aflF	Dehydrogenase	norsolonic acid (NOR) → averabtin (AVN)

Table 2 Importance gene in aflatoxin biosynthesis (contd.)

Gene	Enzyme or product	Pathway
aflG	Cytochrome P450 monooxygenase	averantin (AVN) → hydroxyaverantin (HAVN)
aflH	Alcohol dehydrogenase	hydroxyaverantin (HAVN) → averufin (AVR)
aflI	Averufin monooxygenase	averufin → versiconal hemiacetal aceteate (VHA)
aflJ	Cytosole esterase	versiconal hemiacetal aceteate → versiconal (VAL)
aflK	Versicolorine B synthase	versiconal → versicolorin B (VERB)
aflL	Cytochrome P450 monooxygenase/ desaturase	versicolorin B → versicolorin A (VERA), versicolorin B → demethyl dihydrosterigmatocystin (DMDHST)
aflM	Ketoreductase	versicolorin A → demethylsterigmatocystin (DMST)
aflN	Cytochrome P450 monooxygenase	versicolorin A → demethylsterigmatocystin (DMST)
AflO	O-methyltransferase I /	demethylsterigmatocystin → sterigmatocystin (ST)
	O-methyltransferase B	dihydrodemethylsterigmatocystin → dihydrosterigmatocystin (DHST)
aflP	O-methyltransferase II /	sterigmatocystin → O-methylsterigmatocystin (OMST)

Table 2 Importance gene in aflatoxin biosynthesis (contd.)

Gene	Enzyme or product	Pathway
	O-methyltransferase A	dihydrosterigmatocystin → dihydro-O-methyl sterigmatocystin (DHOMST)
		O-methylsterigmatocystin → aflatoxins B1 and G1
		dihydro-O-methylsterigmatocystin → aflatoxins B2 and G2
aflR	Transcription activator	Pathway regulator
aflS	Transcription enhancer	Pathway regulator

Source: Adapted from Yu *et al.* (2004).

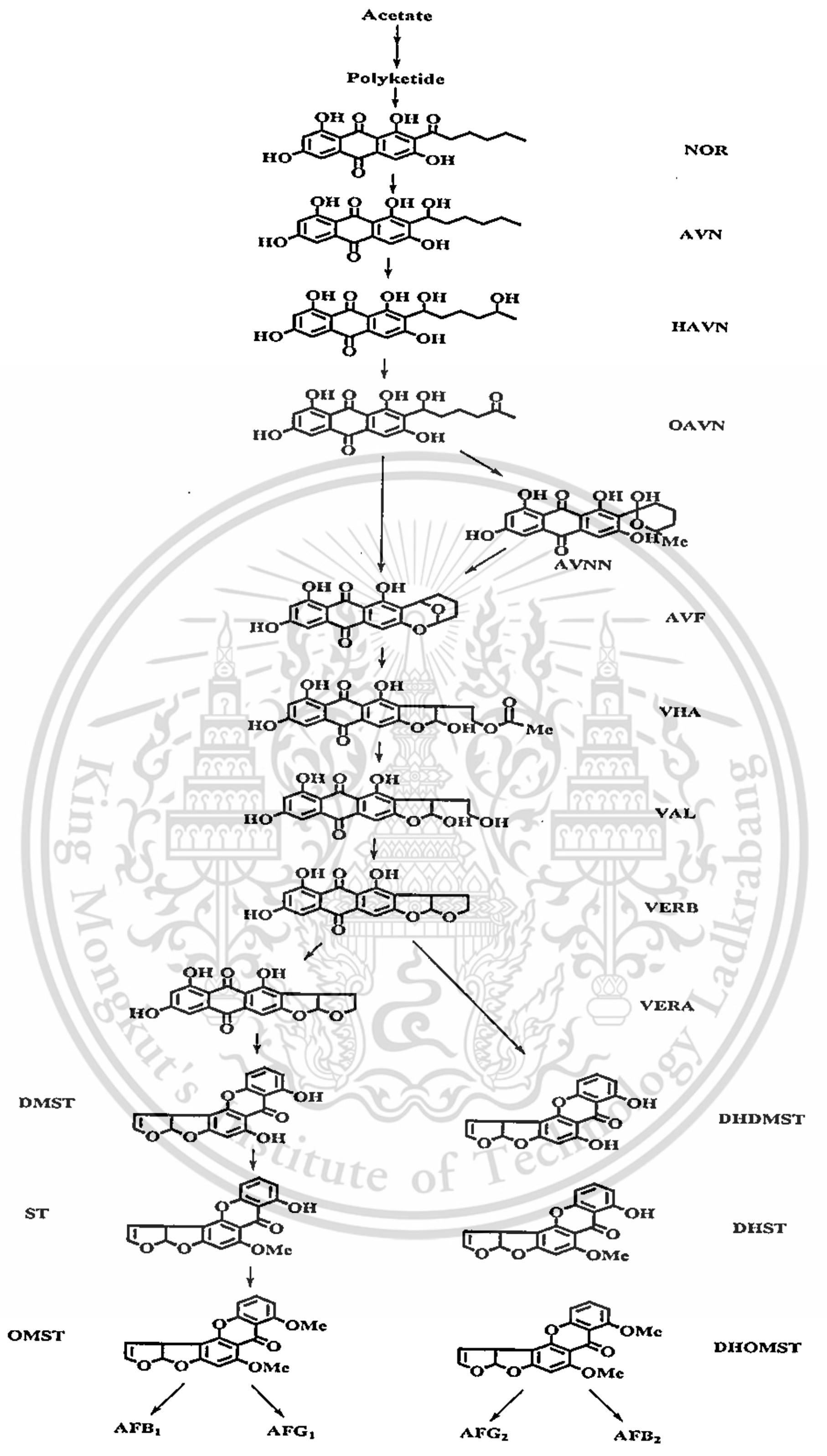

Figure 2 Biosynthesis of aflatoxin

Source: Yu and Cleveland (2004)

2.4 Factors affecting aflatoxin production

Aflatoxigenic fungi are imperfect filamentous fungi, which are saprophytic during most of their life cycle, and grow on a wide variety of substrates including decaying plants and animal debris (Hedayati *et al.*, 2007). The differences in the fungal community are reflected in the relative abundance of AFB and AFG in various regions (Cotty, 1997). The presence of aflatoxins B1, B2, G1, and G2 occurred naturally is about 1.0: 0.1: 0.3: 0.02 (Abbas *et al.*, 2010). Nutritional sources such as carbon or nitrogen source, as well as environmental effects such as high temperature and pH supported the growth of aflatoxigenic strains and subsequently aflatoxin biosynthesis and induction of *AflR* expression (O'Brian *et al.*, 2007). Most carbohydrates can be utilized as the sole carbon source for the growth of *A. flavus* and *A. parasiticus* (Cucullu *et al.*, 1976). Abdollahi and Buchanan (1981) and Mateles and Adye (1965) indicated that the importance of carbon source such as sucrose, fructose and glucose is in particular for aflatoxin production. Lipid substrate is also a good carbon source to support aflatoxin production (Fanelli and Fabbri, 1989). There were significant positive correlations of higher sucrose content in peanuts with their effect on aflatoxin production (Manda *et al.*, 2004). Davis *et al.* (1967) reported that glucose and sucrose were the preferred carbon source, whereas yeast extract and peptone were suitable nitrogen source for aflatoxin production. Reddy *et al.* (1979) also reported that yeast extract was particularly efficient in supporting aflatoxin production. Organic nitrogen source is reported to stimulate aflatoxin synthesis (Luchese and Harrigan, 1993). Nitrate was reported to have a suppressive effect on aflatoxin production (Chang *et al.*, 1995). The amino acid such as tryptophan inhibits aflatoxin formation while tyrosine supports aflatoxin production in *A. flavus* (Wilkinson *et al.*, 2007). Asparagine was found to be essential for aflatoxin production (Reddy *et al.*, 1979). For the trace elements, magnesium, zinc, and iron are the essential metals for aflatoxin production (Wongurai *et al.*, 1990). Diener and Davis (1967) reported that the optimum temperature for aflatoxin production by *A. flavus* was 25 °C on both peanuts and liquid medium but extended from 25 to 30 °C for *A. parasiticus* extended from 25 to 30 °C. The main factors involved are moisture and temperature of these fungi which can grow in the range of temperatures from 12 to 48 °C and the optimal range is between 25 to 42 °C depending on the variety of strain and medium (Gqaleni *et al.*, 1997; Hedayati *et al.*, 2007). The ratio of AFB1 and AFG1 produced by *A. parasiticus* increased with increasing temperature. The range of temperature for the aflatoxin production by *A. flavus* and *A. parasiticus* is reported as 12 to 41 °C, with optimum temperature for production between 28 to 35 °C (Ogundero, 1987). Northolt *et al.* (1976) reported that the optimum water activity (a_w) for aflatoxin B1 production is 0.99, whereas Diener and Davis (1967) indicated that the optimum value is 0.95 and 0.99 depending on the substrate. The a_w for the growth of the *A. flavus* was between 0.86-0.96 (Vujanovic *et al.*, 2001; Gqaleni *et al.*, 1997).

2.5 Prevention and control of aflatoxin

The prevention and control of aflatoxin in agricultural commodities are very important at both pre-and post-harvest processes due to a great impact on quality and quantity of crops as well as nutritious food safety. The growth of aflatoxigenic strains in crops and agricultural products is the main cause of aflatoxin production and many factors are involved such as plant susceptibility to fungal infestation, temperature, climate, moisture content, and physical damage of seeds from insects and pests (Hell and Mutegi, 2011). Kaaya and Kyamuhangire (2006) reported that most food contamination occurred more during the postharvest storage because aflatoxin-producing fungi grow exponentially in the condition with the excessive heat and high humidity, and insect and rodent damages. Generally major crops such as cotton seed, corn and other cereals are infected (Abbas *et al.*, 2009).

Contamination of peanut seed by *A. flavus* and *A. parasiticus* is one of the main factors affecting peanut seed quality and also pre-harvest contamination is the major economic problem in the peanut industry (Assis *et al.*, 2005; Craufurd *et al.*, 2006). The infection of *A. flavus* and *A. parasiticus* in peanuts is from the aggressiveness of the fungus, peanut genotype susceptibility, as well as soil moisture and temperature (Assis *et al.*, 2005) and especially the damage of kernels by insects would increase aflatoxin contamination (Cole *et al.*, 1995). Contamination of groundnuts by *Aspergillus* strains also occurs at both pre-and post-harvest process, leading to aflatoxin contamination. Invasion of groundnut or peanut pods and seeds by *A. flavus* in the field occurs rapidly when kernel moisture content is between 14 to 30%. The optimum relative humidity (RH) for fungal growth is 85% or greater.

Diener and Davis (1970) reported that the lowest RH for aflatoxin production on peanuts was 83% and the significant levels of aflatoxins produced were at 97 to 99% RH and the temperature of 20 to 35 °C. Seed surface lipids are the key factor in supporting the growth of *A. parasiticus* ans aflatoxin formation in oil and starchy seeds (Luca *et al.*, 1989). The hyphae of *Aspergillus* sp. penetrate the peanut pod walls and the seed coat to the nutrient rich cotyledons (Bhatnagar-Mathur *et al.*, 2015). The fungal spores also penetrate either by sudden and extreme changes in weather or during pollination (in maize) can cause damage to the pod wall/kernels (in peanut) (Bhatnagar-Mathur *et al.*, 2015). For aflatoxin management in post-harvest, grain quality can be preserved to minimize aflatoxin accumulation by lower moisture less than 10% and elimination of insect damage and low temperature. Numerous studies have been conducted to prevent and eliminate fungal and aflatoxin contamination in stored agricultural products. Drying process to control fungal contamination in seeds and commodities was suggested at moisture levels lower than 9% for peanut kernel and lower than 13.5% for corn or maize (FAO,1979).

Climate change is threatening for food and feed security in many regions across the globe. Perrone *et al.* (2014) reported the risk of a shift and expanding boundaries in aflatoxin problems toward new territories particular in the United States (Wu *et al.*, 2011), Africa (Bandyopadhyay *et al.*, 2016), Europe (Paterson and Lima, 2010) due to a gradual increase in temperature and changes in atmosphere gas concentration. Increasing temperature to >30°C would support their germination, growth, sporulation and increase of diversity of local fungi population to create the new fungal genotypes with high aggressiveness and increase aflatoxin production (Paterson and Lima, 2010). Changes in environmental temperature influence the expression levels of regulatory genes (*aflR* and *aflS*) to significantly stimulate the aflatoxin production in both *A. flavus* and *A. parasiticus*. (Schmidt-Heydt *et al.*, 2010) and also favor higher increasing of the S strain of *A. flavus* due to increased soil temperature (Jaime-Garcia and Cotty, 2010).

2.6. Methods of aflatoxin decontamination

Contamination of aflatoxin producing fungi and aflatoxin production in agricultural commodities as the raw materials of food and feed have caused problem around the world. The method for aflatoxin decontamination such as physical, chemical and biological methods are applied.

Physical methods of aflatoxin decontamination include sorting, sunlight (Herzallah *et al.*, 2008), ionizing radiation such as gamma-rays can stop growth of food spoilage microorganisms and causes a reduction in the concentration of aflatoxins (Shantha and Sreenivasa, 1977). Roasting (Ogunsanwo *et al.*, 2004: Yazdanpanah *et al.*, 2005: Arzenden and Jinap, 2011) and microwaving (Farag *et al.*, 1996) are used to destroy the aflatoxin contamination. Mobeen (2001) reported a 60% reduction of aflatoxin contamination in peanuts with the effect of microwave process.

Chemical methods have been used as the most effective means for reduction and removal of mycotoxins from contaminated commodities. However, toxic residues and the reagents can reduce the nutritional value of the product or might change the taste of the food quality (Peraica *et al.*, 2002). The common chemicals commonly for detoxification and reduction of aflatoxin are ammonia gas (NH_3) (Gomaa *et al.*, 1997), hydrogen peroxide (H_2O_2) (Cucullu *et al.*, 1976), and sodium bisulphate ($NaHSO_3$). Bankole *et al.* (1995) showed that ethylene oxide and methyl bromide, chemical fumigants, significantly reduced toxigenic moulds. The effective reaction of ammoniation is dependent on temperature, pressure and aflatoxin is detoxified *via* a primary addition of the double bond of the furan ring and oxidation involving phenol formation and opening of the lactone ring. However, FDA do not approve due to possible toxic chemical irritating the eyes, suffocation and death in prolonged inhalation (Klick, 2007). In the presence of strong acid, aflatoxins B and G are converted to 2-hydroxy derivatives and AFB2a, respectively, while the lactone group in aflatoxins can be opened in the alkaline condition (Andrellos and Reid, 1964).

The use of chemical fungicides in the field such as azoxystrobin, pyraclostrobin, propiconazole, terconazole, dithiocarbamate showed effective inhibition of aflatoxin biosynthesis in *A. flavus* (Abbas *et al.*, 2009). However, repeated uses can lead to fungicide-resistant fungi (Feng and Zheng, 2006) and their high and acute residual toxicity have long degradation period causing, environmental pollulation and have adverse effects on food and human health (Unnikrishman and Nath, 2002).

Biological methods have been used to reduce aflatoxin contamination in various crops such the use of bacteria, yeast and non-aflatoxigenic strains to displace the toxigenic strains in the field. Substrate competition by these microorganisms and their production of some metabolites enable the inhibition of aflatoxigenic strains (Bhatnagar *et al.,* 2004). Kimura and Hirano (1988) reported that using *Bacillus subtilis* and *Bacillus pumilus* suppressed fungal growth and aflatoxin production by *A. flavus* in corn and by *A. parasiticus* in peanuts. Thakur *et al.* (2003) reported that *Trichoderma* sp. and *Pseudomonas fluorescens* were also able to reduce *A. flavus* in ground nut kernel infection in the field. However, Chourasia (1995) showed that the high aflatoxin production was marked when *A. flavus* and *Bacillus megaterium* were grown together. While *Flavobacterium odoratum* showed inhibition of aflatoxin formation by *A. flavus*, geocarposphere bacteria stimulated growth and aflatoxin production. In corn field with non-aflatoxigenic strains of *A. flavus* significant reduction of aflatoxin levels was observed (Atehnkeng *et al.,* 2008; Abbas *et al.,* 2006; Pitt and Hocking, 2006). The critical factor of biological methods is the selection of a stable non-toxigenic strain which is also competitive in the field, and preferably persistent (Pitt and Hocking, 2006). In addition, some probiotic bacteria strains such as *Lactobacillus acidophilus*, *L. bulgaricus* and *L. olanatarum* showed the ability to remove AFB1 by their adhesion capability of aflatoxin (Karunaratne *et al.*, 1990).

The possible management strategy for reducing, eliminating or removing aflatoxigenic strains and aflatoxin through food chains are as follows: (Cleveland *et al.*, 2003; Bhatnagar-Mathur *et al.*, 2015; Namazi *et al.,* 2002)

1. Developing of genetically modified crops which are resistant to fungal invasion
2. Destroy mycelia and spores of aflatoxigenic strains which may disperse in the soil and air and further proliferate under the favorable condition.
3. Control the environmental and physiological factors affecting fungal growth and aflatoxin biosynthesis.
4. Prevention of aflatoxin biosynthetic pathway by direct inhibition of gene expression or enzyme activity in the pathway.

2.7 Control of aflatoxinigenic strains and aflatoxin production using essential oils

Essential oils or volatile oils are highly volatile substances extracted from plants by a physical process. Aromatic plant essential oils are valuable natural products used in perfumes, cosmetics, aromatherapy, and the food industry (Buchbauer, 2000). Moreover, most of the essential oils are categorized as generally recognized as safe (GRAS) by the United State Food and Drug Administration (FDA), which are eco-friendly and harmless to humans, and can be used as an alternative to synthetic fungicides to control aflatoxigenic strains (Shukla *et al.*, 2009; Thanoboripat *et al.*, 2016).

Constituents in the form of structure and functional groups of the most abundant compounds in the essential oil are important for their antifungal activities. There is evidence that minor components have a critical factor to play in antimicrobial activity, by a synergic effect between other compounds (Burt, 2004) on their differential capacity to support essential oil to penetrate into the chitin-based cell walls of fungal hyphae (Moghadd *et al.*, 2013). The activity level of essential oil components on antifungal action is as follows: phenols > aldehydes > ketones >alcohols >ethers > hydrocarbons (Kalemba and Kunicka, 2003). The antifungal activity of essential oil is linked to its individual components. Ethyl cinnamate (Abdelgaleil *et al.*, 2008), pentadecane (Essien *et al.*, 2016), ethyl methoxycinnamate (Gupta *et al.*, 1976), bergamotene (Teixeira *et al.*, 2013), caryophyllene oxide (Guillen *et al.*, 1996), guaiazulene (Higa and Sakemi, 1988), β-selinene (Kramer and Abraham, 2012), linalool (Herman *et al.*, 2016; Hsu *et al.*, 2003), cinnamaldehyde (Sun *et al.*, 2015), and β-elemene (Zhu *et al.*, 2013), have been proved to show antifungal activities. Other compounds in relatively low concentrations, such as borneol (Tabanca *et al.*, 2001) and cymene (Ultee *et al.*, 2002), are also known to have effective antimicrobial properties as reported by many researchers.

Various methods for extraction of essential oil such as steam distillation, hydrodistillation or solvent extraction (Nakatsu *et al.*, 2000) are applied according to the stability and activity of the essential oil. Stream distillation or hydrodistillation are the preferred method (Masango, 2005; Sokovic and van Griensven, 2006). However, their need of energy consumption to evaporate volatile compounds and the prolonged contact between the plant and water at high temperature may cause changes in the chemical composition as well as degradation of unsaturated or ester compounds are the disadvantage of this method (Khajeh *et al.*, 2004). For the solvent extraction, using organic solvents may be a very simple way. However, the solvent extraction may include both of volatile and non-volatile compounds and the solvent are also required to be completely eliminated from the extract (Guan *et al.,* 2007). Microwave-assisted extraction is an important alternative method due to its advantage by reducing extraction time and solvent (Sadani and Shakeri, 2016). Supercritical CO_2 is respected in terms of quality and environment. It is limited to high-value products due to the high operational cost (Ouzzar *et al.,* 2015).

Several research have studied on the antifungal properties of essential oils and their mode of action (Sharma and Tripathi 2006; Bluma *et al*., 2009; Rasooli and Abyaneh, 2004). Farag *et al*. (1989) reported the inhibition of growth and aflatoxin formation of *A. flavus* and *A. parasiticus* by spice oil and their active compounds. Thanaboripat *et al.* (2007) studied the inhibitory effects of 16 medicinal essential oils, and found that three essential oils, i.e. white wood (*Melaleuca cajuputi*), cinnamon (*Cinnamomum cassia*) and lavender (*Lavandula officinalis*) showed higher inhibition of *A. flavus* IMI 242684 than the other essential oils. Soliman and Badeaa (2002) reported that thymus and anise essential oils were more effective than cinnamom (*Cinnamomum zeylanicum* L.) and spearmint (*Mentha viridis*). Anise essential oil completely inhibited *A. flavus* and *A. parasiticus* strains at 500 mg/kg in maize meal extract agar. Ozcakmak *et al.* (2010) reported that 250 μL/mL of *Thymus vulgaris* essential oil, 125 μL/mL of thyme essential oil and 500 μL/mL of *Rosmarinus officinalis* L. essential oil had the fungicidal effect on *A. flavus* MAM-200682. Yooussef *et al.* (2016) showed that mycelial growth of *A. parasiticus* was inhibited by cinnamon oil at 1000 ppm or higher, lemongrass and clove oils at 1500 ppm or higher and thyme at 2500 ppm. Phuangri *et al*. (2017) found that essential oil from *Zanthoxylum piperitum* seeds inhibited aflatoxigenic *A. flavus* with the MIC of 4.5 μL/ml in both solid and liquid cultures. Although the antimicrobial mechanism of essential oils from many plant species has been extensively studied, it has not been fully understood. It might be explained that their mixtures of several compounds do act on specific targets and may be synergistic interaction with other active compounds (Adegoke *et al*., 2000).

In addition, the antifungal mechanism of essential oil might be due to their low molecular weight and high lipophilicity of essential oil, which allows them to degrade fungal cell wall and causes increased membrane permeability resulting in leakage of ions and other cell organelles (Uribe *et al.*, 1985; Williams and Barry 1991; *Tian et al.*, 2011, 2012; Romagnoli *et al.*, 2005), as explained in Figure 3.

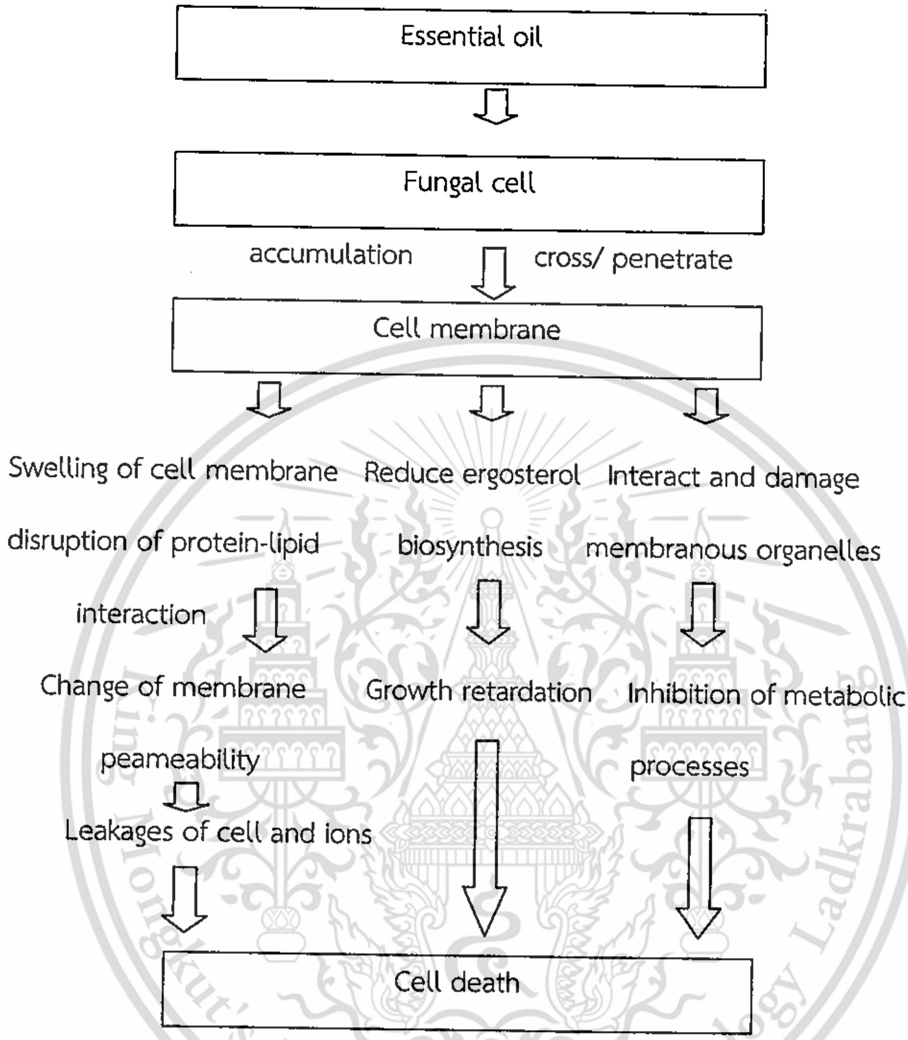

Figure 3. Antifungal mode of action of essential oil

Source: Adapted from Kedia *et al.*, 2015

Sharma and Tripathi (2006) reported that the inhibitory activity of essential oil against fungi attributed to the morphological change in cell wall and interference in enzymatic reaction of cell wall synthesis, which in turn affects fungal growth and morphogenesis. Several researchers have also reported that a greater antifungal activity of essential oil achieved in vapor phase than in aqueous solution or agar contact (Lopez *et al.*, 2005; Laghchimi *et al.*, 2014). Inouye *et al.* (2003) reported that fungi are inhibited more effectively by vapor treatment than by contact treatment with essential oils of cinnamon, thyme, and lavender. These may be related to the variation in the compositions of the two phases, with the vapor phase being higher in more volatile compounds. Due to the hydrophobic nature of compounds that constitute the volatile of essential oil, it could be expected that they may act mainly by accumulation on mycelium rather than the agar and inhibited fungi at three stages, i.e. germination of a conidium, elongation of vegetative mycelium and sporulation of reproductive mycelium. The use of the vapor phase has the additional advantages of ease for application and avoiding direct contact with the oil. Furthermore, a smaller volume of essential oil is required to achieve the same level of inhibition. In addition, the toxicity by vapor contact has been reported more rarely (Feng *et al.*, 2011).

The inhibitory effect of essential oil against sporulation has been previously reported (Mahanta *et al.*, 2007; Tzortzakis and Economakis, 2007). Alverez-Castellanos *et al.* (2001) showed that *Botrytis cinerea, Penicillium digitatum* and *Sclerotinia sclerotiorum* decreased spore production in the presence of essential oil from flowerheads of *Chrysanthemum coronarium*, either by contact or from headspace volatiles. Essential oil may act on the surface mycelial development and/or the perception/ transduction of signals involved in the switch from vegetative to reproductive development (Tzortzakis and Economakis, 2007). Gandomi *et al.* (2009) indicated that *Zataria multifora* essential oil exhibited significant inhibition of fungal growth as well as spore production. Bluma *et al.* (2009) reported that perina and peppermint essential oils completely inhibited sporulation of *Aspergillus* section Flavi. The effect of three Moroccan essential oils on the partial inhibition of spore production could be attributed to mycelial destruction or inhibition of fungal growth (Tataoui-Elaraki *et al.*, 1993). Silva *et al.* (2012) studied the antifungal activity of essential oils of fennel, ginger, mint, and thyme against *A. flavus* and *A. parasiticus* and found that the essential oil of thyme showed the best inhibitory effect on mycelial growth and sporulation of *A. flavus* and *A. parasiticus*. The inhibition of mycelial growth and sporulation of antiaflatoxigenic fungi is one of the main factors for preventing aflatoxin contamination which may proliferate in atmosphere, water and soil (Tzortzakis and Economakis, 2007). *R. officinalis* essential oil at 450 ppm completely inhibited aflatoxin production, whereas at 250 ppm decreased aflatoxin production only by 1.87%. Rasooli *et al*. (2008) also reported that aflatoxin biosynthesis was suppressed by the effect of *R. officinalis* essential oil. Bluma *et al* (2008) showed that 150-500 mg/kg of *Minthosthachys verticillata* (Griseb) Epl., peperina and peppermint essential oils inhibited AFB1 production by 85-90%. The essential oil of cassia and bay leaves caused 98% reduction in aflatoxin B1 but stimulated fungal growth. Patkar *et al.* (1993) reported that essential oil of anise, caraway, and cinnamon exhibited fungistatic and fungicidal activities against *A. flavus* and the inhibitory effect of 1 μl/ml of *Syzygium aromaticum* L. essential oil on growth and aflatoxin B1 production of *A. flavus* was also recorded. At 200 ppm of *Zataria multiflora* Boiss the fungal growth and sporulation of *A. flavus* ATCC 15546 were reduced by 79.4% and 92.5% on PDA while essential oil at 150 ppm in YES broth could reduce the mycelial growth and aflatoxin production by 90% and 99.4%, respectively (Gandomi *et al.*, 2009). The essential oil of *Pelargonium graveolens* exhibited a fungistatic activity at 0.75 g/l and completely inhibited aflatoxin B1 at 0.50 g/l. Patil *et al.* (2000) reported that 0.75 mg/ ml of *Ageratum conyzoides* L. essential oil completely inhibited the growth of *A. parasiticus* and inhibited more than 84% aflatoxin production. El-Habib (2012) reported that essential oils of dill, coriander, basil, marjoram, rosemary, mint and thyme have antifungal activities against *A. flavus* and aflatoxin B1 production. Kedia *et al.* (2014a) showed that spearmint essential oil inhibited aflatoxin B1 production at a concentration lower than its MIC for fungal growth. The essential oil of *Cuminum cyminum* L. seeds inhibited both *A. flavus* LHP (C)-D6 growth and aflatoxin production. The essential oils of *Thymus eriocalyz* and *T. x-porlock* were evidently fungicidal and inhibitory to aflatoxin production (Rasooli and Abyaneh, 2004). Paranagama *et al.* (2003) reported that lemongrass oil was fungistatic and fungicidal against *A. flavus* Link at 0.6 and 1.0 mg/ml, respectively, while at 0.1 mg/ml aflatoxin production was completely inhibited. Essential oil of *Nigella sativa* significantly inhibited aflatoxin B1 production of *A. flavus* by 47.9-58.3% and *A. parasiticus* by 32-48% and no significant effect on the growth of both *Aspergillus* strains (El-Nagerabi *et al.*, 2012). Atanda *et al.* (2007) examined the potential of essential oils from *Ocimum basilicum*, *Cinnamomum cassia*, *Coriandrum sativum* and *Laurus nobilis* for controlling the growth of *A. parasiticus* CFR 223 and aflatoxin production and found that the essential oil of

Ocimum basilicum completely inhibited mycelial growth and aflatoxin production at 5% (v/v). Thanaboripat *et al.* (2004) reported that essential oils of citronella inhibited growth, AFB1 production, and sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 102566 in maize grain. Ansari and Shrivastava (1991) reported that eucalyptus oil showed the effect on growth inhibition of *A. flavus* and aflatoxin production at 6 days of incubation, however at 12 days of incubation acceleration in aflatoxin formation was marked without any further effect on growth. Sinha *et al.* (1993) showed that 50 and 100 μg/mL of clove oil and 50 μg/mL of cinnamon oil did not significantly reduce the growth of *A. flavus* while both compounds above 100 μg/ml significant reduced both mycelial growth and aflatoxin formation. Clove oil and cinnamon at 250 μg/ml reduced aflatoxin formation by 67 and 73%, respectively. In the maize kernels treatment with 1000 mg/ml of clove oil and cinnamon oil was inhibited aflatoxin formation by 76 and 78%, respectively. Sindhu *et al.* (2011) reported that turmeric leaf essential oil at 1.0 % and 1.5% in YES broth exhibited 95.3% and 100% inhibition of aflatoxin production of *A. flavus*, respectively. Prakash *et al.* (2010) showed that MIC of *P. bettle* L. essential oil was 0.7 μl/ml against *A. flavus* while the inhibition of AFB1 production occurred at 0.6 μl/ml. Ferreira *et al.* (2013) showed that essential oil from *Curcuma longa* L. significantly inhibited *A. flavus* sporulation and also aflatoxin production at the concentration above 0.05% which may be related to inhibiting on the ternary steps of aflatoxins biosynthesis involving lipid peroxidation and oxygenation. Vilela *et al.* (2009) reported that essential oil obtained from leaves of *Eucalyptus globules* completely inhibited the growth of *A. flavus* Link and *A. parasiticus* Speare in contact and volatile assays. Mean AFB1 production by both fungi is lower in the vapor assay than in the contact assay. Tian *et al.* (2011) reported the efficacy of essential oil on aflatoxin inhibition may be due to the inhibition of carbohydrate catabolism in *A. flavus* by acting on some key enzymes and reducing its ability to produce aflatoxins. Holmes *et al.* (2006) indicated that factors affecting the inhibition of aflatoxin biosynthesis are environmental and physiological factors, signaling and regulatory up stream and gene expression or enzyme activity affecting aflatoxin biosynthesis. Jermnak *et al.* (2012) showed that methyl syringate from the essential oil of *Betula alba* was an aflatoxin production inhibitor, without significantly inhibiting fungal growth. Awuah (1995) reported that *Cymbopogon citratus, Xyloppia aethipoca* and *Cinnamomum verum* are effective in inhibiting the formation of norsolorinic acid, a precursor of the aflatoxin synthesis.

2.8 Effects of aflatoxins on animals and humans

Food contaminated with mycotoxins especially aflatoxins can cause health hazard to human and farm animals and also has an impact on nationally and internationally economic loss regarding feed supplies and food markets. Consumption of aflatoxin contaminated food are actively metabolized in hepatic microsomal and cytochrome P-450 enzymes (CYP1A2, CYP3A4, CYP3A5, CYP3A7, GSTM1) to at least 9 different forms of metabolites through hydroxylation, hydration, O-demethylation and epoxidation (Bennett and Klich, 2003), as shown in Figure 4.

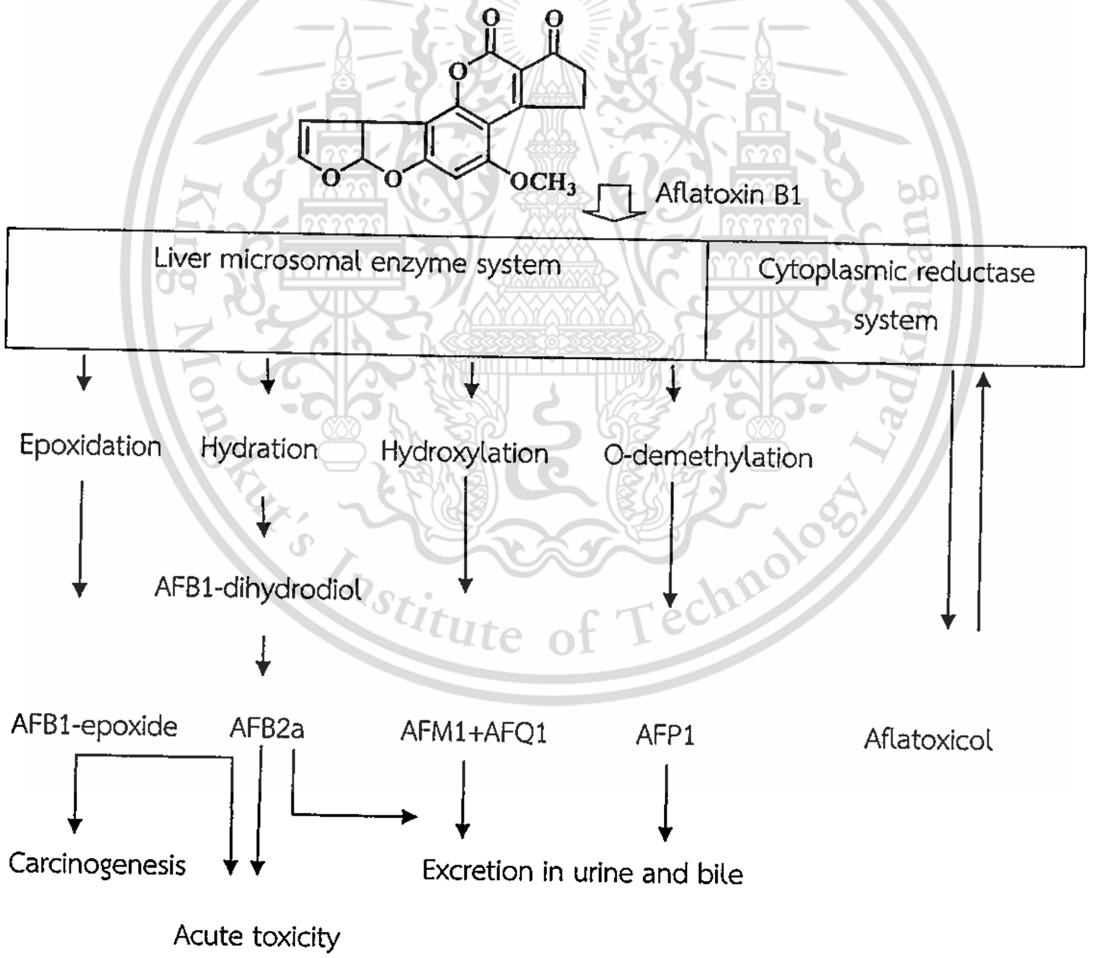

Figure 4 Metabolized aflatoxin B1 in human

Source: Adapted from Suttajit (2003)

AFB1 is metabolized to AFM1 and AFQ1 by hydroxylation at C4 or C22, respectively, and excreted in the urine. AFM1 is also a mutagen and carcinogen commonly found in urine, milk and dairy product. Human exposure to AFM1 occurs mainly through consumption of aflatoxin-contaminated milk, including mother's milk (Choketaworn *et al.,* 1986). AFP1 results from O-demethylation at C15 while AFB1-epoxide is formed by epoxidation at the 2,3 double bond of AFB1. The metabolic activation of AFB1 causes DNA mutation and replication by AFB1 epoxide reacting with the N^7 position of guanine residues in double-stranded DNA in GC to TA transversions and hence induces mutation in p53 tumor suppressor gene at codon 249 to development of hepatocellular carcinoma (Chan *et al.,* 2003). Aflatoxicol is derivative AFB1 by a soluble cytoplasmic reductase enzyme. AFB2a is metabolized by hydration of AFB1 at C2-C3 double bond. Other aflatoxin derivatives are present in liver tissue, blood and urine. Conjugated products or adducts of aflatoxin, such as AFB1-guanine, AFB1-glucuronide, and AFB1-glutathione, can be found in urine and liver samples whereas aflatoxin-albumin adducts are detected in human blood serum (Suttajit, 2006).

Aflatoxicosis is poisoning resulting from ingestion of aflatoxins in contaminated food or food. The symptomatology due to intoxication can be acute or chronic in both animal and human. In human, the acute syndrome is characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma, and death with cerebral edema and fatty involvement of the liver, kidneys, and heart (IARC, 2002).

Aflatoxicosis caused by the presence of aflatoxin in feeds is a serious disease syndrome in poultry. Aflatoxins are capable of producing liver cancer in the most sensitive animal species when fed at a very low concentration of only one ppb. Duckling, chicks, calves, guinea pigs and pig trout are very sensitive to the hepatotoxin effects of AFB1, whereas rat, goat, sheep, and mouse are relatively sensitive to the acute effect of this toxin (Mor and Singh, 1998). Aflatoxin poisoning in animal could be detected at early sign of reduction of growth rate as well as meat milk and egg production and reduction of animal immunity depends on the species of animal, age, dose and duration of aflatoxin exposure. The most important pathological effect is liver injury and is often rapidly followed by death.

2.9 Aflatoxin regulation

Realization on the potential health hazard for humans, threshold levels of aflatoxins in commodities has been established worldwide. The United States Food and Drug Administration (FDA) has established specific guidelines on levels of aflatoxin at 20 ppb in food and feed, and 0.5 ppb of aflatoxin M1 in milk (Table 3). The European Union (EU) has the most strict aflatoxin standard by permitting the limit at 2 ppb for aflatoxin B1 and 4 ppb for total aflatoxins in nuts, peanuts, dried fruits, and cereals. Limits were also established for AFM1 of 0.05 ppb in milk and milk products. However different countries have different accepted levels for aflatoxins to control aflatoxin contamination in food and feed and also to prohibit trade of contamination product. Thai Government through the National Bureau of Agricultural Commodity and Food Standards (ACFS) of Ministry of Agriculture announced the maximum level of aflatoxins at 20 ppb in food (FAO, 2004). As for the food exporter, the agricultural commodities have to meet the regulation of the importing countries. Standard and regulation of aflatoxin is not the only purpose of increasing the safeguard of public's health, but also has an impact on the agricultural economy through the loss of productive and time for expense associated with monitoring and decontamination. The existence of different standards can be considered as trade and socio-economic concern due to trade limitation (Wu, 2004).

Table 3 FDA tolerance levels for total aflatoxins

Aflatoxin	Item	Tolerance level (ppm)
Aflatoxin	Food for human consumption	20
Aflatoxin	Feed for beef cattle and poultry	300
Aflatoxin	Feed for swine	200
Aflatoxin	Feed for breedinf livestock	100
Aflatoxin	Feed for dairy cattle	20
Aflatoxin M1	Milk	0.5

Source: Adapted from FAO, 2004

2.10 Determination of Aflatoxin

Aflatoxins are the toxic and carcinogenic compounds found in nature and affect humans and animals health. Regulation for aflatoxins has been enforced and method for aflatoxin detection must be accurated. Thus, the chemical methods for the analysis of aflatoxin have been well standardized. Irrespective of the method chosen, effective sample preparation, including extraction, and purification as well as the percentage of recovery are necessary.

The most current methods used for quantitative aflatoxin are thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). The traditional TLC method is suitable for screening method. Improvements of the stationary phase layer (HPTLC), and mobile phase application followed by densitometry, resulted in accuracies and precision comparable to HPLC methods (Rahmani *et al.*, 2009). HPLC is the most frequent method with more specificity and sensitivity for identification and quantitative analysis of aflatoxins better than detection by spectrophotometric and fluorescence method (Turner *et al.*, 2009).

The ELISA method involves a specific antigen-antibody complex. Antibodies are immobilized on microplates. An enzyme-labeled antigen conjugate and an enzyme substrate is added to bind to the unbound antibodies. The bound enzyme reacts with the substrate and the resulting color change is measured spectrophotometrically. Usually, ELISA method combines high sensitivity and specificity and also requires minimal sample preparation and reduces the time of sample analysis and the amount of solvent used significantly (Abbas *et al.*, 2004). Colak *et al.* (2006) showed that ELISA assays have consistently greater recovery values when comparing by HPLC and LC/MS method but all methods showed good correlation between the compared methods.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Reagents and laboratory apparatus

Chemical and Media	Company, Country
Demethyl sulfoxide (DMSO)	Merck, Germany
Sodium sulfate anhydrous (Na_2SO_4)	Merck, Germany
Potato Dextrose Broth	Himedia, India
Potato Dextrose Agar (PDA)	Himedia, India
Tween 80	Merck, Germany
Methanol	Merck, Germany
Aflatoxin B1	Sigma, USA.
Ketoconazole	Sigma, USA.

3.2 Equipment, instruments and glasswares

Equipment	Model/ Country
Biological Safety Cabinet	NuAire, USA.
Autocleave	Sanyo, Japan
Balance	Mettler Toledo, Switzerland
Freezer	Sanyo, Japan
Incubator	Memmert, Germany
Heat Mentle	MTOPs, Korea
Microcentrifuge	Daihan, India
Stereomicroscope	Olympus, Japan

3.2 Equipment, Instruments, Plastics and glasswares (contd.)

Equipment	Model/ Country
Vertex Mixter	Scientific Industrial, USA.
Test tube/ Petri dish	Pyrex, USA.
Multichannel pipette/ pipette tips	Rainin, USA.
Blender	Moulinex, France
Hot air oven model BE 500	Schwabach, Germany
Syring-Filter 0.45 μm	Sartorious, Germany
Glass bottle with screw cab	Schott Duran, Germany
Clevenger-type apparatus	SP Glass, Thailand
Microtiter plate 96 with lid	Costa Corning, USA.
Blender	Moulinex, France
Hemacytometer	BOECO, Germany
Effendrof	Costa Corning, USA.
DOA-Aflatoxin ELISA test Kit	Department of Agriculture (DOA), Thailand,
Microplate reader	Tacan, Switzerland
Filter paper	Whatman, England

3.3 Plant materials

The plant materials obtained from a local vegetable and fruit market, Herbal Pharmacy Store in Bangkok, Ayuddhaya, Bueng Kan and Prachinburi Province. Eighty eight plants belongs to 41 Families were listed in Table 4.

Table 4 Plant materials

Family	Scientific Name	Common name/ Local Name	Plant used
Acanthaceae	*Acanthus ebracteatus* Vahl.	Sea Holly	Leave
	Rhinacanthus nasutus (L.) Kurz	Thong Phan Chang	Leave
Alliaceae	*Allium tuberosum* Roxb.	Chinese Chive	Flower
Annonaceae	*Cananga odorata* Lam. Hook.f. and Thomson	Ylang Ylang	Flower
	Melodorum fruticosum Lour.	White Cheesewood	Flower
Araceae	*Acorus calamus* L.	Calamus	Rhizome
	Homolomena aromatics (Roxb.Sims) Schott	Tao-Kiat	Rhizome
Araliaceae	*Polyscias fruticosa* (L.) Harms	Polyscias	Leave
Apiaceae	*Anethum graveolens* L.	Dill	Aerial Plant
	Apium graveolens L.	Celery	Aerial Part
	Carum carvi L.	Caraway	Seed
	Coriandrum sativum L.	Coriander	Seed
	Cuminum cyminum L.	Cumin	Seed
	Eryngium foetidum L.	Long Coriander	Leave
	Foeniculum vulgare Mill.	Fennel	Seed
	Petroselinum crispum (Mill.) A.W.Hill	Parsley	Aerial Part

Table 4 Plant materials (contd.)

Family	*Scientific Name*	Common name/ Local Name	Plant used
Apiaceae	*Pimpinella anisum* L.	Anise	Seed
	Trachyspermum amni (L.) Sprague	Ajowan	Seed
Asteraceae	*Artemisia dracunculus*	Tarragon	Aerial Plant
	Acmella oleracea (L.) R.K. Jansen	Paracress	Leave
	Eupatorium odoratum L.	Siam Weed	Aerial Plant
	Spilanthes acmella Murr.	Toothache	Aerial Plant
	Vernonia cinerea (L.) Less.	Ya-la-ong	Aerial Plant
Basellaceae	*Basella alba* L.	Ceylon Spinach	Leave
Bignoniaceae	*Millingtonia hortensis* L.F.	Indian Cork	Flower
Brassicaceae	*Brassica juncea* L.	Mustard	Seed
Calophyllaceae	*Mesua ferrea* L.	Ironwood	Flower
Clusiaceae	*Mammea siamensis* Kosterm	Negkassar	Flower
	Garcinia cowa Roxb.	Cowa	Leave
Commelinaceae	*Murdannia bracteata*	Angel Grass	Leave
	Commelina benghalensis L.	Spiderwort	Leave
Compositae	*Cathamus tinctorius* L.	Safflower	Flower

Table 4 Plant materials (contd.)

Family	*Scientific Name*	Common name/ Local Name	Plant used
Cruciferae	*Lepidium sativum*	Garden cress	Seed
Cyperaceae	*Cyperus rotundus* L.	Nut Grass	Rhizome
Euphorbiaceae	*Sauropus androgynus* (L.) Merr.	Star Gooseberry	Leave
Illiciaceae	*Illicium verum* Hooker	Star Anise	Seed
Labiatae	*Vitex trifolia* L.	Khonthi-so	Leave
Lamiaceas	*Mentha cordifolia*	Kitchen Mint	Aerial Plant
	Rosmarinus officinalis L.	Rosemary	Twigs
	Salvia officinalis L.	Sage	Leave
Lauraceae	*Cinnamomum porrectum* (Roxb.) Kosterm	Thep Taro	Bark
	Cinnamomum zeylanicum Blume	Cinnamon	Bark
	Laurus nobilis	Bay	Leave
Leguminosae	*Parkia speciosa* Hassk.	Stink Bean	Seed
Magnoloaceae	*Michelia alba* DC.	White Champaka	Flower
	Magnolia champaca L.	Champaka	Flower
Menispermaceae	*Tiliacora triandra* (Colebr.) Diels.	Bamboo Grass	Leave
Mimoseae	*Acacia pennata* L.	Peninslar	Leave
Myristiceae	*Myristica fragrans* Houtt.	Nutmeg	Seed

Table 4 Plant materials (contd.)

Family	*Scientific Name*	Common name/ Local Name	Plant used
Myrtaceae	*Syzygium acromaticum* L.	Clove	Flower Bud
	Psidium guajava L.	Guava	Leave
Pandanaceae	*Pandanus odoratissimus* L.f.	Screw Pine	Flower
	Pandanus amaryllifolius Roxb.	Pandanus	Aerial Plant
Papilionaceae	*Trigonella foenumgraecum* L.	Fenugreek	Seed
Piperaceae	*Piper nigrum* L.	Black Pepper	Seed
	Piper retrofractum Vahl.	Long Pepper	Seed
	Piper sarmentosum Roxb	Wild Pepper	Leave
	Peperomia pellucida (L.) Kunth.	Phak Kra Sang	Leave
Plantaginaceae	*Plantago ovate* Forssk.	Psyllium	Seed
Poaceae	*Cymbopogon citratus* (DC.) Stapf.	Lemon Grass	Leave
	Cymbopogon nardu L.Rendle	Citronella	Leave
	Vetiveria zizanioides L.	Vetiver	Root
Polygonaceae	*Polygonum odoratum* Lour.	Vietnamese Coriander	Aerial Part
Ranunculaceae	*Nigella sativa* L.	Black Cumin	Seed

Table 4 Plant materials (contd.)

Family	*Scientific Name*	Common name/ Local Name	Plant used
Rubiaceae	*Gardenia jasminoides* J.Ellis.	Cape Jasmine	Flower
	Tarenna hoaensis Pitard	Sandal Wood	Wood
	Morinda citrifolia L.	Indian Mulberry	Leave
	Coffea arabica L.	Kofi	Seed
Rutaceae	*Citrus hystrix*	Kaffir Lime	Leave
	Zanthozylum budrunga	Chinese Pepper	Seed
Sapotaceae	*Mimusops elengi* L.	Bullet Wood	Flower
Scrophulariaceae	*Limnophila geoffrayi* Bonati	Finger Grass	Aerial Part
Thunbergiaceae	*Thunbergia laurifolia* L.	Blue Thunbergia	Leave
Verbenaceae	*Nyctanthes abortristis* L.	Night Blooming Jasmine	Flower
Zingiberaceae	*Alpinia conchigera* Griff.	Lesser Alpinia	Rhizome
	Alpinia galangal L.	Galanga	Rhizome
	Boesenbergia rotunda L. Mansf.	Fingerroot	Rhizome
	Curcuma aromatic Salisb.	Wild Turmeric	Rhizome
	Curcuma longa L.	Turmeric	Rhizome
	Curcuma mangga Val.&Zijp.	Mango Ginger	Rhizome
	Curcuma xanthorrhiza Roxb.	Javanese	Rhizome
	Curcuma zedoaria	Zedoary	Rhizome
	Elettaria cardamomum (L.) Maton	Cardamon	Fruit

Table 4 Plant materials (contd.)

Family	*Scientific Name*	Common name/ Local Name	Plant used
Zingiberaceae	*Kaempferia galanga* L.	Galanga	Rhizome
	Kaempferia parviflora Wallich.ex Baker	Black Ginger	Rhizome
	Zingiber montanum Koenig.	Phali	Rhizome
	Zingiber officinale Rosc.	Ginger	Rhizome
	Zingiber zerumbet	Bitter Ginger	Rhizome

3.4 Analytical Method

3.4.1 Plant extraction by hydrodistillation

Eighty-eight plant materials were washed twice with distilled water, subsequently air dried to prevent volatility of the plant constituents and to keep the natural colour of the essential oil. Before homogenizing to the fine powder, plant materials were stored in airtight bottles. Air-dried plant materials (100 g) were placed in a 1 L round-bottom distillation flask and 300 mL double distilled water were added. The essential oils were then obtained by hydrodistillation for 3 h, using a Clevenger-type apparatus according to the method of Clevenger (1928). The oily layer collected on top of the aqueous distillate was separated and dried over anhydrous sodium sulfate, filtered and then stored in a tightly closed dark vial at 4 °C to avoid exposure to light and oxygen until further studies. The oil yields were calculated. The extraction yields were calculated in percentage (%v/w) relative to the starting dry plant material in three replication.

3.4.2 GC-MS analysis of essential oil

The chemical composition of essential oil was analyzed using gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis was performed on

Agilent 6890 gas chromatograph in electron impact (EI, 70 eV) mode coupled to an HP 5973 mass selective detector and fitted with a fused silica capillary column (HP-5MS, 30.0 m x 0.25 mm i.d., 0.25 μm film thickness). The injector and detector temperatures were maintained at 280°C. The oven temperature was raised from 100 to 188°C at a heating rate of 3°C/min, and then 20°C/min to 280°C with the final hold time of 3 min. Helium gas at 1.0 ml/min was used as a carrier. Diluted samples (20%, in dichloromethane) of 0.2 μL was injected in the split mode ratio 1:50. Screening of the chromatograms was performed in scan mode, from *m/z* 50 to 500, at a rate of 3.25 scan/second, with the ionization source temperature set at 200°C. Finally, the constituents of the essential oil were identified using standard and reference by comparison with the mass spectra on mass spectral library database (National Institute of Standards and Technology, NIST 98 and Wiley 7n.1 libraries) by selecting a percent quality match greater than 85%. The relative percentage of the essential oil constituents were expressed as a percentage by peak area normalization.

3.4.3 Preliminary screening of essential oils against *Aspergillus* strains using modified disc diffusion method

3.4.3.1 Preparation of the spore suspension

Two aflatoxigenic strains, i.e. *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 obtained from the International Mycological Institute (Egham, Surrey, UK.) were used throughout this study. The fungal strains were cultured on potato dextrose agar slant for 7–10 days at 28±1°C. Spores were harvested aseptically by adding 10 mL of sterile 0.05% (v/v) Tween 80 to the culture and gently scraping the mycelial surface with a sterile inoculating loop to free spores. The spore concentration was determined using a haemocytometer and diluted to 10^6 spores/mL using 0.05% Tween 80 (Nguefack *et al.,* 2004).

3.4.3.2 Screening of antifungal activity

The essential oils extracted from 56 plants were screened for antifungal activity against *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using modified disc diffusion method (Bauer *et al.*, 1966). The spore suspension (0.1 mL) was spread on the surface of PDA plates. Sterile filter paper discs (6.0 mm in diameter, Whatman

No. 1) were then aseptically placed in the center of the agar plate. The plates were allowed to dry for at least five minutes. Essential oils (5 μL of 100% oil) were immediately added to these paper discs. A disc with distilled water was used as a negative control and with ketoconazole (50μg/ml) as a positive control. The plates were incubated at 30°C for 120 hours. The diameters of the inhibition zones were then measured using a vernier calliper (±0.01 mm) and reported in millimeters for evaluation of antifungal activity. All tests were performed in triplicate as mean±S.D. Growth inhibition zone diameter (disc diameter included) was classified as follows: very strong activity (diameter ≥ 30 mm) ; strong activity (diameter between 21-29 mm) ; weak activity (diameter between 11-15 mm) and small or no activity (diameter less than 10 mm) (Rahmoun *et al.*, 2013).

3.4.4 Determination of the minimum inhibitory concentration and minimum fungicidal concentration

The minimum concentration of essential oils required to completely inhibit *Aspergillus* strains was determined using the broth microdilution method modified from Gulluce *et al.* (2007). Each essential oil was solubilized in 10% dimethyl sulfoxide (DMSO) and diluted to a final concentration of 10 mg/mL. Potato dextrose broth (100 μL) was added to all 96 wells of a sterile microplate. The solubilized essential oils (100 μL) were added to each of the first wells and then performed 2-fold serial dilution. The spore suspension (10^6 spore/ml) prepared in potato dextrose broth (100 μL) was added to each well. The final concentrations of each essential oil were 2.5, 1.25, 0.64, 0.32, 0.16, 0.08, 0.04, and 0.02 mg/mL. A solvent of DMSO: water (1: 9) was used as a negative control, and ketoconazole (50 μg/ml) served as a positive control. All tests were performed in triplicate. Discs containing the spore suspension but lacking essential oils were also used for comparison. The covered microplates were then incubated at 30°C and examined visually every 24 h up to 72 h. The minimum concentrations at which no visible growth was observed were defined as the MICs (in mg/ml). After MIC determination, the microplates were shaken automatically for 10 seconds, and 100 μL of solution from each well that showed no growth was placed on PDA. These plates were incubated at 30°C for 72 h. The minimum concentrations of essential oils capable of inhibiting fungal growth completely were considered to be the MFCs. The criteria for MIC classification of plant essential oil *in vitro* categorized by Aligiannis *et al.* (2001) was applied. Plants show MICs of <0.5, 0.5-1.5, and >1.5 mg/ml are defined as strong, moderate, and weak inhibitors, respectively.

3.4.5 Determination of the different concentration of essential oils against *Aspergillus* strains by contact and vapor treatments

The antifungal activity of essential oils against both *Aspergillus* strains was studied using two experiments. For the first experiment, selected essential oils at 300 ppm were tested for the inhibitory of mycelial growth, sporulation and AFB1 production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 by comparison between contact and vapor treatments. Essential oils at 100, 200, 300, 600 and 1000 ppm were then prepared by the same method and analyzed for IC50.

The contact treatment was modified from Soliman *et al.* (2002) and vapor treatment was modified from Soylu *et al.* (2010). For the contact treatment, determination of essential oil activities was done by poisoned food technique prepared by adding appropriate volumes of essential oil (in 0.05% Tween-80) to the sterilized molten PDA medium (20 mL) and then poured into sterilized Petri dishes (90 mm diameter). A 6 mm sterile diameter Whatman No 1 filter paper disc was placed at the center of each PDA dish and inoculated with 10 µl of spore suspension (10^6 spore/ml). The plates were immediately sealed with parafilm to prevent leakage of essential oil vapor. All PDA plates with the same concentration were then sealed in polyethylene bags and incubated for 5 days at $30\pm1^{\circ}C$ in the darkness. All treatments were performed in triplicate.

For the vapor treatment, the determination of essential oil activities was determined by a similar manner to the contact treatment. Only various amounts of essential oil were applied to the paper disc (Whatman No 1, 6 mm. diameter) and placed in the center of the lid of each PDA plate after the addition of 20 ml of PDA medium, with 80 ml air space. Sterile blank disc served as a control. The Petri plates were then sealed and incubated as described above. All treatments were performed in triplicate.

3.4.6. Determination of growth inhibition of *Aspergillus* strains

The efficacy of essential oil was evaluated daily by measuring the average of two perpendicular diameters of each fungal colony using a vernier caliper. The mean values of growth were obtained and then converted into the percent inhibition (I, %) of mycelial growth in relation to control treatment by using the following equation: I (%) = [(Dc-Dt)/Dc] x 100, where Dc is the diameter of fungal colony in the control Petri dish and Dt is the diameter of fungal colony in the essential oil-treated Petri dish. A linear regression analysis of percent growth inhibition versus essential oil concentration, estimated as 50% inhibition (IC50), was determined from the regression equation. The fungitoxicity (fungistatic/fungicidal activity) of the essential oil was determined by using the modification technique of Thompson (1989). All treatments were performed in triplicate. The inhibited fungal discs of the essential oil-treated sets were reinoculated into fresh PDA medium and revival of their growth was recorded. After growth was evaluated, all samples were then analyzed for sporulation and aflatoxin B1 qualification.

3.4.7 Determination of inhibitory effect on sporulation of *Aspergillus* strains

Spore production was determined using the modified method of Tzortzakis and Economakis (2007). Spores from colonies of both *Aspergillus* strains previously incubated for 5 days, which exposed to essential oil by both contact and vapor treatments, were collected by adding 5 ml 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. The spore suspension was collected and then centrifuged and estimated using a haemocytometer. The percent inhibition of spore production was calculated using the following equation: Inhibition of sporulation (%) = [(Nc - Ns) / Nc] x 100, where Nc is the number of spores in the control sample and Ns is the number of spores in the treated sample.

3.4.8 Determination of inhibitory effect on AFB1 production of *Aspergillus* strains.

The antiaflatoxigenic efficacy of essential oils was studied after sporulation was determined. All sample were extracted with 10 mL of 70% MeOH, shaking for 5 min before filtering through Whatman no 4 and analysed for aflatoxin B1 by ELISA analysis using DOA-Aflatoxin ELISA Test Kit (Chinaphuti *et al.*, 2002). 50 μl of AFB1 standards was added into the antibody coated wells and 50 μl of diluted sample was added into the other wells followed by adding 50 μl of AFB1-horseradish peroxidase conjugate to each well, slightly shaken and incubated at room temperature for 30 min. The contents of the well were discarded into the appropriate waste container and the plate was washed 3-5 times with 0.5% Tween 20 in 0.01 M phosphate buffer saline. 100 μl of tetramethylbenzidine substrate was added to the well, incubated for 10 min at room temperature before adding 100 μl of stopping solution (0.3M phosphoric acid). The solution was then read at 450 nm using the automated MicroELISA reader. The concentration of AFB1 of each sample was calculated from the slope between % maximum binding and standard AFB1 concentration. Any sample having more than 40 ppb was diluted further and re-tested. Percent of AFB1 inhibition was evaluated as follows: Inhibition of AFB1 production (%) = (AFB1 concentration in a control sample - AFB1 concentration in a treatment sample) × 100/AFB1 concentration in a control sample. The concentrations of AFB1 in the sample are calculated in ppb. The detection is limit to a range of quantitation between 4-40 ppb.

3.5 Experimental design

3.5.1 Screening of antifungal activity of essential oils against *Aspergillus* strains using the disc diffusion method were designed and analyzed in Completely Randomized Design with three replications by two factors: (1) essential oils (56 essential oils) and (2) fungal strains (*A. flavus* IMI 242684 and *A. parasiticus* IMI 283883).

3.5.2 MICs and MFCs were analysed based on two factors: (1) essential oils (19 essential oils) and (2) fungal strains (*A. flavus* IMI 242684 and *A. parasiticus* IMI 283883).

3.5.3 Inhibitory effects of essential oils at 300 ppm on mycelial growth, sporulation and AFB1 production were designed and analyzed in Completely Randomized Design with three replications based on three factors: (1) essential oils

(12 essential oils), (2) contact and vapor treatments, and (3) fungal strains (*A. flavus* IMI 242684 and *A. parasiticus* IMI 283883).

3.5.4 Inhibitory effects of different concentration of essential oils on mycelial growth, sporulation and AFB1 production were designed and analyzed in Completely Randomized Design with three replications based on three factors: (1) concentration of essential oil (0, 100, 200, 300, 600, and 1000 ppm), (2) contact and vapor treatments, and (3) fungal strains (*A. flavus* IMI 242684 and *A. parasiticus* IMI 283883).

3.6 Statistical analysis

All analyses were performed in triplicate. Data were recorded as means ± standard deviations and analysed for normality and then subjected to an analysis of variance (ANOVA). Factor interactions were also investigated and significant differences between mean values were determined using a multiple comparison tests (Tukey's post hoc test). P-values less than 0.05 were considered statistically significant.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Yield of essential oils

In the first experiment, 88 plants from 42 families were investigated. Fifty six plants were found to contain varied amount of essential oil by hydrodistillation, based on 100 g of dry plants, as shown in Table 5. The yields of essential oils on dry weight from different plants varied between 0.02 to 4.50% (v/w). The yield from *Cymbopogon citratus* (DC.) Stapf. (4.50%) was the highest, followed by those from *Syzygium acromaticum* L. (4.13%), *Trachyspermum amni* (L.) Sprague (3.57%), *Cinnamomum porrectum* (3.43%), and *Zingiber montanum* Koenig. (3.13%), respectively. The yield from *Coriandrum sativum* L., *Piper nigrum* L. and *Piper sarmentosum* Roxb. were the lowest (0.02%).

Table 5 Yield of essential oils

No	Family	Scientific Name	Yield (%, v/w)
1.	Acanthaceae	*Acanthus ebracteatus* Vahl.	nd
2.		*Rhinacanthus nasutus* (L.) Kurz	nd
3.	Alliaceae	*Allium tuberosum* Roxb.	nd
4.	Annonaceae	*Cananga odorata* (Lam.) Hook.f. and Thomson	0.53±0.03
5.		*Melodorum fruticosum* Lour.	0.48±0.06
6.	Araceae	*Acorus calamus* L.	0.42±0.03
7.		*Homolomena aromatics* (Roxb.Sims) Schott	0.30±0.00

Table 5 Yield of essential oils (contd.)

No	Family	Scientific Name	Yield (%, v/w)
8.	Araliaceae	*Polyscias fruticosa* (L.) Harms	nd
9.	Apiaceae	*Anethum graveolens* L.	2.60±0.00
10.		*Apium graveolens* L.	0.03±0.00
11.		*Carum carvi* L.	2.01±0.02
12.		*Coriandrum sativum* L.	0.02±0.00
13.		*Cuminum cyminum* L.	1.20±0.00
14.		*Eryngium foetidum* L.	0.04±0.00
15.		*Foeniculum vulgare* Mill.	2.43±0.12
16.		*Petroselinum crispum* (Mill.) A.W.Hill.	0.13±0.01
17.		*Pimpinella anisum* L.	1.13±0.12
18.		*Trachyspermum amni* (L.) Sprague	3.57±0.06
19.	Asteraceae	*Artemisia dracunculus*	0.70±0.00
20.		*Acmella oleracea* (L.) R.K. Jansen	nd
21.		*Eupatorium odoratum* L.	0.10±0.00
22.		*Spilanthes acmella* Murr.	0.08±0.00
23.		*Vernonia cinerea* (L.) Less.	nd
24.	Basellaceae	*Basella alba* L.	nd

Table 5 Yield of essential oils (contd.)

No	Family	Scientific Name	Yield (%, v/w)
25.	Bignoniaceae	*Millingtonia hortensis* L.f.	nd
26.	Brassicaceae	*Brassica juncea* L.	0.70±0.00
27.	Calophyllaceae	*Mesua ferrea* L.	0.09±0.01
28.	Clusiaceae	*Mammea siamensis* Kosterm	nd
29.		*Garcinia cowa* Roxb.	nd
30.	Commelinaceae	*Murdannia bracteata*	nd
31.		*Commelina benghalensis* L.	nd
32	Compositae	*Carthamus tinctorius* L.	nd
33.	Cruciferae	*Lepidium sativum* L.	nd
34.	Cyperaceae	*Cyperus rotundus* L.	0.27±0.03
35.	Euphorbiaceae	*Sauropus androgynus* (L.) Merr.	nd
36.	Illiciaceae	*Illicium verum* Hooker	0.80±0.00
37.	Labiatae	*Vitex trifolia* L.	nd
38.	Lamiaceas	*Mentha cordifolia*	0.20±0.00
39.		*Rosmarinus officinalis* L.	0.12±0.03
40.		*Salvia officinalis* L.	1.20±0.0
41.	Lauraceae	*Cinnamomum porrectum* (Roxb.) Kosterm	3.43±0.16
42.		*Cinnamomum zeylanicum* Blume	1.57±0.06
43.		*Laurus nobilis* L.	nd
44.	Leguminosae	*Parkia speciosa* Hassk.	nd

Table 5 Yield of essential oils (contd.)

No	Family	Scientific Name	Yield (%, v/w)
45.	Magnoloaceae	*Michelia alba* DC.	0.12±0.03
46.		*Magnolia champaca* L.	0.10±0.00
47.	Menispermaceae	*Tiliacora triandra* (Colebr.) Diels.	nd
48.	Mimoseae	*Acacia pennata* L.	nd
49.	Myristiceae	*Myristica fragrans* Houtt.	2.12±0.10
50.	Myrtaceae	*Syzygium acromaticum* L.	4.13±0.12
51.		*Psidium guajava* L.	nd
52.	Pandanaceae	*Pandanus odoratissimus* L.f.	nd
53.		*Pandanus amaryllifolius* Roxb.	nd
54.	Papilionaceae	*Trigonella foenumgraecum* L.	nd
55.	Piperaceae	*Piper nigrum* L.	0.02±0.0
56.		*Piper retrofractum* Vahl.	1.42±0.14
57.		*Piper sarmentosum* Roxb.	0.02±0.00
58.		*Peperomia pellucida* (L.) Kunth.	nd
59.	Plantaginaceae	*Plantago ovate* Forssk.	nd
60.	Poaceae	*Cymbopogon citratus* (DC.) Stapf.	4.50±0.00
61.		*Cymbopogon nardu* L. Rendle.	0.50±0.00
62.		*Vetiveria zizanioides*	0.58±0.08

Table 5 Yield of essential oils (contd.)

No	Family	Scientific Name	Yield (%, v/w)
63.	Polygonaceae	*Polygonum odoratum* Lour.	0.12±0.03
64.	Ranunculaceae	*Nigella sativa* L.	0.32±0.03
65.	Rubiaceae	*Gardenia jasminoides* J.Ellis	nd
66.		*Tarenna hoaensis* Pitard	nd
67.		*Morinda citrifolia* L.	nd
68.		*Coffea arabica* L.	nd
69.	Rutaceae	*Citrus hystrix*	1.82±0.03
70.		*Zanthozylum budrunga*	0.83±0.06
71.	Sapotaceae	*Mimusops elengi* L.	nd
72.	Scrophulariaceae	*Limnophila geoffrayi* Bonati	0.63±0.03
73.	Thunbergiaceae	*Thunbergia laurifolia* L.	nd
74.	Verbenaceae	*Nyctanthes abortrististis* L.	nd
75.	Zingiberaceae	*Alpinia conchigera* Griff.	0.32±0.03
76.		*Alpinia galangal* L.	0.14±0.00
77.		*Boesenbergia rotunda* L. Mansf.	0.53±0.06
78.		*Curcuma aromatic* Salisb.	0.38±0.03
79.		*Curcuma longa* L.	0.48±0.03
80.		*Curcuma mangga* Val. & Zijp.	0.57±0.06
81.		*Curcuma xanthorrhiza* Roxb.	0.05±0.00
82.		*Curcuma zedoaria*	1.20±0.00

Table 5 Yield of essential oils (contd.)

No	Family	Scientific Name	Yield (%, v/w)
83.	Zingiberaceae	*Elettaria cardamomum* (L.) Maton	0.60±0.00
84.		*Kaempferia galanga* L.	1.83±0.06
85.		*Kaempferia parviflora* Wallich.ex Baker	0.20±0.00
86.		*Zingiber montanum* Koenig	3.13±0.12
87.		*Zingiber officinale* Rosc.	0.50±0.00
88.		*Zingiber zerumbet*	2.52±0.03

nd = not detected

4.2 GC-MS analysis of plant essential oils

The chemical composition of 12 plant essential oils was analysed by GC-MS. The identified chemical components, retention times, formula, molecular weight and percent compositions are presented in Appendix A (Tables 1-12). The total oil compositions and the main constituents of the twelve plants are summarized in Table 6. Essential oils with main compounds higher than 10% according to van Vuuren and Viljoen (2008) were selected.

A total of 7 compounds in the seed essential oil of *Cuminum cyminum* L., were identified and represented 99.99% of the detected compounds, of which 2-methyl-3-phenyl-propanal (78.87%) was the main compound. Seven compounds identified in the seed essential oil of *Foeniculunm vulgare* Mill. were anethol (53.13 %) and anisole (24.97 %) as the main conposition. Thymol (92.77%) was the main compound of *Trachyspermum amni* (L.) Sprague seed essential oil. A total of 7 compounds was identified in *Brassica juncea* L. essential oil, accounting for 96.8 % of which 2-isothiocyanatoethyl benzene (65.59%) was the major compound. Safrole (93.92 %) was the main compound in *Cinnamomum porrectum* (Roxb.) Kosterm essential oil. A total of 3 compounds was identified in *Cinnamomum zeylanicum* Blume essential oil, accounting for 100%., and cinnamaldehyde (96.56) was the major compound. Thirteen compounds in the flower essential oil of *Michelia alba* DC. (96.35%) were identified and linalool (50.14 %) was the main compound.

Safrone (42.50%) and 4-terpineol (23.81%) were the main compounds of *Myristica fragrans* Houtt. (99.96%) from the total 7 compounds. Among 25 compounds (98.88%) identified in the leave essential oil of *Cymbopogon citratus* (DC.) Stapf., geranial (37.29%) was the main compound, followed by neral (24.63%). *Citrus hystrix* essential oil exhibited 10 compounds, representing 99.41% of citronella (84.22%) as the main compound. Twenty-two compounds of *Limnophila geoffrayi* Bonati essential oil were indentified. Pulegone (22.5%) and isolimonene (19.06%) were the main compounds. Ethyl cinnamate (65.28%) and pentadecane (20.36%) were identified as the main compounds of *Kaempfer galangal* L. essential oil.

Table 6 The total oil composition and the major component of the essential oils

Essential oil	Total Compound	Main composition[a]
Cuminum cyminum L.	7	2-methyl-3-phenyl-propanol (78.87%)
Foeniculum vulgare Mill.	7	anethole (53.13%), estragole (24.97%)
Trachyspermum amni (L.) Sprague	4	thymol (92.77%)
Brassica juncea L.	7	2-phenylethyl isothiocyanate (65.59%)
Cinnamomum porrectum (Roxb.) Kosterm	3	safrole (93.92%)
Cinnamomum zeylanicum Blume	3	cinnamaldehyde (96.56%)
Michelia alba DC.	13	linalool (50.14%)
Myristica fragrans Houtt.	7	safrole (42.50%), 4-terpineol (23.81%)
Cymbopogon citratus (DC.) Stapf	25	geranial (37.29%), neral (24.63%)
Citrus hystrix	10	citronellal (84.22%)
Limnophila geoffrayi Bonati	22	pulegone (22.50%), isolimonene (19.06%)
Kaempferia galanga L.	9	ethyl cinnamate (65.28%), pentadecane (20.36%)

[a] the main compositions found are more than 10%

4.3 Screening of essential oils for antifungal activity against the aflatoxigenic strains

Among these essential oils, *Cinnamomum zeylanicum* Blume. essential oil showed the highest antifungal activity with the diameter of inhibition zone of 63.8-68.3 mm., followed by *Trachyspermum amni* L. Sprague (61.3-65.0 mm), *Foeniculum vulgare* Mill. (60.2-65.0 mm.), *Michelia alba* DC. (54.2-60.0 mm), *Cinnamomum porrectum* (Roxb.) Kosterm (55.7-58.0 mm), *Kaempferia galangal* L. (52.3-57.5 mm), *Cuminum cyminum* L. (50.3-54.5 mm) and *Cymbopogon citratus* Stapf. essential oil (50.7-51.0 mm), respectively (Table 7).

Table 7 Inhibition zones of essential oils of the aflatoxigenic strains

No	Essential oil[a]	Inhibition zone (mm)[b]	
		Aspergillus flavus	*Aspergillus parasiticus*
	Annonaceae:		
1	*Cananga odorata* (Lam.) Hook.f.and Thomson.	31.0±1.0 r	36.8±0.8 opq
2	*Melodorum fruticosum* Lour.	35.3±0.6 q	37.0±0.9 opq
	Araceae		
3	*Acorus calamus* L.	28.7±0.3 stuvwx	24.0±0.0 z
4	*Homolomena aromatics* (Roxb.Sims)Schott.	24.8±0.3 y	21.3±0.6 z
	Apiaceae:		
5	*Anethum* graveolens L.	25.2±0.3 y	26.7±0.6 xy
6	*Apium graveolens* L.	10.7±0.6 z	8.3±0.3 z
7	*Carum carvi* L.	47.7±1.2 k	41.0±1.0 m
8	*Coriandrum sativum* L.	28.8±0.8 stuvw	22.0±0.0 z
9	*Cuminum cyminum* L.	54.5±0.5 g	50.3±0.6 ij
10	*Eryngium foetidum* L.	25.0±0.0 z	27.5±0.5 vwx
11	*Foeniculum vulgare* Mill.	60.2±0.3 c	65.0±0.0 b
12	*Petroselinum crispum* Mill. A.W.Hill.	20.5±0.5 z	18.3±0.6 z
13	*Pimpinella anisum* L.	22.0±0.0 z	21.3±0.6 z
14	*Trachyspermum amni* (L.) Sprague.	65.0±0.0 b	61.3±0.6 c

Table 7 Inhibition zones of essential oils of the aflatoxigenic strains (contd.)

No	Essential oil[a]	Inhibition zone (mm)[b]	
		Aspergillus flavus	*Aspergillus parasiticus*
	Asteraceae:		
15	*Artemisia dracunculus*	13.3±1.2 z	13.5±0.5 z
16	*Eupatorium odoratum* L.	10.5±0.5 z	12.0±1.0 z
17	*Spilanthes acmella* Murr.	11.8±0.8 z	13.0±0.0 z
	Brassicaceae		
18	*Brassica juncea* L.	46.3±0.6 kl	49.8±0.8 j
	Calophyllaceae		
19	*Mesua ferrea* L.	20.8±1.5 z	26.8±0.3 wxy
	Cyperaceae		
20	*Cyperus rotundus* L.	30.3±0.6 rs	36.0±0.0 pq
	Illiciaceae		
21	*Illicium verum* Hooker	20.3±0.6 z	18.0±0.0 z
	Lamiaceas		
22	*Mentha cordifolia*	9.0±0.0 z	11.7±0.6 z
23	*Rosmarinus officinalis* L.	11.0±1.0 z	17.3±0.6 z
24	*Salvia officinalis* L.	19.5±0.5 z	18.2±0.3 z
	Lauraceae:		
25	*Cinnamomum porrectum* (Roxb.) Kosterm	55.7±0.6 fg	58.0±0.0 de
26	*Cinnamomum zeylanicum* Blume.	63.8±1.0 b	68.3±0.6 a
27	*Laurus nobilis* L.	20.5±0.5 z	23.0±0.0
	Magnoloaceae:		
28	*Michelia alba* DC.	54.2±0.3 gh	60.0±0.9 cd
29	*Magnolia champaca* L.	25.0±0.0 yz	20.2±0.3 z
	Myristiceae:		
30	*Myristica fragrans* Houtt.	50.0±0.0 j	44.3±0.6 l
	Myrtaceae:		
31	*Syzygium acromaticum* L.	50.8±0.8 ij	45.0±0.0 l

Table 7 Inhibition zones of essential oils of the aflatoxigenic strains (contd.)

No	Essential oil[a]	Inhibition zone (mm)[b]	
		Aspergillus flavus	*Aspergillus parasiticus*
	Piperaceae:		
	Piper nigrum L.	21.0±0.0 z	27.7±0.3 vwx
	Piper retrofractum Vahl.	27.3±0.3 wx	28.0+0.0 uvwx
34	*Piper sarmentosum* Roxb.	30.7±0.6 rs	38.0±0.3 nop
	Poaceae:		
35	*Cymbopogon citratus* (DC.) Stapf	50.7±0.6 ij	51.0±0.0 ij
36	*Cymbopogon nardu* L. Rendle.	30.0±0.0 rstu	35.5±0.5 q
37	*Vetiveria zizanioides* L.	21.0±0.0 z	19.0±1.0 z
	Polygonaceae:		
38	*Polygonum odoratum* Lour.	29.5±1.5 rstuv	28.2±0.3 tuvwx
	Ranunculaceae:		
39	*Nigella sativa* L.	28.8±0.8 stuvw	29.5±1.5 rstuv
	Rutaceae:		
40	*Citrus hystrix*	38.0+0.0 nop	35.2+0.3 q
41	*Zanthozylum budrunga*	28.2±0.3 tuvwx	27.5±0.5 vwx
	Scrophulariaceae:		
42	*Limnophila geoffrayi* Bonati	37.7±0.3 op	40.0±0.0 mn
	Zingiberaceae:		
43	*Alpinia conchigera* Griff.	28.0±0.0 uvwx	28.2±0.3 tuvwx
44	*Alpinia galangal* L.	20.5±0.9 z	15.0±0.0 z
45	*Boesenbergia rotunda* L. Mansf.	10.0±0.0 z	11.8±0.8 z
46	*Curcuma aromatic* Salisb.	20.7±0.6 z	25.0±0.0 y
47	*Curcuma longa* L.	11.8±0.3 z	15.0±0.0 z
48	*Curcuma mangga* Val. & Zijp.	28.0±0.0 uvwx	25.0±0.0 y
49	*Curcuma xanthorrhiza* Roxb.	8.0±0.0 z	10.0±0.0 z
50	*Curcuma zedoaria*	12.0±0.0 z	10.0±0.0 z
51	*Elettaria cardamomum* (L.) Maton	9.7±0.3 z	8.0±0.0 z

Table 7 Inhibition zones of essential oils of the aflatoxigenic strains (contd.)

No	Essential oil[a]	Inhibition zone (mm)[b]	
		Aspergillus flavus	*Aspergillus parasiticus*
52	*Kaempferia galanga* L.	52.3±2.1 hi	57.5±0.5 ef
53	*Kaempferia parviflora* Wallich.ex Baker.	8.5±0.9 z	8.0±0.0 z
54	*Zingiber montanum* Koenig.	12.7±0.2 z	14.7±0.2 z
55	*Zingiber officinale* Rosc.	10.0±0.0 z	12.3±0.3 z
56	*Zingiber zerumbet*	16.0±0.0 z	14.3±0.3 z

[a] Essential oil (test volume 5 μl/disc)

[b] Inhibition zone: values are given as mean ± SD (n = 3)

The means followed by the same letter are not significantly different according to ANOVA and Tukey's multiple comparison tests ($p < 0.05$)

Preliminary screening of the antifungal activity of 56 essential oils against the aflatoxigenic strains was evaluated using the disc diffusion method. The results were recorded as the zone of inhibition including the diameter (6 mm) of the paper disc. There was a significant difference ($p<0.05$) for the potential essential oils against both *Aspergillus* strains (Appendix B, Table 13). The diameter of inhibition zone varied between 8.0 to 68.3 mm. Nineteen essential oils of *Cananga odorata* (Lam.) Hook.f. and Thomson, *Melodorum fruticosum* Lour., *Carum carvi* L., *Cuminum cyminum* L., *Foeniculum vulgare* Miller., *Trachyspermum amni* (L.) Sprague, *Brassica juncea* L., *Cyperus rotundus* L., *Cinnamomum porrectum* (Roxb.) Kosterm, *Cinnamomum zeylanicum* Blume., *Michelia alba* DC., *Myristica fragrans* Houtt., *Syzygium acromaticum* L., *Piper sarmentosum* Roxb., *Cymbopogon citratus* (DC.) Stapf., *Cymbopogon nardu* L. *Rendle., Citrus hystrix, Limnophila geoffrayi* Bonati., and *Kaempferia galangal* L. gave very strong activity (≥ 30 mm zone of inhibition) against both *Aspergillus* strains and were selected for determination of MICs and MFCs.

4.4 Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The antifungal activity of 19 essential oils was quantified in terms of MIC and MFC values. The results were showed in Table 8. The MICs values of both *Aspergillus* strains were in the range of 0.32 to 1.25 mg/ml, while the MFC values were in the range between 1.25 to >2.5 mg/ml. Essential oils exhibited MICs of 0.16 and 0.32 mg/ml which were lower than 0.5 mg/ml were considered to be a strong inhibitor, i.e. the essential oils of *Cuminum cyminum* L., *Foeniculum vulgare* Mill., *Trachyspermum amni* (L.) Sprague, *Brassica juncea* L., *Cinnamomum porrectum* (Roxb.) Kosterm., *Cinnamomum zeylanicum* Blume, *Michelia alba* DC., *Myristica fragrans* Houtt., *Cymbopogon citrates* (DC.) Stapf., *Citrus hystrix, Limnophila geoffrayi* Bonati. and *Kaempferia galangal* L. The other essential oils were considered as moderate inhibitors.

After the determination of the MICs, the fungicidal effect on *Aspergillus* strains was investigated. The MFCs values of the essential oils varied between >2.5 – 0.32 mg/ml, where the MFCs of essential oils corresponded about 1.95 to 3.9 times higher than MICs. Thus, twelve essential oils showing strong inhibition were selected for further study for the inhibitory effect on mycelial growth, sporulation, and aflatoxin B1 production of the aflatoxigenic strains by comparison between the contact and the vapor treatments.

Table 8 MIC and MFC of *Aspergillus* strains

Essential oil	*A. flavus* IMI 242684		*A. parasiticus* IMI 283883	
	MIC (mg/ml)	MFC (mg/ml)	MIC (mg/ml)	MFC (mg/ml)
Cananga odorata (Lam.) Hook.f. and Thomson	0.64	>2.5	0.64	>2.5
Melodorum fruticosum Lour.	0.64	2.5	0.64	2.5
Carum carvi L.	0.64	2.5	0.64	2.5
Cuminum cyminum L.	0.32	1.25	0.32	1.25
Foeniculum vulgare Mill.	0.16	0.32	0.16	0.32
Trachyspermum amni (L.) Sprague	0.16	0.32	0.16	0.32
Brassica juncea L.	0.32	1.25	0.32	1.25
Cyperus rotundus L.	0.64	>2.5	0.64	>2.5
Cinnamomum porrectum (Roxb.) Kosterm	0.32	1.25	0.32	1.25
Cinnamomum zeylanicum Blume	0.32	2.5	0.32	2.5
Michelia alba DC.	0.32	2.5	0.32	2.5
Myristica fragrans Houtt.	0.32	0.64	0.32	0.64
Syzygium acromaticum L.	0.64	2.5	0.64	2.5
Piper sarmentosum Roxb.	1.25	>2.5	1.25	>2.5
Cymbopogon citratus (DC.) Stapf.	0.32	1.25	0.32	1.25
Cymbopogon nardu L. Rendle.	0.64	2.5	0.64	2.5
Citrus hystrix	0.32	1.25	0.32	1.25
Limnophila geoffrayi Bonati	0.32	1.25	0.32	1.25
Kaempferia galanga	0.32	1.25	0.32	1.25

4.5 Inhibitory effect of essential oils against *Aspergillus* strains

4.5.1 Inhibitory effect of essential oils on mycelial growth of *Aspergillus* strains by contact and vapor treatments

Twelve essential oils selected from previous study were investigated for the inhibitory effect on *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 by comparison between contact and vapor treatments at concentration of 300 ppm.

Statistical analyses indicated that single, two and three factors, as well as their interactions also had a significant ($p < 0.05$) impact on the inhibitory effects of these essential oils on mycelial growth of both *Aspergillus* strains and the statistical result was shown in Appendix B (Table 14). If was found that all essential oils significantly reduced ($p < 0.05$) the mycelial growth of both *Aspergillus* strains in comparison with the control. Mycelial growth inhibition in contact system was in the range of 15.5-66.2%, while in the vapor system was in the range of 15.5-79.9%, as shown in Table 9.

Among 12 essential oils, *Cinnamomum* zeylanicum Blume, and *Kaempferia galanga* L. essential oils showed the significantly ($p < 0.05$) highest mycelial growth inhibition of *Aspergillus* strains in the range of 75.0-79.9% and 76.8-79.6%, respectively, in vapor treatment when compared with others essential oils.

In contact treatment, essential oils of *Cinnamomum zeylanicum* Blume and *Kaempferia galanga* L. showed more than 60% growth inhibition of *A. flavus* IMI 242684. However, in vapor treatment, these essential oils show more significantly effective ($p < 0.05$) on mycelial growth inhibition than contact treatment about 1.2-2.0 folds, with the exception of *Foeniculum vulgare* and *Cymbopogon citratus* (DC.) Stapf. essential oils which showed the same effective growth inhibition against *A. flavus* IMI 242684 by both treatments ($p <0$).

Table 9 Mycelial growth inhibition *of Aspergillus* strains at 300 ppm of essential oil

Essential oil	Mycelial growth inhibition (%)[a]			
	A. flavus IMI 242684		*A. parasiticus* IMI 283883	
	Contact	Vapor	Contact	Vapor
Cuminum cyminum L.	16.5±0.5 x	41.2±2.2 op	34.8±0.1 q	45.0±1.0 m
Foeniculum vulgare Mill.	15.5±0.6 y	15.5±0.0 y	16.6±0.0 x	21.9±0.6 w
Trachyspermum amni (L.) Sprague	21.4+1.0 w	39.3±1.1 p	35.0±0.5 q	45.6±0.5 lm
Brassica juncea L.	27.1+0.5 stu	53.1±0.0 gh	40.1±1.1 op	44.4+0.5 mn
Cinnamomum porrectum (Roxb.) Kosterm	50.8±0.5 hij	60.0±1.1 de	48.6±0.5 jk	58.6±1.0 e
Cinnamomum zeylanicum Blume.	61.9±1.0 d	79.9±1.1 a	48.0±1.0 kl	75.0±1.6 b
Michelia alba DC.	30.0±0.0 r	51.8±0.5 hi	29.0±0.5 rs	55.5±0.0 fg
Myristica fragrans Houtt.	24.6±0.5 uv	48.4±0.0 jk	25.9±0.0 tu	40.5±0.5 op
Cymbopogon citratus (DC.) Stapf.	57.6±0.0 ef	58.1±1.1 ef	54.7±0.0 g	66.3±0.5 c
Citrus hystrix	16.2±0.0 x	28.1±1.1 rst	18.7±0.5 x	21.8±1.9 w
Limnophila geoffrayi Bonati	22.7±0.5 vw	29.7±0.6 rs	28.4±0.6 rst	42.0±0.0 no
Kaempferia galanga L.	66.2±0.5 c	79.6±0.6 a	50.4±1.0 ijk	76.8±0.0 b

[a] Values with different letters are statistically significantly different ($p< 0.05$).

4.5.2 Inhibitory effect of essential oils on sporulation of *Aspergillus* strains by contact and vapor treatments

The inhibitory effects of 12 essential oils at 300 ppm on sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 were compared between contact and vapor treatments. Statistical analyses indicated that single, two and three factors as well as their interactions also had a significant ($p < 0.05$) impact on the inhibitory effects of these essential oils on sporulation of both *Aspergillus* strains as shown in Appendix B (Table 14). The results showed that all essential oils significantly reduced the sporulation in comparison with the control. Sporulation inhibition in the contact treatment was in the range of 17.8-77.1%, while in the vapor system was in the range of 32.3- 92.2%, as shown in Table 10.

In contact treatment, three essential oils, i.e. *Cinnamomum zeylanicum* Blume, *Cymbopogon citratus* (DC.) Stapf., and *Kaempferia galanga* L. showed more than 60% sporulation inhibition of both *Aspergillus* strains. However, *Cuminum cyminum* L., *Brassica juncea* L, and *Cinnamomum porrectum* (Roxb.) Kosterm. only inhibited sporulation of *A. parasiticus* IMI 283883.

In vapor treatment, three essential oils of *Cinnamomum zeylanicum* Blume., *Myristica fragrans* Houtt., and *Kaempferia galangal* L. showed more significant effect on both *Aspergillus* strains than by contact treatment. *Cinnamomum porrectum* (Roxb.) Kosterm., *Michelia alba* DC., *Cymbopogon citratus* (DC.) Stapf., and *Limnophila geoffrayi* Bonati. essential oils in vapor treatment showed more significant inhibition on *A. flavus* IMI 242684 than in contact treatment. *Brassica juncea* L essential oil showed more significant inhibition against *A. parasiticus* IMI 283883 showed significant more sporulation inhibition in vapor treatment than contact treatment. Other essential oils showed the same effective of sporulation inhibition on the both treatments ($p <0.05$). *Cinnamomum zeylanicum* Blume., and *Kaempferia galanga* L. essential oils showed the significantly ($p < 0.05$) highest sporulation inhibition of both *Aspergillus* strains with the range of 91.4-92.2% in vapor treatment when compared with other essential oils.

Table. 10 Sporulation inhibition *of Aspergillus* strains at 300 ppm of essential oil

Essential oil	Sporulation inhibition (%)[a]			
	A. flavus IMI 242684		*A. parasiticus* IMI 283883	
	Contact system	Vapor system	Contact system	Vapor system
Cuminum cyminum L.	43.7±5.0 jklmnop	45.8±4.8 ijklmnop	77.1±5.5 abc	77.3±2.5 abc
Foeniculum vulgare Mill.	23.7±7.6 qrs	33.6±8.2 opqr	36.7±4.8 mnopqr	41.6±6.8 klmnop
Trachyspermum amni (L.) Spragu	51.2±8.2 ghijklm	51.8±7.0 ghijklm	58.1±5.3 efghij	72.8+8.9 bcde
Brassica juncea L.	51.6±0.8 ghijklm	53.1±0.8 ghijkl	62.7±1.2 cdefg	80.1±3.9 ab
Cinnamomum porrectum (Roxb. Kosterm	55.8±5.6 fghijk	80.0±3.4 ab	70.9±2.3bcdef	71.6±3.9 bcde
Cinnamomum zeylanicum Blum	70.8±2.6 bcdef	92.2±2.9 a	70.9±2.0 bcdef	91.4±1.3 a
Michelia alba DC.	30.8±3.1 pqrs	50.4±2.1 ghijklmn	39.5±3.1 lmnop	46.9±1.4 hijklmno
Myristica fragrans Houtt.	21.6±4.0 rs	83.1±10.6 ab	17.8±4.4 s	60.7±3.7 defghi
Cymbopogon citratus (DC.) Stapf	63.3±9.0 cdefg	80.0±3.4 ab	61.6±2.3 defgh	71.2±2.5 bcde
Citrus hystrix	31.3±2.2 pqrs	38.1±4.8 lmnopq	35.2±4.1 nopqr	32.3±3.1 opqrs
Limnophila geoffrayi Bonati	47.1±4.4 hijklmno	68.6±2.8 bcdef	44.5±5.9 jklmnop	58.2±5.5 efghij
Kaempferia galanga L.	75.4±2.6 bcd	91.8±2.8 a	75.1±5.3 bcd	91.6±2.7 a

[a] Values with different letters are statistically significantly different ($p < 0.05$).

4.5.3 Inhibitory effect of essential oils on aflatoxin B1 production of *Aspergillus* strains by contact and vapor treatments

The inhibitory effects of 12 essential oils at 300 ppm on aflatoxin B1 production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 were compared between contact and vapor treatments. Statistical analyses indicated that single, two and three factors as well as their interactions also had a significant ($p < 0.05$) impact on the inhibitory effects of these essential oils on sporulation of both *Aspergillus* strains as shown in Appendix B (Table 14). The results showed that all essential oils significantly reduced the aflatoxin B1 production in comparison with the control. aflatoxin B1 production inhibition in the contact treatment was in the range of 16.3-85.8%, while in the vapor system was in the range of 28.0- 100%, as shown in Table 11.

In contact treatment, *Cinnamomum zeylanicum* Blume., *Cinnamomum porrectum* (Roxb.) Kosterm., and *Kaempferia galangal* L. essential oils showed more than 60% AFB1 production inhibition of both *Aspergillus* strains. *Brassica juncea* L and *Cymbopogon citratus* (DC.) Stapf. essential oil showed more than 60% inhibition in *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883, respectively.

In vapor treatment, *Foeniculum vulgare* Mill., *Cinnamomum porrectum* (Roxb.) Kosterm., *Cinnamomum zeylanicum* Blume., *Michelia alba* DC., *Myristica fragrans* Houtt., and *Kaempferia galangal* L. essential oils in vapor treatment showed significant AFB1 inhibition of both *Aspergillus* strains more than contact treatment about 1.2-2.3 times. *Citrus hystrix* essential oil in vapor treatment showed significant AFB1 inhibition of *A. flavus* IMI 242684 more than contact treatment. Other essential oils showed the same effective inhibition on AFB1 by both treatments ($p <0$). *Cinnamomum porrectum* (Roxb.) Kosterm., *Cinnamomum zeylanicum* Blume., and *Kaempferia galanga* L. essential oils showed complete inhibition of AFB1 production by both *Aspergillus* strains in vapor treatment.

Table 11 Inhibition of AFB1 production by *Aspergillus* strains at 300 ppm of essential oil

Essential oil	Percent inhibition of AFB1 production (%)[a]			
	A. flavus IMI 242684		*A. parasiticus* IMI 283883	
	Contact system	Vapor system	Contact system	Vapor system
Cuminum cyminum L.	59.8±3.9 ghijkl	50.4±0.7 klmnop	49.7±3.9 lmnopq	49.2±4.8 lmnopq
Foeniculum vulgare Mill.	30.3±4.2 st	49.7±4.7 lmnopq	39.5±5.3 pqrs	51.8±1.5 jklmno
Trachyspermum amni (L.) Spragı	51.2±3.5 klmno	61.2±2.7 ghijk	46.8±3.3 nopqr	53.6±7.4 ijklmno
Brassica juncea L.	62.3±4.7 fghij	68.9±2.3 defgh	48.7±4.2 mnopq	59.3±4.4 hijklm
Cinnamomum porrectum (Roxb.) Kosterm	82.9±2.2 bc	100±0.0 a	73.5±1.3 cde	100±0.0 a
Cinnamomum zeylanicum Blum	82.2±1.9 bc	100±0.0 a	82.3±2.3 bc	100±0.0 a
Michelia alba DC.	31.8±4.5 st	46.8±1.5 nopqr	16.3±3.0 u	28.0±3.7 t
Myristica fragrans Houtt.	35.9±3.2 rst	72.8+2.3 cdef	29.9±4.2 st	70.4±7.3 defg
Cymbopogon citratus (DC.) Stapf	57.2±2.7 ijklmn	64.2±1.9 efghi	85.8±2.3 b	90.6±0.5 ab
Citrus hystrix	31.2±1.3 st	46.2±3.7 nopqr	38.8±1.8 qrst	46.2±2.1 nopqr
Limnophila geoffrayi Bonati	50.2±2.3 klmnop	51.5±2.9 jklmno	43.2±2.5 opqr	44.3±5.0 opqr
Kaempferia galanga L.	79.7±3.9 bcd	100±0.0 a	82.7±1.7 bc	100±0.0 a

[a] Values with different letters are statistically significantly different ($p < 0.05$).

4.5.4 Effect of different concentrations of essential oils on *Aspergillus* strains and IC50 using the contact and vapor treatments

The mycelial growth, sporulation and AFB1 production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 resulting from the exposure to the five different concentrations (100, 200, 300, 600 and 1000 ppm) of each essential oil during 5 days of incubation at 30 °C by the contact and vapor treatments were statistical analysed. The results of the inhibitory effects by 12 essential oils were presented in Tables 15-26.

4.5.4.1 Effect of *Cumium cymium* L. essential oil on *Aspergillus* strains

The results showed that mycelial growth inhibition was significantly increased with increasing concentration of *Cumium cymium* L. essential oil (Table 12).

Table 12 Effect of different concentrations of *Cumium cymium* L. essential oil on the mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50
	100	200	300	600	1000	(ppm)
A. flavus IMI 242684						
Contact	9.1±2.1 k	14.7± 1.1 j	20.5±0.5 hi	33.1±1.1 g	54.0+0.0 c	923.5
Vapor	23.0±1.9 h	32.4±0.0 g	42.4±2.2 e	47.4±0.0 d	65.2±0.9 a	656.1
A. parasiticus IMI 283883						
Contact	10.6±1.0 k	19.1±1.0 i	33.0±0.0 g	47.5±0.0 d	52.3+1.0 c	779.1
Vapor	32.7 ±1.1 g	37.0±0.0 f	46.3±0.0 d	60.4±1.1 b	65.4±1.1 a	359.2

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 ($p < 0.05$).

When compared between contact and vapor treatments, the result indicated that vapor treatment was significantly effective ($p < 0.05$) on mycelial growth inhibition when using the same concentration of essential oils. The percent growth inhibition of both *Aspergillus* strains was 9.1-54.0% and 23.0-65.4% in contact and vapor treatments, respectively, with IC50 values of 779.1-923.5 ppm and 359.2-656.1 ppm, respectively.

Sporulation inhibition of *Aspergillus* strains increased with increasing concentration of *Cumium cymium* L. essential oil, as shown in Table 13.

Table 13 Effect of different concentrations of *Cumium cymium* L. essential oil on the sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	20.4±2.9 i	2.9±2.9 h	46.7±1.9 fg	57.3±3.4 e	76.4±2.8 cd	493.1
Vapor	17.7±0.8 i	37.7±5.7 gh	49.1±2.8 ef	56.3±2.7 e	86.4±1.4 bc	331.3
A. parasiticus IMI 283883						
Contact	30.5±5.8 h	42.2±2.9 fg	78.3±5.2 cd	86.4±2.4 bc	100 a	231.2
Vapor	38.3±1.9 gh	44.9±3.1 fg	83.0±2.4 bcd	90.7±4.9 b	100 a	287.8

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 ($p < 0.05$).

The sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 in contact treatment was inhibited in the range of 20.4-76.4% and 30.5-100%, respectively, when compared with control. In vapor treatment, the inhibition of sporulation was not significant different from the contact treatment, when the same concentration of essential oil was compared. At 1000 ppm of *Cumium cymium* L. essential oil, sporulation of *A. parasiticus* IMI 283883 was completely inhibited in both treatments while sporulation of *A. flavus* IMI 242684 was inhibited by 76.4 and 86.4% in contact and vapor treatments, respectively. The IC50 values of *Cumium cymium* L. essential oil against sporulation of *A. flavus* IMI 242684 in contact and vapor treatment were 493.1 and 331.3 ppm, while IC50 values of *A. parasiticus* IMI 283883 were 231.2 and 287.8 ppm, respectively.

The AFB1 production of both *Aspergillus* strains was inhibited by 11.9-100%, when compared with control, and the inhibitory effects between the contact and vapor treatments were not significant ($p < 0.05$) when comparing at the same concentration of essential oil.

Table 14 Effect of different concentrations of *Cumium cymium* L. essential oil on the aflatoxin B1 of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentration of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	11.9±1.2 h	30.7±2.5 g	57.1±1.3 de	62.1+3.0 bc	100a	273.1
Vapor	16.1±1.3 h	29.4±1.8 g	54.9±1.9 de	60.5±3.8 bc	100a	282.0
A. parasiticus IMI 283883						
Contact	15.2±1.1 h	37.4±2.8 f	55.1±0.9 de	64.4+0.7 b	100a	286.0
Vapor	12.7±2.5 h	34.8±1.3 fg	53.3+1.3 e	59.2+1.9 bcd	100a	287.4

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 ($p < 0.05$).

The results in Table 14 showed that the inhibition of AFB1 production significantly ($p < 0.05$) increased as the concentration of essential oil increased. At 1000 ppm of *Cumium cymium* L. essential oil, AFB1 production of both *Aspergillus* strains in both treatment was completely inhibited.

The IC50 values of *Cumium cymium* L. essential oil against AFB1 production of *A. flavus* IMI 242684 in contact and vapor treatment were 273.1 and 282.0 ppm, while IC50 values of *A. parasiticus* IMI 283883 were 286.0 and 287.4 ppm, respectively.

4.5.4.2 Effect of *Foeniculum vulgare* Mill. essential oil on *Aspergillus* strains

The results in Table 15 showed that percent inhibition on the growth of both *Aspegillus* strains was 5.8-28.2% and 6.1-35.1% in contact and vapor treatment, respectively, and significantly ($p < 0.05$) increased as the concentration of essential oil increased except at concentrations of 100 ppm and 200 ppm.

Table 15 Effect of different concentrations of *Foeniculum vulgare* Mill. essential oil on the mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	7.6±0.5jk	9.1± 1.1ij	16.5±0.5g	20.8±0.0f	28.2+0.0c	>1000
Vapor	6.1±0.0k	8.6±2.2j	16.8±1.1g	23.0+0.0ef	29.3+1.1bc	>1000
A. parasiticus IMI 283883						
Contact	5.8±0.0k	12.7±0.5h	18.2+0.5g	21.5±1.0f	27.5+0.0cd	>1000
Vapor	11.1 ±0.0hi	18.5±0.0g	25.3+1.1de	30.8+1.1b	35.1+0.0a	>1000

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 ($p < 0.05$).

The vapor treatment was significantly ($p < 0.05$) higher than contact treatment when the same concentration of essential oil was applied against *A. parasiticus* IMI 283883 while the inhibition on mycelial growth of *A. flavus* IMI 242684 was not sifnificant different in both treatments. The IC50 values of *Foeniculum vulgare* Mill. essential oil against mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 were more than 1000 ppm in both contact and vapor treatments, respectively.

The effect of different concentrations of *Foeniculum vulgare* Mill. essential oil on sporulation inhibition was shown in Table 16. The sporulation of both *Aspergillus* strains was inhibited in the range of 20.0-53.5% and 19.5-69.2% by contact and vapor treatments, respectively, when compared with control.

Table 16 Effect of different concentrations of *Foeniculum vulgare* Mill. essential oil on the sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	20.0±1.3i	24.2±1.4hi	28.8±2.2h	37.6±0.8ef	54.5±4.4b	891.2
Vapor	19.5±0.0i	27.2±2.8h	40.9±3.4ef	43.6±2.1de	63.6±2.8a	650.3
A. parasiticus IMI 283883						
Contact	24.4±0.0hi	29.8±2.7gh	38.4±2.0ef	47.7±2.3cd	53.5±2.0bc	762.8
Vapor	25.5±1.9hi	36.0±1.9fg	40.1±2.5ef	51.8±3.1bc	69.2±1.4a	558.4

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 ($p < 0.05$).

At 1000 ppm, essential oil significantly ($p <0$) exhibited higher sporulation inhibition in vapor treatment than in the contact treatment. The IC50 values of

Foeniculum vulgare Mill. essential oil in contact and vapor treatments against sporulation of *A. flavus* were 891.2 and 650.3 ppm, while IC50 values against *A. parasiticus* were 762.8 and 558.4 ppm, respectively.

The AFB1 production of both *Aspergillus* strains was inhibited by 1.3-100% when compared with control. The results in Table 17 showed that AFB1 production significantly increased with increase concentration of *Foeniculum vulgare* essential oil.

Table 17 Effect of different concentrations of *Foeniculum vulgare* Mill. essential oil on the aflatoxin B1 production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	1.3±4.4j	23.8±3.6h	31.2±1.0fg	53.3±4.5cd	100 a	517.8
Vapor	4.4±2.8j	30.4±1.2fgh	47.3±3.2d	62.0±2.7b	100 a	333.5
A. parasiticus IMI 283883						
Contact	6.1±1.5j	26.5±3.1gh	38.3±1.0e	51.3±1.7cd	100 a	496.0
Vapor	13.9±0.7i	36.5±2.0ef	54.2±2.1c	64.9±1.1b	100 a	292.6

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 ($p < 0.05$).

At 100, 300, and 600 ppm of *Foeniculum vulgare* essential oil, AFB1 production inhibition was significantly ($p < 0$) higher in vapor treatment than contact treatment except at 100 and 300 ppm against *A. flavus* IMI 242684. At 1000 ppm of *Foeniculum* *vulgare* Mill. essential oil, AFB1 production of both *Aspergillus* strains was completely inhibited in both of treatment. The IC50 values of *Foeniculum vulgare* Mill. essential oil in contact and vapor treatments against AFB1 production of *A. flavus* IMI 242684 were 517.8 and 333.5 ppm, while IC50 values against *A. parasiticus* IMI 283883 were 496.0 and 292.6 ppm, respectively.

4.5.4.3 Effect of *Trachyspermum amni* (L.) Sprague essential oil on *Aspergillus* strains

Mycelial growth inhibition significantly increased with increasing concentration of *Trachyspermum amni* (L.) Sprague. essential oil. The results in Table 18 showed that mycelial growth inhibition in vapor treatment was significantly ($p < 0.05$) higher than contact treatment when the same concentration was compared.

Table 18 Effect of different concentration of *Trachyspermum amni* (L.) Sprague essential oil on the mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	12.8±1.1m	16.5±1.1l	25.4±0.9j	40.5±1.1g	57.6±0.0c	821.3
Vapor	23.7±1.1jk	33.0±1.1i	43.0±1.1f	48.4+0.9e	66.5±0.5a	635.3
A. parasiticus IMI 283883						
Contact	13.0±0.0m	22.1±0.0l	36.9±1.4h	40.8±1.0fg	54.4±0.5d	801.4
Vapor	32.4 ±0.9i	37.3±0.5h	46.6±0.5e	61.7+1.1b	68.2±0.5a	340.6

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 ($p < 0.05$).

Percent of growth inhibition of both *Aspergillus* strains was between 12.8-57.6% and 23.7-68.2% in contact and vapor treatments, respectively, with IC50 values of mycelial growth of *A. flavus* IMI 242684 at 821.3 and 801.4 ppm, respectively, in contact treatment and 635.3 and 340.6 ppm, respectively, in vapor treatment.

Sporulation inhibition significantly increased with increased concentration of *Trachyspermum amni* (L.) Sprague essential oil except at 100 and 200 ppm against *A. parasiticus* in contact treatment. The sporulation of both *Aspergillus* strains was significantly inhibited by 18.6-100% when compared with control and there was no significant ($p < 0.05$) inhibition between the contact and vapor treatments when the same concentration was compared as shown in Table 19. At 1000 ppm, sporulation of both *Aspergillus* strains was completely inhibited in both treatments.

Table 19 Effect of different concentrations of *Trachyspermum amni* (L.) Sprague essential oil on the sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	25.4±1.4k	37.1± 0.7j	51.3±2.2ghi	68.2±2.8cde	100 a	292.8
Vapor	18.6±2.1k	40.9±6.7ij	51.8±4.8gh	64.5±5.9def	100 a	279.3
A. parasiticus IMI 283883						
Contact	37.2±2.0j	45.7±5.5hij	63.6±5.9ef	77.5±2.9bc	100 a	208.4
Vapor	43.2±4.0hij	57.1±6.2fgh	74.5±3.2de	87.0±1.9b	100 a	134.7

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 ($p < 0.05$).

The IC50 values of *Trachyspermum amni* (L.) Sprague essential oil on sporulation inhibition of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 were 360.5 and 191.7 ppm, respectively, in contact treatment and 292.8 and 208.4 ppm, respectively, in vapor treatment.

The AFB1 production inhibition of *Trachyspermum amni* (L.) Sprague essential oil was presented in Table 20. It was indicated that AFB1 production inhibition significantly increased with increased concentration of essential oil against both *Aspergillus* strains. Both contact and vapor treatments showed no significant difference of AFB1 production inhibition against *Aspergillus* strains. Both *Aspergillus* strains were significantly inhibited by 12.8-100%, when compared with control. At 1000 ppm, AFB1 production of both *Aspergillus* strains was completely inhibited in both treatments.

Table 20 Effect of different concentrations of *Trachyspermum amni* (L.) Sprague essential oil on aflatoxin B1 production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	21.2±1.7gh	33.5±4.8f	51.5±1.0e	64.1±2.0cd	100 a	294.5
Vapor	24.2±5.6g	34.4±4.6f	62.3+2.3de	73.7±0.7bc	100 a	250.4
A. parasiticus IMI 283883						
Contact	12.8±1.2i	33.3±2.4f	47.1±2.2e	70.7±1.7bc	100 a	324.1
Vapor	14.8±3.4hi	38.1±1.7f	60.4±4.2de	70.3±2.4bc	100 a	269.9

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 ($p < 0.05$).

The IC50 values of *Trachyspermum amni* (L.) Sprague essential oil on AFB1 production inhibition of *A. flavus* IMI 242684 were 294.5 and 250.4 ppm, respectively while IC50 values against *A. parasiticus* IMI 283883 were 324.1 and 269.9 ppm in contact and vapor treatments, respectively.

4.5.4.4 Effect of *Brassica juncea* L essential oil on *Aspergillus* strains

. The results in Table 21 showed that the inhibition of mycelial growth of *Aspergillus* strains significantly ($p < 0.05$) increased as the concentration of essential oil increased in both treatments. Mycelial growth inhibition in vapor treatment was significantly ($p <0.05$) higher than contact treatment when the same concentration of essential oil was compared.

Table 21 Effect of different concentrations of *Brassica juncea* L. essential oil on the mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	15.0±0.5n	26.3± 0.0lm	29.7±0.5k	48.4±0.0f	64.4±1.1c	684.0
Vapor	36.8±1.1i	42.1±0.5gh	53.7±1.1e	63.1±1.1c	75.3±0.5a	253.9
A. parasiticus IMI 283883						
Contact	13.3±0.5n	25.1±1.0m	40.5±0.5h	52.6±0.5e	60.4±0.5d	555.2
Vapor	28.7±0.9kl	33.02±0.5j	42.9±0.5g	66.6±0.0b	73.4±1.1a	390.8

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 ($p < 0.05$).

The growth inhibition of both *Aspergillus* strains was 60.4-64.4% and 73.4-75.3% in contact and vapor treatments, respectively, with IC50 values of mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 at 684.0 and 555.2 ppm, respectively, in contact treatment and 253.9 and 390.8 ppm, respectively, in vapor treatment.

The sporulation of both *Aspergillus* strains was inhibited by 26.3-100%, when compared with control and significantly ($p < 0$) increased as the concentration of essential oil increased in both treatments (Table 22).

Table 22 Effect of different concentrations of *Brassica juncea* L. essential oil on the sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	26.3+2.5k	38.8±0.0j	52.9±0.7gh	72.3±0.8d	100 a	280.1
Vapor	26.3±3.6k	42.7±3.6j	58.6±2.8fg	76.4±2.8cd	100 a	232.1
A. parasiticus IMI 283883						
Contact	38.1±1.8j	49.6±1.8hi	65.5±3.7e	79.5±1.3c	100 a	206.4
Vapor	44.4±3.7ij	59.9±2.1ef	80.2±2.5c	90.3±1.2b	100 a	121.3

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 ($p < 0.05$).

At 1000 ppm of *Brassica juncea* L essential oil, sporulation of both *Aspergillus* strains in both treatments was completely inhibited. The IC50 values of *Brassica juncea* L essential oil against *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 were 280.1 and 206.4 ppm, in contact treatment and 232.1 and 121.3 ppm, respectively, in vapor treatment.

The results in Table 23 showed that the inhibition of AFB1 production of *Aspergillus* strains significantly ($p < 0.,05$) increased when the concentration increased in both treatments and the aflatoxin B1 inhibition of both *Aspergillus* strains was 16.8-100% and 26.8-100% in contact and vapor treatments, respectively, with IC50 values against *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 at 235.9 and 270.6 ppm, respectively, in contact treatment and 203.7 and 208.4 ppm, respectively, in vapor treatment. At 1000 ppm of *Brassica juncea* L. essential oil, AFB1 production of both *Aspergillus* strains in both treatments was completely inhibited.

Table 23 Effect of different concentrations of *Brassica juncea* L. essential oil on the aflatoxin B1 production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	21.9±0.9i	41.4±2.3g	60.1±0.9d	80.1±0.5b	100a	235.9
Vapor	28.9±0.9h	43.5±1.1fg	69.7±3.0c	83.2±1.0b	100a	203.7
A. parasiticus IMI 283883						
Contact	16.8±2.0j	40.8±2.1g	54.7±1.3e	69.7±3.2c	100a	270.6
Vapor	26.8±0.6hi	47.5±3.7f	64.5±0.6d	79.7±1.7b	100a	208.4

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 ($p < 0.05$).

4.5.4.5 Effect of *Cinnamomum porrectum* (Roxb.) Kosterm essential oil on *Aspergillus* strains

Mycelial growth of both *Aspergillus* strains was significantly inhibited with increasing concentration of *Cinnamomum porrectum* (Roxb.) Kosterm essential oil. When contact and vapor treatments were compared, it was found that vapor treatment showed significantly effective ($p <0.05$) on mycelial growth inhibition when using the same concentration of essential oil at 100 to 600 ppm, except at 100 ppm against *A. flavus* IMI 242684 (Table 24).

Table 24 Effect of different concentrations of *Cinnamomum porrectum* (Roxb.) Kosterm essential oil on the mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50
	100	200	300	600	1000	(ppm)
A. flavus IMI 242684						
Contact	13.1±0.5 j	22.3±0.5 h	51.8±1.9 e	77.6±0.5 c	100 a	293.8
Vapor	16.8±1.1 ij	33.7±1.1 g	62.4±5.0 d	83.1±0.0 b	100 a	251.1
A. parasiticus IMI 283883						
Contact	13.3±0.5 j	20.9±1.0 hi	52.3±3.8 e	75.5±1.6 c	100 a	292.6
Vapor	24.0±1.9 h	43.8±1.1 f	60.4±1.1 d	84.8±0.5 b	100 a	222.3

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 ($p < 0.05$).

Both treatments showed that the essential oil at 1000 ppm completely inhibited both *Aspergillus* strains. The IC50 values of *Cinnamomum porrectum* (Roxb.) Kosterm essential oil on mycelial growth inhibition of *A. flavus* IMI 242684 were 293.8 and 292.6 ppm while of *A. parasiticus* IMI 283883 were 375.9 and 222.3 ppm in contact and vapor treatments, respectively.

The inhibitory activity of *Cinnamomum porrectum* (Roxb.) Kosterm essential oil on sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 was shown in Table 25.

Table 25 Effect of different concentrations of *Cinnamomum porrectum* (Roxb.) Kosterm essential oil on the sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50
	100	200	300	600	1000	(ppm)
A. flavus IMI 242684						
Contact	16.7±3.6 gh	33.3±3.6 ef	54.2±3.8 cd	100 a	100 a	284.7
Vapor	28.6±3.4 fg	60.0±11.1 c	87.7±2.7 ab	100 a	100 a	170.0
A. parasiticus IMI 283883						
Contact	8.8±1.7 hi	34.9±9.1 ef	66.3±6.5 c	100 a	100 a	236.2
Vapor	44.4±4.5 de	57.9±6.1 cd	80.2±8.6 b	100 a	100 a	128.4

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 ($p < 0.05$).

It was found that sporulation of *Aspergillus* strains was significantly ($p < 0.05$) inhibited with increased concentration of essential oil. Sporulation inhibition at 100 and 200 ppm against *A. parasiticus* IMI 283883 was not different in vapor treatment ($p < 0.05$). At 600 and 1000 ppm essential oil completely inhibited sporulation of both *Aspergillus* strains. However, the sporulation inhibition by vapor treatment was more significantly effective than the contact treatment when the same concentrtaion of essential oil (100, 200 and 300 ppm) was applied, except at 100 ppm against *A. flavus* IMI 242684. The IC50 values of *Cinnamomum porrectum* (Roxb.) Kosterm on sporulation of *A. flavus* IMI 242684 were 284.7 and 170.0 ppm, respectively while *A. parasiticus* IMI 283883 were 236.2 and 128.4 ppm in contact and vapor treatments, respectively.

The inhibitory activity of *Cinnamomum porrectum* (Roxb.) Kosterm essential oil on aflatoxin B1 production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 was presented in Table 26.

Table 26 Effect of different concentrations of *Cinnamomum porrectum* (Roxb.) Kosterm essential oil on the aflatoxin production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	19.6±1.0 g	41.4±3.9 ef	81.9±7.9 b	100 a	100 a	200.8
Vapor	34.6±6.9 f	68.6±5.0 c	100 a	100 a	100 a	148.2
A. parasiticus IMI 283883						
Contact	20.0±4.1 g	53.7±3.7 d	75.4±7.2 bc	100 a	100 a	182.5
Vapor	48.5±2.5 de	66.2±3.3 c	100 a	100 a	100 a	145.5

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 ($p < 0.05$).

Aflatoxin B1 production inhibition of both fungal strains ($p<0.05$) increased as the concentration of essential oil increased. Treatment of essential oil at 600 and 1000 ppm by both contact and vapor methods completely inhibited AFB1 production by both *Aspergillus* strains. At 300 ppm, vapor treatment with *Cinnamomum porrectum* (Roxb.) Kosterm essential oil completely inhibited AFB1 of both *Aspergillus* strains, while contact treatment inhibited only 81.9 and 75.4% of *A. flavus* and *A. parasiticus*, respectively. The IC50 values of *Cinnamomum porrectum* (Roxb.) Kosterm on AFB1 production of *A. flavus* IMI 242684 were 200.8 and 148.2 ppm, while of *A. parasiticus* IMI 283883 were 182.5 and 145.5 ppm, in contact and vapor treatments, respectively.

4.5.4.6 Effect of *Cinnamomum zeylanicum* Blume essential oil on *Aspergillus* strains

Mycelial growth inhibition was significantly increased with increased concentration of *Cinnamomum zeylanicum* Blume essential oil against *Aspergillus* strains in both treatments (Table 27).

Table 27 Effect of different concentrations of *Cinnamomum zeylanicum* Blume essential oil on the mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	22.7±1.8 j	48.1±0.5 h	57.6±0.0 g	70.5±0.0 e	100 a	237.9
Vapor	47.4±0.0 h	70.2±0.5 e	81.8±1.1 b	100 a	100 a	107.7
A. parasiticus IMI 283883						
Contact	13.0±0.0 k	21.5±0.5 j	47.5±0.0 h	74.6±0.0 c	100 a	382.3
Vapor	45.3 ±1.6 i	59.8±1.1 f	72.5±0.5 d	100 a	100 a	119.8

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 ($p < 0.05$).

When compared between contact and vapor treatments, the result showed that vapor treatment was significantly effective ($p<0.05$) on mycelial growth inhibition when the same concentration of essential oils at 100 to 600 ppm was applied. Essential oil at 600 ppm in vapor treatment completely inhibited mycelial growth of both *Aspergillus* strains, while in contact treatment the inhibition was only about 70 - 74%. Essential oil at 1,000 ppm completely inhibited both *Aspergillus* strains in both treatments. The IC50 values of *Cinnamomum zeylanicum* Blume on mycelial growth of *A. flavus* IMI 242684 were 237.9 and 107.7 ppm, while of *A. parasiticus* IMI 283883 were 382.3 and 119.8 ppm in contact and vapor treatments, respectively.

The sporulation of both *Aspergillus* strains was significantly inhibited by 27.3-100%, when compared with control (Table 28). At 300 ppm, sporulation inhibition by *Cinnamomum zeylanicum* Blume essential oil was significantly higher in vapor treatment than in contact treatment. At 600 and 1000 ppm, *Cinnamomum zeylanicum* Blume essential oil completely inhibited sporulation of both *Aspergillus* strains in both treatments. The IC50 values of *Cinnamomum zeylanicum* Blume essential oil on sporulation inhibition of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 were 207.8 and 208.0 ppm, respectively, in contact treatment and 148.9 and 136.5 ppm, respectively, in vapor treatment.

Table 28 Effect of different concentrations of *Cinnamomum zeylanicum* Blume essential oil on the sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	27.9±6.9 e	44.2±6.9 cd	72.5±4.5 b	100 a	100 a	207.8
Vapor	34.5±10.8 de	68.2±9.3 b	91.4±0.8 a	100 a	100 a	148.9
A. parasiticus IMI 283883						
Contact	27.3±1.5 e	39.9± 6.8 cde	76.4±4.7 b	100 a	100 a	208.0
Vapor	46.3 ±5.1 cd	53.4± 5.1 c	90.3±0.0 a	100 a	100 a	136.5

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 ($p < 0.05$).

The AFB1 production of both *Aspergillus* strains was significantly inhibited by 30.5-100% when compared with control and significantly ($p < 0.05$) increased as the *Cinnamomum zeylanicum* Blume essential oil increased in the range of 100 to 300 ppm in both treatments, as shown in Table 29.

Table 29 Effect of different concentrations of *Cinnamomum zeylanicum* Blume essential oil on the aflatoxin B1 production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50
	100	200	300	600	1000	(ppm)
A. flavus IMI 242684						
Contact	36.0±5.2 e	54.5± 7.4 d	80.1±3.5 c	100 a	100 a	153.1
Vapor	50.1±1.1 d	89.4±0.5 b	100 a	100 a	100 a	97.8
A. parasiticus IMI 283883						
Contact	30.5±5.7 e	52.5±2.9 d	86.2±1.7 bc	100 a	100 a	164.0
Vapor	47.3±6.4 d	87.9±0.9 bc	100 a	100 a	100 a	102.8

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 ($p < 0.05$).

At 100, 200, and 300 ppm of *Cinnamomum zeylanicum* Blume essential oil, AFB1 production inhibition was significantly higher in vapor treatment than in contact treatment. At 600 and 1000 ppm, *Cinnamomum zeylanicum* Blume essential oil completely inhibited aflatoxin B1 production of both *Aspergillus* strains in both treatments. The IC50 values of *Cinnamomum zeylanicum* Blume essential oil werre 153.1 and 164.0 ppm on AFB1 production inhibition of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883, respectively, in contact treatment and 97.8 and 102.8 ppm, respectively, in vapor treatment.

4.5.4.7 Effect of *Michelia alba* DC. essential oil on *Aspergillus* strains

Mycelial growth inhibition of both *Aspergillus* strains significantly increased as the concentration of *Michelia alba* DC. essential oil increased in both treatments except at 100 and 200 ppm against *A. parasiticus* in vapor treatment (Table 30).

Table 30 Effect of different concentrations of *Michelia alba* DC. essential oil on the mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	10.3±2.1j	18.0±2.4i	28.5±1.4h	40.5±4.6e	68.1+1.1bc	710.2
Vapor	30.6±1.9gh	38.7±1.1ef	53.1±0.0d	72.5±1.1b	100 a	282.3
A. parasiticus IMI 283883						
Contact	2.4±0.5k	18.7±1.3i	29.0±0.5h	34.4±0.5 fg	66.7+2.7c	748.5
Vapor	35.8±1.1ef	38.3±1.1ef	54.6±1.6d	70.7±0.5 bc	100 a	280.4

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 ($p < 0.05$).

When compared between contact and vapor treatments, it indicated that vapor treatment was significantly effective ($p<0.05$) on mycelial growth inhibition when using the same concentration of essential oils at 100 to 1000 ppm. At 1000 ppm, essential oil in vapor treatment completely inhibited mycelial growth of both *Aspergillus* strains, while the inhibition was only about 66.7- 68.1% in contact treatment. Essential oil at 1000 ppm was found to inhibit both *Aspergillus* strains (100%) in vapor treatments. The IC50 values of *Michelia alba* DC. on mycelial growth of *A. flavus* IMI 242684 were 710.2 and 282.3 ppm, respectively while of *A. parasiticus* IMI 283883 were 748.5 and 280.4 ppm in contact and vapor treatments, respectively.

The inhibition of sporulation of both *Aspergillus* strains was not significant ($p < 0.05$) between the contact and vapor treatments (Table 31). At 100 ppm, the inhibition effect was not significantly different when compared with control in both treatments.

Table 31 Effect of different concentrations of *Michelia alba* DC. essential oil on the sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	12.0±6.2ghi	25.8±7.1fgh	36.2±8.9defg	77.5±10.9ab	100 a	462.1
Vapor	15.8±4.0ghi	35.0±7.6efgh	60.4±1.5bcd	78.3±5.9ab	100 a	261.6
A. parasiticus IMI 283883						
Contact	11.2±3.7hi	35.3±5.9efgh	44.2±1.2def	70.5±3.6 bc	100 a	457.8
Vapor	22.5±7.7fghi	36.4±4.1defg	51.6±2.4cde	70.5±3.5bc	100 a	289.4

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 ($p < 0.05$).

At 1000 ppm, *Michelia alba* DC. essential oil completely inhibited sporulation of both *Aspergillus* strains in both treatments. The IC50 values of *Michelia alba* DC. on sporulation of *A. flavus* IMI 242684 were 462.1 and 261.6 ppm while IC50 values of *A. parasiticus* IMI 283883 were 457.8 and 289.4 ppm in contact and vapor treatment, respectively.

The AFB1 production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 was inhibited by 10.7 to 86.2% and 4.7 to 75.3% inhibition, respectively (Table 32).

Table 32 Effect of different concentrations of *Michelia alba* DC. essential oil on the aflatoxin B1 production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	10.7+0.9ghi	24.14±5.1fg	25.1±4.4fg	42.5±4.6de	80.2±3.6a	628.9
Vapor	17.3±5.1fgh	30.0±8.7ef	46.5±3.6cd	61.1±1.8bc	80.2±3.6a	361.2
A. parasiticus IMI 283883						
Contact	4.7±9.1hi	13.1±3.7ghi	20.5±4.1fg	23.0±2.6fg	72.6±5.6ab	782.4
Vapor	4.7±9.1hi	22.1±1.7fg	32.6±1.9def	40.7±1.5de	75.3±1.4ab	654.6

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 ($p < 0.05$).

There was no significant difference at 100 ppm when compared with contact and vapor treatments. The IC50 values of *Michelia alba* DC. on AFB1 production inhibition of *A. flavus* IMI 242684 were 628.9 and 361.2 ppm, while of *A. parasiticus* IMI 283883 were 782.4 and 654.6 ppm in contact and vapor treatments, respectively.

4.5.4.8 Effect of *Myristica fragrans* Houtt. essential oil on *Aspergillus* strains

Mycelial growth inhibition significantly increased with increasing concentration of *Myristica fragrans* Houtt. essential oil ($p < 0.05$) (Table 33).

Table 33. Effect of different concentrations of *Myristica fragrans* Houtt. essential oil on the mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	3.6±1.0kl	8.8± 2.4ij	25.4±0.0g	36.7±1.0e	72.6±1.4b	717.3
Vapor	19.9±2.1h	29.3±1.0fg	48.0±2.1d	55.9±0.9c	100 a	452.4
A. parasiticus IMI 283883						
Contact	5.1±1.0jk	10.3±2.4i	26.9±0.5fg	37.2±1.0e	71.9±0.9b	715.3
Vapor	17.5±0.9h	30.8±1.0f	38.2±0.5e	47.5±1.9d	100 a	618.8

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 ($p < 0.05$).

When compared between contact and vapor treatments, it indicated that vapor treatment was significantly effective ($p<0.05$) on mycelial growth inhibition when using the same concentration of essential oils at 100 to 1000 ppm, At 1000 ppm, essential oil in vapor treatment completely inhibited mycelial growth of both *Aspergillus* strains, while in contact treatment the inhibition was only 71-72%. The IC50 values of *Myristica fragrans* on mycelial growth of *A. flavus* IMI 242684 were 717.3 and 452.4 ppm, while of *A. parasiticus* IMI 283883 were 715.3 and 618.8 ppm in contact and vapor treatments, respectively.

Vapor treatment with essential oil at 600 ppm completely inhibited sporulation of both *Aspergillus* strains, while in contact treatment only 35.8 and 36.8% of

A. flavus IMI 242684 and *A. parasiticus* IMI 283883 was inhibited, respectively (Table 34).

Table 34 Effect of different concentrations of *Myristica fragrans* Houtt. essential oil on the sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50
	100	200	300	600	1000	(ppm)
A. flavus IMI 242684						
Contact	4.1±3.6gh	12.5±6.2fgh	17.9±1.4fg	35.8±5.2de	100 a	688.4
Vapor	25.0±6.2ef	56.2±10.6c	82.9±10.1b	100a	100 a	168.7
A. parasiticus IMI 283883						
Contact	14.7±3.4fgh	18.2±6.4fg	22.1±4.0ef	36.8±10.8de	100 a	683.5
Vapor	26.7±5.1ef	51.9±7.7cd	62.8±5.3c	100a	100 a	189.3

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 ($p < 0.05$).

At 1000 ppm, *Myristica fragrans* Houtt. essential oil completely inhibited sporulation of both *Aspergillus* strains in both treatments. Sporulation was significantly inhibited ($p < 0.05$) by vapor treatment more than by contact treatment when compared at the same concentrations of 100, 200, 300 and 600 ppm, expect at 100 ppm against *A. parasiticus* IMI 283883.

The IC50 values of *Myristica fragrans* essential oil on sporulation inhibition of *A. flavus* IMI 242684 were 688.4 and 168.7 ppm, respectively while of *A. parasiticus* IMI 283883 were 683.5 and 189.36 ppm in contact and vapor treatments, respectively.

The results showed that AFB1 production was significantly inhibited ($p < 0.05$) by vapor treatment more than by contact treatment when compared at the same concentrations of 200, 300 and 600 ppm against both *Aspergillus* strains except at 200 ppm against *A. parasiticus* IMI 283883 as shown in Table 35. At 1000 ppm, *Myristica fragrans* essential oil completely inhibited AFB1 production of both *Aspergillus* strains in both treatments.

Table 35 Effect of different concentrations of *Myristica fragrans* Houtt. essential oil on the aflatoxin production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	6.4±3.4gh	25.2±8.0ef	36.4±3.7de	53.2±5.0c	100 a	504.5
Vapor	12.8±8.0fg	41.5±3.7cd	74.4±2.3b	100a	100 a	222.9
A. parasiticus IMI 283883						
Contact	15.0±3.2fg	24.7±8.3ef	29.6±4.5de	35.5±5.3de	100 a	689.7
Vapor	15.0±3.2fg	30.7±3.0de	67.4±2.4b	74.3±4.8b	100 a	246.6

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 ($p < 0.05$).

The IC50 values of *Myristica fragrans* Houtt essential oil on AFB1 production inhibition of *A. flavus* IMI 242684 were 504.5 and 222.9 ppm, respectively while of *A. parasiticus* IMI 283883 were 689.7 and 246.6 ppm in contact and vapor treatments, respectively.

4.5.4.9 Effect of *Cymbopogon citratus* (DC.) Stapf. essential oil on *Aspergillus* strains

The mycelial growth inhibition of both *Aspergillus* strains significantly increased as the concentration of essential oil increased. When compared between contact and vapor treatments, it indicated that vapor treatment was significantly effective ($p<0.05$) on mycelial growth inhibition when using the same concentration of essential oils at 200 to 600 ppm. At 1000 ppm, essential oil completely inhibited mycelial growth of both *Aspergillus* strains in both treatments.

Table 36 Effect of different concentrations of *Cymbopogon citratus* (DC.) Stapf. essential oil on the mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50
	100	200	300	600	1000	(ppm)
A. flavus IMI 242684						
Contact	21.4±1.1l	36.8± 1.1i	65.0±0.0f	76.1±0.0d	100 a	244.0
Vapor	20.5±1.1l	35.9±0.5j	59.9±1.1g	78.4±0.9c	100 a	251.6
A. parasiticus IMI 283883						
Contact	13.0±0.0 m	25.1±1.0k	55.3±1.0h	72.8±0.0e	100 a	298.1
Vapor	12.9±0.0 m	38.8±0.0i	66.9±0.5f	80.8±1.1b	100 a	247.1

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 ($p < 0.05$).

The IC50 values of *Cymbopogon citratus* (DC.) Stapf. on mycelial growth of *A. flavus* IMI 242684 were 244.0 and 251.6 ppm, respectively while of *A. parasiticus* IMI 283883 were 298.1 and 247.1 ppm in contact and vapor treatments, respectively.

The results in Table 37 showed that the sporulation of both *Aspergillus* strains was significantly inhibited by 25.0-100% when compared with control. The sporulation inhibition of *Aspergillus* strains was significantly increased as the concentration of essential increased. At 200, 300, and 600 ppm of *Cymbopogon citratus* (DC.) Stapf. essential oil, the inhibition was significantly higher in vapor treatment than in contact treatment when the same concentration of essential oil was applied. At 1000 ppm, *Cymbopogon citratus* (DC.) Stapf. essential oil completely inhibited sporulation of both *Aspergillus* strains in both treatments. The IC50 values of *Cymbopogon citratus* (DC.) Stapf. essential oil were 188.0 and 161.8 ppm of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883, respectively, in contact treatment and 211.3 and 150.4 ppm, respectively, in vapor treatment.

Table 37 Effect of different concentrations of *Cymbopogon citratus* (DC.) Stapf. essential oil on the sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	25.0±3.3h	53.8±1.3e	63.8±2.5d	78.6+1.6c	100 a	188.0
Vapor	27.7±3.6gh	61.8±3.6d	78.6±2.8c	100a	100 a	161.8
A. parasiticus IMI 283883						
Contact	27.4±2.6gh	42.2±2.9f	66.7±2.9d	85.7±2.7b	100 a	211.3
Vapor	32.6 ±1.7g	65.6±3.7d	74.5±3.6c	100a	100 a	150.4

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 ($p < 0.05$).

The AFB1 production of both *Aspergillus* strains was inhibited by 17.6-100% when compared with control as shown in Table 38. *Cymbopogon citratus* (DC.) Stapf. essential oil did not show significant inhibition ($p < 0$) of both *Aspergillus* strains when contact and vapor treatments at 100 ppm and 200 ppm were applied. At 1000 ppm, *Cymbopogon citratus* (DC.) Stapf. essential oil completely inhibited AFB1 production of both *Aspergillus* strains and at 600 ppm against *A. parasiticus* IMI 283883 in both treatments.

Table 38 Effect of different concentrations of *Cymbopogon citratus* (DC.) Stapf. essential oil on the aflatoxin production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50
	100	200	300	600	1000	(ppm)
A. flavus IMI 242684						
Contact	17.6±3i	38.7±1.3g	57.6±1.3f	75.0±0.6d	100a	260.8
Vapor	19.3±2.5i	42.7±3.1g	65.4±0.7e	82.6±1.7c	100a	230.3
A. parasiticus IMI 283883						
Contact	33.9±0.8h	55.3±2.4ef	86.0±1.6c	100a	100a	155.0
Vapor	38.3±1.9gh	68.5±1.5e	91.0±0.4b	100a	100a	139.8

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 ($p < 0.05$).

The IC50 value of *Cymbopogon citratus* (DC.) Stapf. essential oil were 260.8 and 155.0 ppm of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883, respectively, in contact treatment and 230.3 and 139.8 ppm, respectively, in vapor treatment.

4.5.4.10 Effect of different concentrations of *Citrus hystrix* essential oil on *Aspergillus* strains inhibitory

The results in Table 39 showed that the inhibition of mycelial growth was significantly ($p<0.05$) increased as the concentration of essential oil increased. When compared between contact and vapor treatments, it indicated that vapor treatment was significantly effective ($p<0.05$) on mycelial growth inhibition when using the same amount of essential oils at 100 to 1000 ppm except at 100 ppm against *A. parasiticus* IMI 283883.

Table 39 Effect of different concentration of *Citrus hystrix* essential oil on the mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	6.1±0.0n	6.7± 1.1n	13.4±0.0l	20.8±0.0j	37.4±0.0d	>1000
Vapor	15.5±0.0k	23.0±0.0i	26.8±0.0gh	33.0±1.1f	45.5±0.0b	>1000
A. parasiticus IMI 283883						
Contact	9.4±0.0m	16.7±0.0k	20.0±0.5j	25.1±1.0h	35.4±1.0e	>1000
Vapor	11.1±0.0m	19.1±1.1j	27.7±0.0g	39.5±1.1c	68.5±0.0a	745.0

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 ($p < 0.05$).

The IC50 values of *Citrus hystrix* on mycelial growth of *A. flavus* IMI 242684 were >1000 and >1000 ppm, respectively while *A. parasiticus* IMI 283883 were >1000 and 712.1 ppm in contact and vapor treatments, respectively.

The sporulation of both *Aspergillus* strains was not significantly ($p < 0.05$) inhibited between the contact and vapor treatments when compared at the same concentration of *Citus hystrix* essential oil except at 1000 ppm against both *Aspergillus* strains (Table 40).

Table 40. Effect of different concentrations of *Citrus hystrix* essential oil on the sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	17.9±1.4j	20.8± 1.4j	35.0±0.0fg	38.2±0.8efg	57.3±0.8b	800.9
Vapor	20.4±1.6j	28.1±2.1hij	41.3±2.7ef	44.1±1.4de	65.9±1.4a	642.4
A. parasiticus IMI 283883						
Contact	24.4±0.0ij	32.2±5.9gh	39.1±1.8ef	48.4±2.7cd	55.4±2.9b	679.0
Vapor	23.9±0.7ij	36.4±1.4fg	38.4±4.9efg	51.4±3.2bc	69.6±1.2a	565.7

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 ($p < 0.05$).

The IC50 values of *Citrus hystrix* on sporulation of *A. flavus* IMI 242684 were 800.9 and 642.4 ppm, respectively while of *A. parasiticus* IMI 283883 were 679.0 and 565.7 ppm in contact and vapor treatments, respectively.

The AFB1 production of both *Aspergillus* strains was inhibited by 5.4-69.1% when compared with control (Table 41). At 1000 ppm, *Citrus hystrix* essential oil significantly inhibited ($p < 0.05$) AFB1 production in vapor treatment higher than in contact treatment.

Table 41 Effect of different concentrations of *Citrus hystrix* essential oil on the aflatoxin production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	8.8±2.4jk	27.1±4.3h	32.0±0.4gh	58.3±0.0cd	63.7±2.8bc	518.3
Vapor	19.0±2.0i	30.1±0.8h	47.3±3.2e	63.9±1.6abc	70.5±2.4a	380.5
A. parasiticus IMI 283883						
Contact	5.4±2.4kl	28.4±2.3h	39.6±1.9f	50.1±3.8e	58.2±1.7cd	582.6
Vapor	13.9±0.7ij	37.7±1.4fg	53.9±2.5de	64.8±1.0abc	69.1±2.6ab	273.1

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 ($p < 0.05$).

The IC50 values of *Citrus hystrix* on AFB1 production of *A. flavus* IMI 242684 were 518.3 and 380.5 ppm, respectively while of *A. parasiticus* IMI 283883 were 582.6 and 273.1 ppm in contact and vapor treatments, respectively.

4.5.4.11 Effect of *Limnophilia geoffrayi* Bonati essential oil on *Aspergillus* strains

The results in Table 42 showed that the inhibition of mycelial growth was significantly ($p<0.05$) increased as the concentration of essential oil increased. The mycelial growth of both *Aspergillus* strains was inhibited by 10.4-70.7% when compared with control.

Table 42 Effect of different concentrations of *Limnophilia geoffrayi* Bonati essential oil on the mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	10.7±0.9l	16.8±1.9k	26.0±0.5i	48.4±1.8e	58.3±1.1cd	700.8
Vapor	10.4±1.1l	20.8±0.0j	30.9±0.9h	50.6+0.5e	60.1±0.5bc	573.1
A. parasiticus IMI 283883						
Contact	12.4±1.0l	21.5±1.0j	30.6±1.0h	55.9±1.0d	62.0±3.1b	562.7
Vapor	19.7±1.0jk	37.5±0.9g	41.7±0.5f	60.4+0.5bc	70.7±0.5a	390.3

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 ($p < 0.05$).

At 1000 ppm, *A. parasiticus* IMI 283883 was significantly inhibited by vapor treatment more than by contact treatment, while *A. flavus* IMI 242684 did not show significant effect. The IC50 values of *Limnophilia geoffrayi* Bonati essential oil on mycelial growth of *A. flavus* IMI 242684 were 700.8 and 573.1 ppm, respectively while of *A. parasiticus* IMI 283883 were 562.7 and 390.3 ppm in contact and vapor treatments, respectively.

The results in Table 43 showed that the inhibition of sporulation was significantly ($p<0.05$) increased as the concentration of essential oil increased. When compared between contact and vapor treatments, it indicated that vapor treatment was significantly effective ($p<0.05$) more than contact treatment when the same concentrations of essential oils at 100 to 300 ppm were applied except at 100 ppm against *A. flavus* IMI 242684.

Table 43 Effect of different concentrations of *Limnophilia geoffrayi* Bonati essential oil on the sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	13.3±5.1fg	32.5±4.3e	52.1±8.0cd	100a	100 a	289.5
Vapor	16.3±5.4fg	45.8±3.1d	71.3±3.3b	100a	100 a	216.7
A. parasiticus IMI 283883						
Contact	9.7±2.7gh	20.9±4.2f	46.5±6.2d	100a	100 a	330.6
Vapor	33.7±2.3e	47.7±3.5 d	61.6±4.0 bc	100a	100 a	225.2

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 ($p < 0.05$).

At 600 and 1000 ppm, essential oil completely inhibited sporulation of both *Aspergillus* strains in both treatments. The IC50 values of *Limnophilia geoffrayi* Bonati on sporulation of *A. flavus* IMI 242684 were 289.5 and 216.7 ppm, respectively while *A. parasiticus* IMI 283883 were 330.6 and 225.2 ppm in contact and vapor treatments, respectively.

The AFB1 production of both *Aspergillus* strains was inhibited by 10.3-100% when compared with control (Table 44).

Table 44 Effect of different concentrations of *Limnophilia geoffrayi* Bonati essential oil on aflatoxin production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50
	100	200	300	600	1000	(ppm)
A. flavus IMI 242684						
Contact	19.1±8.4hi	31.4± 2.7fg	53.0±3.2d	82.9±2.5bc	100 a	266.8
Vapor	22.8±2.8gh	33.2±2.7fg	56.0+3.8d	80.0±3.5bc	100 a	256.1
A. parasiticus IMI 283883						
Contact	10.3±2.2i	35.8±6.3ef	46.4±6.5de	91.4±0.2ab	100 a	325.2
Vapor	11.5±1.2i	40.9±1.3ef	52.2±3.1d	93.6±0.6 ab	100 a	258.2

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 ($p < 0.05$).

The inhibition of AFB1 was not significant ($p < 0.05$) different between the contact and vapor treatments when compared at the same concentration of essential oil. At 1000 ppm, *Limnophilia geoffrayi* Bonati essential oil completely inhibited AFB1 production of both *Aspergillus* strains in both treatments. The IC50 values of *Limnophilia geoffrayi* Bonati essential oil on AFB1 production inhibition of *A. flavus* IMI 242684 were 266.8 and 256.1 ppm, respectively while of *A. parasiticus* IMI 283883 were 256.1 and 325.2 ppm in contact and vapor treatments, respectively

4.5.4.12 Effect of *Kaempferia galanga* L. essential oil on *Aspergillus* strains

The results in Table 45 showed that the inhibition of mycelial growth was significantly ($p<0.05$) increased as the concentration of essential oil increased from 100 ppm to 600 ppm.

Table 45 Effect of different concentrations of *Kaempferia galanga* L. essential oil on mycelial growth of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	22.7±1.8i	31.9±0.0h	66.9±0.0d	100 a	100 a	251.2
Vapor	42.4±1.1g	68.1±0.0d	80.3±0.9b	100 a	100 a	120.8
A. parasiticus IMI 283883						
Contact	13.3±0.5j	20.9±1.0i	52.3±3.8e	100 a	100 a	289.7
Vapor	47.5+1.1f	68.2±0.5d	75.9±0.0c	100 a	100 a	111.8

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 ($p < 0.05$).

In vapor treatment, the effect on mycelial growth inhibition was significant ($p<0.05$) when using the same concentration of essential oil at 100 to 300 ppm. At 600 and 1000 ppm of *Kaempferia galanga* L. essential oil, mycelial growth of both *Aspergillus* strains was completely inhibited in both treatments. The IC50 values of *Kaempferia galanga* L. on mycelial growth of *A. flavus* IMI 242684 were 251.2 and 120.8 ppm, respectively while *A. parasiticus* IMI 283883 were 289.7 and 111.8 ppm in contact and vapor treatments, respectively.

The inhibitory effect of *Kaempferia galanga* L. essential oil on sporulation of *Aspergillus* strains was shown in Table 46.

Table 46 Effect of different concentrations of *Kaempferia galanga* L. essential oil on the sporulation of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50
	100	200	300	600	1000	(ppm)
A. flavus IMI 242684						
Contact	24.6±5.6 e	42.9±5.1 d	77.5±4.5 b	100 a	100 a	207.4
Vapor	35.4±5.5d e	67.3±10.7 bc	92.3±1.6 a	100 a	100 a	147.9
A. parasiticus IMI 283883						
Contact	27.1±2.4 e	39.5±3.5 d	74.8±4.1 b	100 a	100 a	210.4
Vapor	44.4±5.7 d	59.5±3.7 c	91.1±3.5 a	100 a	100 a	135.3

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 ($p < 0.05$).

In vapor treatment there was significant effect ($p<0.05$) on sporulation inhibition when using the same concentration of essential oil at 100 to 300 ppm except at 100 ppm against *A. flavus* IMI 242684. .At 600 and 1000 ppm, *Kaempferia galanga* L. essential oil completely inhibited sporulation of both *Aspergillus* strains in both treatments. The IC50 values of *Kaempferia galanga* L. on sporulation of *A. flavus* IMI 242684 were 207.4 and 147.9 ppm, respectively while of *A. parasiticus* IMI 283883 were 210.4 and 135.3 ppm in contact and vapor treatments, respectively.

The inhibition effects of AFB1 production of *Aspergillus* strains by various concentrations of *Kaempferia galanga* L. essential oil are presented in Table 47.

Table 47 Effect of different concentrations of *Kaempferia galanga* L. essential oil on the aflatoxin production of *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using contact and vapor treatments

Mode of Inhibition	Percent inhibition (%) at various concentrations of essential oil (ppm)					IC50 (ppm)
	100	200	300	600	1000	
A. flavus IMI 242684						
Contact	36.0±5.2 e	56.1±4.4 c	82.8±8.4 b	100 a	100 a	164.4
Vapor	44.6±2.2 d	82.1±1.5 b	100 a	100 a	100 a	109.3
A. parasiticus IMI 283883						
Contact	13.3±0.6 f	52.5±2.9 cd	86.2±1.7 b	100 a	100 a	192.7
Vapor	51.3±2.5 cd	85.0±5.4 b	100 a	100 a	100 a	96.0

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 ($p < 0.05$).

In vapor treatment, there was significant effect ($p<0.05$) on AFB1 production inhibition when using the same concentration of essential oil at 100 to 300 ppm against both of *Aspergillus* strains, when comparison with contact treatment. At 600 and 1000 ppm, *Kaempferia galanga* L. essential oil completely inhibited AFB1 of both *Aspergillus* strains in both treatments. The IC50 values of *Kaempferia galanga* L. on AFB1 production of *A. flavus* IMI 242684 were 164.4 and 109.3 ppm, respectively while of *A. parasiticus* IMI 283883 were 192.7 and 96.0 ppm in contact and vapor treatments, respectively.

4.6 Comparison of IC50 of essential oils against *Aspergillus* strains

The IC50 values of different essential oils against *Aspergillus* strains were calculated according to the linear relation between inhibition and logarithm of concentration. The results are presented in Tables 48-50.

4.6.1 Comparison of IC50 of essential oils on mycelial growth of *Aspergillus* strains by contact and vapor treatments

The results in Table 48 showed that IC50 of mycelial growth inhibition in contact system was in the range of 237- >1,000 ppm, while in the vapor system was in the range of 107->1,000 ppm, respectively with the coefficient of determination (R^2) between 0.9000 to 0.9980.

In contact treatment, *Cinnamomum zeylanicum* Blume *and Cymbopogon citratus* (DC.) Stapf. essential oil showed IC50 < 250 ppm against mycelial growth of *A. flavus* IMI 242684. In vapor treatment, mycelial growth inhibition was significantly effective ($p < 0.05$) more than contact treatment about 1.2-2.0 fold, except for *Cymbopogon citratus* (DC.) Stapf. and *Citrus hystrix* essential oil against *A. flavus* IMI 242684 and *Foeniculum vulgare* Mill. in both *Aspergillus* strains.

Among 12 essential oils, *Cinnamomum* zeylanicum Blume and *Kaempferia galanga* L. essential oils showed the significant ($p < 0.05$) lowest IC50 against mycelial growth of *Aspergillus* strains in the range of 107.7-119.8 ppm and 111.8- 120.8 ppm, respectively, in vapor treatment when compared to other essential oils.

Table 48. IC50 against mycelial growth *of Aspergillus* strains

Essential oil	IC50 (ppm)			
	Aspergillus flavus IMI 242684		*Aspergillus parasiticus* IMI 283883	
	Contact	Vapor	Contact	Vapor
Cuminum cyminum	923.5 C	656.1 s	779.1 z	359.2 j
Foeniculum vulgare	>1000 D	>1000 D	>1000 D	>1000 D
Trachyspermum amni	821.3 B	635.3 r	801.4 A	340.6 i
Brassica juncea L.	684.0 t	253.9 e	555.2 n	390.8 l
Cinnamomum porrectum	293.8 g	251.1 e	292.6 g	222.3 d
Cinnamomum zeylanicum	237.9 d	107.7 a	382.3 k	119.8 c
Michelia alba	710.2 v	282.3 f	748.5 y	280.4 f
Myristica fragrans	717.3 w	452.4 m	715.3 w	618.8 q
Cymbopogon citratus	244.0 e	251.6 e	298.1 h	247.1 d
Citrus hystrix	>1000 D	>1000 D	>1000 D	745.0 x
Limnophila geoffrayi	700.8 u	573.1 p	562.7 o	390.3 l
Kaempferia galanga	251.2 e	120.8 c	289.7 g	111.8 b

[a] Values with different letters are statistically significantly different ($p < 0.05$).

The statistical analysis for IC50 against sporulation of *Aspergillus* strain (Appendix B, Table 27) showed that the single factor ($p < 0.05$) affecting sporulation and all two-, and three- factor interactions were significant ($p<0.05$). In contact treatment, *Cinnamomum zeylanicum* Blume, *Cymbopogon citratus* (DC.) Stapf., and *Kaempferia galangal* L. essential oils were significant effective at lower IC50 values on *A. flavus* IMI 242684 sporulation inhibitionthan other essential oils, followed by *Brassica juncea* L., *Cinnamomum porrectum* (Roxb.) Kosterm, and *Limnophila geoffrayi* Bonati. essential oil while *Brassica juncea* L., *Cinnamomum zeylanicum* Blume, and *Trachyspermum amni* (L.) Sprague. essential oil was significant effective at lower IC50 values on *A. .parasiticus* IMI 283883, followed by *Kaempferia galangal* L. and *Cymbopogon citratus* (DC.) Stapf. essential oil, respectively (Table 49). When compared between contact and vapor treatments, It indicated that vapor treatment was significantly effective ($p <0.05$) at lower IC50 values against sporulation of *Aspergillus* strains except for *Cuminum cyminum* L.. In vapor treatment, *Kaempferia galangal* L. and *Cinnamomum zeylanicum* Blume. essential oils showed significant effect at lower IC50 values on *A. flavus* IMI 242684, followed by *Cymbopogon citratus* (DC.) Stapf. and *Myristica fragrans* Houtt. while *Brassica juncea* L. essential oil was significantly effective at lower ($p<0.05$) IC50, followed by *Cinnamomum porectum* (Roxb.) Kosterm, *Kaempferia galangal* L. and *Cinnamomum zeylanium* Blume essential oil, respectively, in *A. parasiticus* IMI 283883.

Table 49. IC50 against sporulation *of Aspergillus* strains

Essential oil	IC50 (ppm)			
	Aspergillus flavus IMI 242684		*Aspergillus parasiticus* IMI 283883	
	Contact	Vapor	Contact	Vapor
Cuminum cyminum L.	493.1 v	331.3 s	231.2 l	287.8 pq
Foeniculum vulgare Mill.	891.2 F	650.3 z	762.8 D	558.4 w
Trachyspermum amni (L.) Sprague	292.8 r	279.3 o	208.4 hi	134.7 c
Brassica juncea L.	280.1 o	232.1 l	206.4 h	121.3 a
Cinnamomum porrectum (Roxb.) Kosterm	284.7 p	170.0 f	236.2 m	128.4 b
Cinnamomum zeylanicum Blume.	207.8 hi	148.9 d	208.0 hi	136.5 c
Michelia alba DC.	462.1 u	261.6 n	457.8 t	289.4 qr
Myristica fragrans Houtt.	688.4 C	168.7 f	683.5 B	189.3 g
Cymbopogon citratus (DC.) Stapf.	188.0 h	161.8 e	211.3 i	150.4 d
Citrus hystrix	800.9 E	642.8 y	679.0 A	565.7 x
Limnophila geoffrayi Bonati	289.5 qr	216.7 j	330.6 s	225.2 k
Kaempferia galanga L.	207.4 hi	147.9 d	210.4 i	135.3 c

[a] Values with different letters are statistically significantly different ($p < 0.05$).

Statistical analyses indicated that single, two, three factors, as well as their interactions, also had a significant ($p < 0.05$) inhibitory effect on IC50 of aflatoxin B1 production against both *Aspergillus* strains in both treatments. The results in Table 50. showed that IC50 of aflatoxin B1 production inhibition in contact system was in the range of 153.1- 782.4 ppm, while the inhibition in the vapor system was in the range of 96.0-654.6 ppm, respectively with the coefficient of determination (R^2) between 0.9100 to 0.9950. In vapor treatment, IC50 of aflatoxin B1 production inhibition was significantly less than contact treatment, except for *Cuminum cyminum* L. essential oil against *Aspergillus flavus* IMI 242684. In contact treatment, *Cinnamomum zeylanicum* Blume essential oil was more significantly by effective at lower IC50 values on aflatoxin B1 than other essential oils, followed by *Kaempferia galangal* L. and *Cinnamomum porectum* (Roxb.) Kosterm essential oil against A. *flavus* IMI 242684 while *Cymbopogon citratus* (DC.) Stapf. essential oil was significantly effective at lower IC50 values followed by *Cinnamomum zeylanicum* Blume, *Cinnamomum porectum* (Roxb.) Kosterm., and *Kaempferia galangal* L. essential oil, respectively, against *A. parasiticus* IMI 283883. In vapor treatment, *Cinnamomum zeylanicum* Blume. was more significantly effective at lower IC50 values on aflatoxin B1 than other essential oils, followed by *Kaempferia galangal* and *Cinnamomum porectum* (Roxb.) Kosterm. essential oil, respectively, against *A. flavus* IMI 242684 while *Kaempferia galangal* L. essential oil was significant at lower IC50 values, followed *Cinnamomum zeylanicum* Blume, *Cymbopogon citratus* (DC.) Stapf., and *Cinnamomum porectum* (Roxb.) Kosterm essential oil, respectively, against *A. parasiticus* IMI 283883.

Table 50. IC50 against AFB1 production *of Aspergillus* strains

Essential oil	IC50 (ppm)			
	Aspergillus flavus		*Aspergillus parasiticus*	
	Contact	Vapor	Contact	Vapor
Cuminum cyminum L.	273.1 s	282.0 t	286.0 v	287.4 u
Foeniculum vulgare Mill.	517.8 C	333.5 x	496.0 u	292.6 v
Trachyspermum amni (L.) Sprague	294.5 v	250.4 o	324.1 w	269.9 r
Brassica juncea L.	235.9 m	203.7 j	270.6 s	208.4 j
Cinnamomum porrectum (Roxb.) Kosterm	200.8 j	148.2 e	182.5 h	145.5 e
Cinnamomum zeylanicum Blume.	153.1 f	97.8 a	164.0 g	102.8 b
Michelia alba DC.	628.9 E	361.2 y	782.4 H	654.6 F
Myristica fragrans Houtt.	504.5 B	222.9 k	689.7 G	246.6 n
Cymbopogon citratus (DC.) Stapf.	260.8 q	230.3 l	155.0 f	139.8 d
Citrus hystrix	518.3 C	380.5 z	582.6 D	273.1 s
Limnophila geoffrayi Bonati	266.8 r	256.1 p	325.2 w	258.2 qp
Kaempferia galanga L.	164.4 g	109.3 c	192.7 i	96.0 a

[a] Values with different letters are statistically significantly different ($p < 0.05$).

CHAPTER 5
DISCUSSION

The efficacy of 56 plant essential oils was screened for their antifungal activity against aflatoxigenic strains, *A. flavus* IMI 242684 and *A. parasiticus* IMI 283883 using the disc diffusion assay which is a standard method widely used for the rapid screening of natural products for antifungal activity. Nineteen essential oils were found to have very strong activity (≥30 mm zone of inhibition) with MIC values in the range between >2.5 – 0.32 mg/ml and MFC values in the range of 2.5 – 1.25 mg/ml. Twelve essential oils regarding MIC less than 0.5 mg/ml were selected and tested for the inhibitory effect on mycelial growth, sporulation, and AFB1 production and compared between contact and vapor treatments.

For *Cuminum cyminum* L. essential oil, the results showed the extracted yield of 1.20% and according to the GC-MS analysis the main component was 2-methyl-3-phenyl-propanol (78.87%). Li and Jiang (2004) found that cumin seed contained cuminal (36-31%), cominic alcohol (16.92%) and γ-terpinene (11.14%) as major components. *Cuminum cyminum* essential oil from Iran consisted of thymol (40.65%) and γ-terpinene (24.51%) as the main components (Moghadom *et al.*, 2015). Gachkar *et al.* (2007) reported that pinene (29.1%) and limonene (21.5%) were the main components while Choudhary *et al.* (2014) showed that transdihydrocarvone (31.11%), γ-terpinene (23.22%), β-cymeme (15.8%), and α-pheilandrene (12.01%) were the main components.

The result of *Cuminum cyminum* L. (cumin) essential oil in this study showed that IC50 of sporulation and aflatoxin B1 production were less than IC50 of mycelial growth. In vapor treatment IC50 of growth and sporulation of both *Aspergillus* strains was less than in contact treatment while IC50 of aflatoxin B1 production in contact treatment was lower than in vapor treatment. At 1000 ppm, cumin essential oil completely inhibited aflatoxin B1 production in both treatments while mycelial growth was inhibited at more than 50%. Sporulation of *A. parasiticus* IMI 283883 was completely inhibited whereas sporulation of *A. flavus* IMI 242684 was inhibited at more than 75%. Kedia *et al.* (2014) showed that cumin essential oil completely inhibited mycelial growth and aflatoxin B1 production of *A. flavus* strain LHP(C)-D6 at concentrations of 0.8 and 0.6 µl/ml (800 and 600 ppm), in poisoned food method, respectively. Fakoor *et al.* (2013) reported that *Cuminum cyminum* L. at lower concentration of 400 ppm increased aflatoxin production to over four-fold when comparison with control while high concentraion of essential oil reduced aflatoxin production.

In this study the main components of *Foeniculum vulgare* Mill. essential oil were anethol (53.13%) and estragole (24.97%). Anethol (65.59%) and estragole (13.11%) of essential oil from Egyptian fennel were reported by Viuda-Martos *et al.* (2007). Hammouda *et al.* (2013) also reported that anethol (65%) is the main component and Zeng *et al.* (2015) reported 63.30% of anethol and 11.11% of pinene. The IC50 of sporulation and aflatoxin B1 production inhibition by *Foeniculum vulgare* Mill. essential oil was less than IC50 of mycelial growth and IC50 of both *Aspergillus* strains in vapor treatment was less than in contact treatment. At 1000 ppm, essential oil completely inhibited aflatoxin B1 production in both treatments while mycelial growth was inhibited by less than 32%. Sporulation of both *Aspergillus* strains was inhibited by more than 55%. Rody *et al.* (2013) showed that MIC at 10 µg/l (1000 ppm) of *Foeniculum vulgare* against *A. flavus* in broth dilution method and essential oil was more effective by the method of vapor than the direct contact method (Inouye *et al.*, 2006). Singh *et al.* (2006) reported that the trans-anethol is the component responsible for the antifungal activity.

For *Trachyspermum amni* (L.) Sprague. essential oil, the results of this study showed that the yield was 3.57% and thymol (92.77%), γ-terpinene (4.90%) were the major components while Paul *et al.* (2011) reported the yield of 2.36% of *Trachyspermum ammmi* essential oil by hydrodistillation and thymol (49.64%) and β-cymene (16.33%) were the main compounds. Gemeda *et al.* (2014) reported thymol (51.5%) as the main compound. Thymol (63.4%), p-cymene (19%) and γ-terpinene (16.9%) were found as the major components of *Trachyspermum ammi* essential oil and MIC values of 2000 and 3000 ppm against *A. flavus* and *A. parasiticus*, respectively (Gandomi *et al.*, 2013). Kedia *et al* (2015) showed that at 0.8 µl/ml (800 ppm) of *Trachyspermum ammi* essential oil completely inhibited aflatoxin production by poisoned food method.

In this study IC50 of sporulation and aflatoxin B1 production by *Trachyspermum amni* (L.) Sprague essential oil was less than IC50 of mycelial growth in both treatments. IC50 of mycelial growth, sporulation and aflatoxin B1 production in vapor treatment was lower than in contact treatment of both *Aspergillus* strains. At 1000 ppm, essential oil completely inhibited sporulation and aflatoxin B1 production in both treatments while less than 70% of mycelial growth was inhibited. Thymol has been reported to have antifungal activity (Nagalakshmi *et al.,* 2000). Thymol has strong antimicrobial activity by perturbation of the lipid fraction of the microbial plasma membrane, resulting in alterations of the membrane permeability and leakage of intracellular materials (Trombetta *et al.,* 2005).

For *Brassica juncea* L., the results of this study showed that the yield of *Brassica juncea* L. was 0.70% and the main components was 2-phenyl ethyl isothiocyanate (65.59%) while Khan *et al.* (2016) reported 0.4% yield of *Brassica juncea* L. essential oil. The yield in the range of 0.4-0.7% (v/w) which was separated by steam-distilled method was reported (Yu *et al.,* 2003). Allyl isothiocyanate was reported as the main compound of the mustard essential oil from Hebei (54.8%), Shaanxi (68.8%) and Shandong Province (61.8%) (Yu *et al.,* 2003).

The essential oil of *Brassica juncea* L. can be used to suppress various microorganism. Kim and Lee (2009) showed the antibacterial activity of 2-phenylethyl isothiocyanate by disc agar diffusion. Jan *et al.* (2010) showed that isothiocyanate and their derivatives affected Gram-positive and Gram negative bacteria. Defour *et al.* (2015) showed the inhibitory activity of 2-phenylethyl isothiocyanate against *Aspergillus* species, *Colletrotrichum* and *Gibberella* spp. Benzyl and 2-phenyl ethyl isothiocyanate indicated higher activity against most of the pathogenic bacteria than 3-butenyl and 4-pentenyl isothiocyanate (aliphatic) by paper disc agar method.

In this study, IC50 of sporulation and aflatoxin B1 production was less than IC50 of mycelial growth. IC50 of mycelial growth, sporulation and aflatoxin B1 production of both *Aspergillus* strains in vapor treatment was lower than in contact treatment. At 1000 ppm, *Brassica juncea* L. essential oil completely inhibited sporulation and aflatoxin B1 production in both system but mycelial growth was inhibited by less than 76%. The antimicrobial mode of action of isothiocyanates has been attributed to the binding of sulphydryl groups on specific enzyme to microbial growth and survival (Wilson *et al.*, 2013; Aires *et al.*, 2009) and also lead to random modification of proteins, which can disturb biochemical synthesis (Dufour *et al.,* 2015).

For *Cinnamomum porrectum* (Roxb.) Kosterm essential oil, there are a few reports on the chemical components of *Cinnamomum porrectum* (Roxb.) Kosterm essential oil. In this study, the yield of *Cinnamomum porrectum* (Roxb.) Kosterm essential oil was 3.25-3.55%, which was similar to the yield of 4.0% reported by Buru *et al.* (2014). Safrole (93.9%) was the main composition followed by elemicin (4.3%) and methyl eugenol (1.7%). Very few data are available regarding the antimicrobial activity of the essential oil of *Cinnamomum porrectum* (Roxb.) Kosterm essential oil. In particular, there are no data of the activity on mycelial growth, sporulation, and aflatoxin B1 production. Essential oil of *Cinnamomum porrectum* (Roxb.) Kosterm had a high antimicrobial activity against *Candida allbicans* with a MIC of 0.063% (Palanuvej *et al.,* 2006) and showed significant antibacterial activity against both Gram positive and Gram negative bacteria including methicillin-resistant *Staphylococcus aureus* (Buru *et al.,* 2014).

In this study, *Cinnamomum porrectum* (Roxb.) Kosterm essential oil showed that IC50 values of sporulation and aflatoxin B1 production were lower than IC50 of mycelial growth and IC50 of mycelial growth, sporulation and aflatoxin B1 production of both *Aspergillus* strains in vapor treatment was less than in contact treatment. At 600 and 1000 ppm, essential oil completely inhibited sporulation and aflatoxin B1 production in both treatments and at 1000 ppm complete inhibition was found on mycelial growth in both treatments.

Safrole, which was phenylpropene, has been proved to show antifungal activities (Kubo *et al.,* 1993). Elemicin and methyl eugenol, compounds found in relatively low concentrations, are also known to have effective antimicrobial properties (Kubo *et al.,* 1993; Doughari, 2006). Methyl eugenol was also reported to inhibit *A. flavus* and aflatoxin production on peanut pods and kernels (Sudhakar *et al.,* 2009).

For *Cinnamomum zeylanicum* Blume essential oil, the yield of 1.57% was found and the highest percent of cinnamaldehyde (96.56%) was observed in this study. Pawar and Thake (2006) reported cinnamaldehyde (64.13%) and linalool (10.25%) in *Cinnamomum zeylanicum* Blume. essential oil. Cinnamaldehyde with approximate 70% was reported by Unlu *et al*. (2010) while Duarte *et al*. (2015) reported cinnamaldehyde (88.39%) in *Cinnamomum zeylanicum* Blume. essential oil.

Previous research has revealed interesting antimicrobial effect in *Cinnamomum zeylanicum* (Mishra *et al.*, 2000). Bullerman (1974) reported that aflatoxin was inhibited by cinnamon without fungal growth. Patkar *et al*. (1993) studied the effect of cinnamon oil on the growth of *A. flavus* and its aflatoxin B1 production which was inhibited by 0.5 µl/ml (500 ppm) of cinnamon in the medium. The MFC of *Cinnamomum zeylanicum* essential oil was 1000 ppm, while at ≥ 400 ppm aflatoxin production by *A. flavus* was completely inhibited (Jayaratne *et al.,* 2002).

In this study, *Cinnamomum zeylanicum* Blume essential oil showed that IC50 values of sporulation and aflatoxin B1 production were lower than IC50 of mycelial growth in both treatments. IC50 values of mycelail growth, sporulation and aflatoxin B1 production of both *Aspergillus* strains in vapor treatment were lower than in contact treatment. At 600 and 1000 ppm, essential oil completely inhibited mycelial growth, sporulation and AFB1 production in both treatments except at 600 ppm against growth of both *Aspergillus* strains in contact treatment.

For *Michelia alba* DC. essential oil, the major component has also been also identified as linalool (59.12%) (Sanimah *et al.*, 2008) which is similar to this study (50.14% linalool). Euyama *et al*. (1992) and Shang *et al*. (2002) also reported that linalool was the major compound.

In this study, *Michelia alba* DC. essential oil showed that IC50 of sporulation was lower than IC50 of mycelial growth and aflatoxin production and IC50 of mycelial growth, sporulation and aflatoxin B1 production of both *Aspergillus* strains in vapor treatment was also lower than in contact treatment. In vapor treatment, IC50 of mycelial growth showed remarkably lower than in contact treatment by 2.5 fold.

Very few data are available regarding the antimicrobial activity of *Michelia alba* DC. (Luangnarumitchai *et al.*, 2007), in particular, there are no data regarding its effects on growth, sporulation, and AFB1 production of *Aspergillus* strain. Although the antimicrobial activity of essential oils is mainly attributed to their major compounds, linalool (Herman *et al.*, 2016; Hsu *et al.*, 2013), caryophyllene oxide (Guillen *et al.*, 1996), β-selinene (Kramer and Abraham, 2012), cinnamaldehyde (Sun *et al.,* 2015), and β-elemene (Zhu *et al.*, 2013), have been proved to show antimicrobial activities.

For *Myristica fragrans* Houtt. essential oil, hydrodistillation of *Myristica fragrans* Houtt. essential oil achieved a yield of 2.12–2.22% in this study, which was in contrast with the yield of 6.85% reported by Muchtaridi *et al.* (2010). The chemical composition of *Myristica fragrans* essential oil in this study contained 7 components and 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 that the major compounds were sabinene (21.38%), 4-terpineol (13.92%), and myristicin (13.57%). Pal *et al.* (2011) showed that sabinene (41.7%) and β-pinene (7.3%) were the main component of nutmeg from Addaman, Island.

Very few data are available regarding the antimicrobial activity of *Myristica fragrans* Houtt. essential oil, in particular, there are no data regarding its effects on sporulation and AFB1 production. Valente *et al.* (2014) reported that treatment with 0.1% of *Myristica fragrans* essential oil, the growth of *A. flavus* and *Aspergillus ochraceus* was inhibited by 43% and 65%, respectively. At a concentration of 0.3%, the growth of *A. flavus* and *A. ochraceus* was inhibited by 84% and 79%, respectively.

In this study IC50 of sporulation and aflatoxin B1 production of both *Aspergillus* strains was lower than IC50 of mycelial growth and IC50 in vapor treatment was also lower than in contact treatment. At 1000 ppm, sporulation and aflatoxin B1 production was completely inhibited in both treatments and at 600 ppm the inhibition of sporulation and aflatoxin B1 production in vapor treatment was markedly increased more than two-fold when compared with contact treatment.

The antifungal effect of *Myristica fragrans* Houtt. (nutmeg) essential oil is related to its main components such as 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). 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).

For *Cymbopogon citratus* (DC.) Stapf. essential oil, the major chemical constituents of *C. citratus* containing geranial (28.93%) and neral (18.49%) were reported (Wannissom *et al.*, 2010). Pawar and Thaker (2006) also reported geranial (29.4%) and neral (21.39%) and Ewansiha *et al.* (2012) reported geranial (48.1%) and neral (34.6%) as the main components. A natural mixture of geranial and neral, which are two isomeric acyclic monoterpene aldehyde, is referred to as citral. Geranial and neral are actually trans and cis- citral, respectively. Limongrass oil contains citral at concentrations of approximately 65-85% (Wilson *et al.*, 2002) and De Billerbeck *et al.* (2001) reported that citral accounted for more than 70% exhibited the antifungal activity. The results from this study showed that the yield of *Cymbopogon citratus* L. essential oil was 4.50% and the main components were geranial (37.29%) and neral (24.63%). Matasyoh *et al.* (2011) showed that geranial (39.53%) and neral (33.31%) exhibited antifungal activities against mycotoxigenic species (*A. flavus, A. parasiticus, A. ochraceus, A. niger* and *A. fumigatus*).

In this study IC50 values of mycelial growth, sporulation and aflatoxin B1 production of both *Aspergillus* strains in vapor treatment were lower than in contact treatment of both *Aspergillus* strains except IC50 of mycelial growth against *A. flavus*. At 1000 ppm, complete inhibition of mycelial growth, sporulation and aflatoxin B1 in both treatments was found and at 600 ppm the inhibition was at more than 70%. Koffi *et al.* (2010) showed that MIC of the *Cymbopogon citratus* L. essential oil against the toxigenic strain, *A. flavus* was 750 ppm while at 500 ppm aflatoxin B1 production was completely inhibited. The antifungal activity of *Citrus citratus* L. essential could be due to the presence of several components known to have biological activities. These major components of the essential oil, geranial, neral, and geraniol have been reported to have high antifungal activity (Lee *et al.*, 2008; Onawunmi, 1989). Paranagama *et al.* (2003) reported that the sporulation of *A. flavus* was completely inhibited by *Cymbopogon citratus* (2800 ppm) when used as fumigant whereas aflatoxin production was inhibited when treated with *Cymbopogon citratus* essential oil at 100 ppm.

For *Citrus hystrix* essential oil, the results of this study showed that the yield of *Citrus hystrix* essential oil was 1.82% and citronellal (84.22%) was the main component. Wongsariya *et al.* (2014) reported that citronellal (78.11%) was the main component. Essential oil of *Citrus hystrix* exhibited fungicidal activity against *A. flavus*, *A funidatus*, *A. parasiticus*, and *Sacharomyces cereviae* (Chanthaphon *et al.*, 2008; Rammanee and Hongpattarakere, 2011; Thanaboripat *et al.*, 2006).

In this study IC50 of *Citrus hystrix* essential oil on aflatoxin B1 production of both *Aspergillus* strains was lower than IC50 of sporulation and mycelial growth in both treatments and IC50 of mycelial growth, sporulation and aflatoxin B1 production in vapor treatment was lower than in contact treatment. Rammanee and Hongpattarakere (2011) reported that the plasma membranes of *A. flavus* hyphae treated with acid lime essential oil became rough and irregular with continuous folding into the cytoplasm and detached from the fibrillar layer.

For *Limnophila geoffrayi* Bonati essential oil, the extracted yield was 0.63% and pulegone (22.50%) and isolimonene (19.06%) were the main compounds, while Thongdon-A and Inprakhon (2009) reported a yield of 0.72% and the main components in *Limnophila geoffrayi* Bonati essential oil were pulegone (22.14%), perillaldehyde (19.13%) and limonene (9.00%). The main compounds also have antimicrobial activities (Thongdon-A and Inprakhon, 2009). In this study IC50 of sporulation and aflatoxin B1 production was lower than IC50 of mycelial growth. At 1000 ppm, sporulation and aflatoxin B1 in both treatments completely inhibited. At 600 ppm of *Limnophila geoffrayi* Bonati essential oil, sporulation was completely inhibited while mycelial growth and aflatoxin B1 production were inhibited at more than 40% and 80%, respectively.

For *Kaempferia galangal* L. essential oil, no documented reports on the antifungal activity against the aflatoxigenic strains are available. However, there were quite a few reports on the chemical components of *Kaempferia galanga* L. essential oil. In this study, the yield of *Kaempferia galanga* L. essential oil was 1.80-1.90% and ethyl cinnamate (65.28%) was the main component while Tewtrakul *et al.* (2005) reported the yield of 1.11% and ethyl-p-methoxy cinnamate (31.77%) and methyl cinnamate (23.23%) were the main compositions. Kumer *et al.* (2014) reported that a yield of 3.01% of *Kaempferia galanga* L. essential oil from steam distillation and ethylcinnamate (29.48%) and ethyl-methoxy cinnamate (18.42%) were the main components. Li *et al.* (2017) showed that ethyl-methoxy cinnamate (34.79%) and ethyl cinnamate (20.72%) were the main components. Very few data are available regarding the antimicrobial activity of this essential oil, in particular, there is no data of the activity on mycelial growth, sporulation, and aflatoxin B1 production. Essential oil of *Kaempferia galanga* L. exhibited antimicrobial activity against *Candida allbicans* and *Mycobacterium tuberculosis* (Kanjanapothi *et al*, 2004), antibacterial (Arambewela *et al.*, 1999) and antifungal activities (Bin Jantan *et al.,* 2003). In this study IC50 of aflatoxin B1 was lower than IC50 of mycelial growth and sporulation. IC50 of mycelial growth, sporulation and aflatoxin B1 production of both *Aspergillus* strains in vapor treatment was lower than in contact treatment. At 600 and 1000 ppm, mycelial growth, sporulation and aflatoxin B1 production of both *Aspergillus* strains was completely inhibited.

The differences in the chemical composition of essential oils in some literature depend on several parameters including genetic variability, geographical location, environmental and agronomic conditions, and the extraction method (Runyora *et al.,* 2010; Shukla *et al.*, 2009). Comparison of the data obtained in this study with previously published results is problematic. First, the composition of plant oils and extraction methods are known to vary according to local climate and environmental conditions. Furthermore, some essential oils within the same species may be different in subspecies. Secondly, the detail of method used to assess antimicrobial activity as well as the difference of microbial test, are varied between publications.

Finally, when contact and vapor treatments of essential oil were compared, the results indicate that most of essential oils in the vapor treatment showed higher inhibition of mycelial growth, sporulation, and aflatoxin B1 production than the contact treatment and can also be achieved with a smaller amount of essential oil. These could be attributed to the variation in the relative composition of the essential oil and vapors as the latter was not analysed in that study, which can be related to many other factors, such as essential oil volatility, water solubility and general chemical complexity for the higher antifungal activity of the volatiles. Several researchers also reported that a greater antifungal activity of essential oil achieved in vapor phase than in aqueous solution or agar contact (Bluma *et al.*, 2009; Inouye *et al.*, 2003; Laghchimi *et al.*, 2014). Due to the hydrophobic nature of compounds that constitute the volatile of essential oil, it could be expected that they may act mainly by accumulation on mycelium rather than the agar and inhibited three stages on germination of a conidium.

CHAPTER 6

CONCLUSION AND SUGGESTION

6.1 Conclusion

Plant essential oils have shown great potentials for control of aflatoxingenic strains. Plants essential oils at concentration of 300 ppm were screened for MIC of mycelial growth, sporulation, and aflatoxin production of *Aspergillus flavus* IMI 242684 and *A parasiticus* IMI 283883. Essential oils with MIC less than 0.5 mg/ml were selected for further study. IC50 values of mycelial growth, sporulation, and aflatoxin production against both *Aspergillus* strains in contact treatment were in the range of 237.9->1000 ppm, 191.7-891.2 ppm, and 153.1-782.4 ppm, respectively, while in vapor treatment were in the range of 111.8- >1000 ppm, 121.3- 642.8 ppm, and 97.8- 654.6 ppm, respectively. Moreover, most essential oils in vapor treatment showed better antifungal activity than in contact treatment. Overall, *Cinnamomum porrecrum* (Roxb.) Kosterm, *Cinnamomum zeylanicum* Blume, *Myristica fragrans* Houtt., and *Kaempferia galangal* L. also showed remarkably high inhibition against both *Aspergillus* strains in both treatments with IC50 values range from 107.7-383.3 ppm against mycelial growth, 128.4-284.7 ppm against sporulation, and 96.0-200.0 ppm against aflatoxin B1 production.

In conclusion, it could be suggested that 4 essential oils from *Cinnamomum porrecrum* (Roxv.) Kosterm, *Cinnamomum zeylanicum* Blume, *Myristica fragrans* Houtt., and *Kaempferia galangal* L. could be used as an alternative agent to control antiaflatoxigenic strains contaminated on food and agricultural commodities and may become useful tools for further application on food and agricultural commodities.

6.2 Suggestion

In order to further investigation, several factors can be examined as follows:

-Cytotoxicity test of the essential oils should be done to confirm their safety when applied at IC50.

-Application of essential oils on cereals or other agriculture products should be tested *in vitro* and *in vivo.*

-The field experiments should be conducted if needed

-Other methods of essential oil extraction should be investigated in order to increase yields and more bioactive components.

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APPENDICE

APPENDIX A: Chemical composition of essential oil

Table 1. Chemical composition of *Cuminum cyminum* essential oil

No	Compound	RT(min)	Area %	Formula	MW
1	Benzene, 1-methoxy-4-(2-propenyl)-	7.29	1.20	$C_{10}H_{12}O$	148
2	Phenol, 4-(1-methylethyl)-	7.93	1.19	$C_9H_{12}O$	136
3	2-Methyl-3-phenyl-propanol	8.71	78.87	$C_{10}H_{12}O$	148
4	Phenyl-1-propanol	10.15	2.19	$C_9H_{12}O$	136
5	Thiophene,3,4-dimethyl-	11.22	3.25	C_6H_8S	112
6	Cumic acid	14.94	11.59	$C_{10}H_{12}O_2$	164
7	Dehydrocostuslactone	33.74	1.70	$C_{15}H_{18}O_2$	230

Table 2. Chemical composition of *Foeniculum vulgare* essential oil

No	Compound	RT(min)	Area %	Formula	MW
1	Fenchone	4.80	5.81	$C_{10}H_{16}O$	152
2	Tarragon	7.30	24.97	$C_{10}H_{12}O$	148
3	Carvone	8.64	1.46	$C_{10}H_{14}O$	150
4	Benzaldehyde,4-methoxy-	9.00	11.66	$C_8H_8O_2$	136
5	Anethol	10.02	53.13	$C_{10}H_{12}O$	148
6	2-Propanone,1-(4-methoxyphen yl)-	13.26	0.82	$C_{10}H_{12}O_2$	164
7	Dillapiole	21.95	2.15	$C_{12}H_{14}O_4$	222

Table 3. Chemical composition of *Trachyspermum amni* essential oil

No	Compound	RT(min)	Area %	Formula	MW
1	γ-Terpinene	4.18	4.90	$C_{10}H_{16}$	136
2	Thymol	10.10	92.77	$C_{10}H_{14}O$	150
3	Carvacrol	10.30	1.16	$C_{10}H_{14}O$	150
4	Ethyl p-methoxycinnamate	26.85	1.17	$C_{12}H_{14}O_3$	206

Table 4. Chemical composition of *Brassica juncea* L. essential oil

No	Compound	RT(min)	Area %	Formula	MW
1	Linalool	4.74	1.71	$C_{10}H_{18}O$	154
2	Cyclohexanone,2-(2-nitro-2-propenyl)-	6.89	5.29	$C_9H_{13}NO_3$	183
3	2-Cyclopentene-1-thione,2,3-dimethyl-	10.96	1.67	$C_7H_{10}S$	126
4	Benzene, (2-isothiocyanatoethyl)-	15.97	65.59	C_9H_9NS	163
5	Phenol,2,4-bis(1,1-dimethylethyl)-	17.54	8.81	$C_{14}H_{22}O$	206
6	Methanone,diphenyl-	22.03	7.46	$C_{13}H_{10}O$	182
7	2-Cyclohexen-1-ol,2-methyl-5-(1-methylethenyl)-	23.41	6.27	$C_{10}H_{16}O$	152

Table 5. Chemical composition of *Cinnamomum porrectum* (Roxb.) *Kosterm* essential oil

No	Compound	RT(min)	Area %	Formula	MW
1	Safrole	10.26	93.92	$C_{10}H_{10}O_2$	162
2	Methyleugenol	13.89	1.79	$C_{11}H_{14}O_2$	178
3	Elemicine	19.36	4.30	$C_{12}H_{16}O_3$	220

Table 6. Chemical composition of *Cinnamomum zeylanicum* essential oil

No	Compound	RT(min)	Area %	Formula	MW
1	Cinnamaldehyde	9.76	96.56	C_9H_8O	132
2	Bornyl acetate	9.95	1.35	$C_{12}H_{20}O_2$	196
3	Cinnamyl acetate	15.57	2.09	$C_{11}H_{12}O_2$	176

Table 7. Chemical composition of *Michelia alba* DC.essential oil

No	Compound	RT(min)	Area %	Formula	MW
1	Linalool oxide	4.39	5.69	$C_{10}H_{18}O_2$	170
2	Linaloloxide	4.67	5.36	$C_{10}H_{18}O_2$	170
3	Linalool L	4.87	50.14	$C_{10}H_{18}O$	154
4	Cinnamaldehyde	9.57	12.52	C_9H_8O	132
5	Beta elemene	13.54	7.17	$C_{15}H_{24}$	204
6	Methyleugenol	13.88	4.75	$C_{11}H_{14}O_2$	178
7	Varatraldehyde	16.80	0.58	$C_9H_{10}O_3$	166
8	β-Selinene	17.20	1.91	$C_{15}H_{24}$	204
9	Caryophyllene oxide	20.61	4.34	$C_{15}H_{24}O$	220
10	Humulene Oxide	21.63	1.16	$C_{15}H_{24}O$	220
11	Alpha Cadinol	22.91	1.31	$C_{15}H_{26}O$	222
12	Beta selinene	23.40	1.42	$C_{15}H_{24}$	204

Table 8. Chemical composition of *Myristica fragrans* essential oil

No	Compound	RT(min)	Area %	Formula	MW
1	Trans-Sabinene hydrate	4.31	7.70	$C_{10}H_{18}O$	154
2	Cis-Sabinene hydrate	4.89	5.88	$C_{10}H_{18}O$	154
3	4-Terpineol	6.71	23.81	$C_{10}H_{18}O$	154
4	Alpha Terpineol	7.09	2.59	$C_{10}H_{18}O$	154
5	Safrole	9.92	42.50	$C_{10}H_{10}O_2$	162
6	Methyleugenol	13.68	11.14	$C_{11}H_{14}O_2$	178
7	Elemicin	19.12	6.34	$C_{12}H_{16}O_3$	208

Table 9. Chemical composition of *Cymbopogon citratus* essential oil

No	Compound	RT(min)	Area %	Formula	MW
1	Linalool L	4.73	0.85	$C_{10}H_{18}O$	154
2	Citronella	5.88	0.19	$C_{10}H_{18}O$	154
3	3-Methyl-1,4-heptadiene	6.56	0.50	C_8H_{14}	110
4	Beta-Citronellol	7.82	1.20	$C_{10}H_{20}O$	156
5	Neral	8.26	24.63	$C_{10}H_{16}O$	152
6	Geraniol	8.55	4.74	$C_{10}H_{18}O$	154
7	Citral	9.18	37.29	$C_{10}H_{16}O$	152
8	Geranic acid	11.78	0.57	$C_{10}H_{16}O_2$	168
9	Geranyl acetate	12.86	0.81	$C_{12}H_{20}O_2$	196
10	Beta-elemene	13.28	0.30	$C_{15}H_{24}$	204
11	Caryophyllene	14.39	1.76	$C_{15}H_{24}$	204
12	Alpha-Bergamotene	14.85	0.98	$C_{15}H_{24}$	204
13	Alpha-Humulene	15.69	0.28	$C_{15}H_{24}$	204
14	Valencene	17.18	0.27	$C_{15}H_{24}$	204
15	Beta-Humulene	17.39	0.19	$C_{15}H_{24}$	204
16	Beta-bisabolene	17.64	0.37	$C_{15}H_{24}$	204
17	Alpha-Amorphen	17.81	0.97	$C_{15}H_{24}$	204
18	Delta-Cadinene	17.99	1.25	$C_{15}H_{24}$	204
19	Caryophyllene oxide	20.33	1.06	$C_{15}H_{24}O$	220
20	Selina-6-en-4-ol	21.86	11.77	$C_{15}H_{26}O$	222

Table 9. Chemical composition of *Cymbopogon citratus* essential oil (contd.)

No	Compound	RT(min)	Area %	Formula	MW
21	Delta-Cadinene	22.54	0.91	$C_{15}H_{24}$	204
22	Torreyol	22.60	1.08	$C_{15}H_{26}O$	222
23	Copaene	22.71	0.36	$C_{15}H_{24}$	204
24	Alpha-cadinol	23.01	3.37	$C_{15}H_{26}O$	222
25	Beta-neoclovene	23.32	2.28	$C_{15}H_{24}$	204
26	Juniper camphor	24.51	0.90	$C_{15}H_{26}O$	222

Table 10. Chemical composition of *Citrus hystrix* essential oil

No	Compound	RT(min)	Area %	Formula	MW
1	Linalool L	4.73	4.33	$C_{10}H_{18}O$	154
2	Citronella	5.89	84.22	$C_{10}H_{18}O$	154
3	Isopulegol	6.18	1.25	$C_{10}H_{18}O$	154
4	Benzene,1-methoxy-4-(1-propenyl)-	7.13	1.15	$C_{10}H_{12}O$	148
5	Beta-Citronella	7.81	6.39	$C_{10}H_{20}O$	156
6	Citronellyl acetate	11.82	1.26	$C_{12}H_{22}O_2$	198
7	Geranyl acetate	12.85	0.49	$C_{12}H_{20}O_2$	196
8	Trans-Caryophyllene	14.38	0.20	$C_{15}H_{24}$	204
9	Beta-cadinene	17.98	0.05	$C_{15}H_{24}$	204
10	Nerolidol	19.61	0.07	$C_{15}H_{26}O$	222

Table 11. Chemical composition of *Limnophila geoffrayi* essential oil

No	Compound	RT(min)	Area %	Formula	MW
1	Linalool L	4.85	0.43	$C_{10}H_{18}O$	154
2	4-Isopropenylcyclohexanone	6.25	0.65	$C_9H_{14}O$	138
3	Tarragon	7.29	0.64	$C_{10}H_{12}O$	148
4	Cyclohexane, (1-methylethylidene)-	7.42	4.66	C_9H_{16}	124
5	Pulegone	7.61	22.50	$C_{10}H_{16}O$	152
6	1,3,6-Heptatriene, 2,5,6-trimethyl-	9.58	10.72	$C_{10}H_{16}$	136
7	Bicyclo[5.1.0]octane, 8-(1-ethylethylidene)-	9.68	3.92	$C_{11}H_{18}$	150
8	Isobornyl acetate	9.92	0.52	$C_{12}H_{20}O_2$	196
9	Cyclohexane, 1-methylene-3-(1-methylethenyl)	10.05	12.22	$C_{10}H_{16}$	136
10	Cyclohexanol,2-methyl-5-(1-methylethenyl)-	10.27	0.58	$C_{10}H_{18}O$	154
11	1,2-exo-Trimethlene-cis-bicyclo[3.3.0]octane	10.40	1.57	$C_{11}H_{18}$	150
12	1-Methyl-6-ethylenebicyclo[3.2.0]heptane	10.72	0.45	C_9H_{14}	122
13	1-(2-Cyclohexenyl)-2-propanol	12.94	0.71	$C_9H_{16}O$	140
14	Isolimonene	14.38	19.06	$C_{10}H_{16}$	136
15	Trans-Caryophyllene	14.67	3.74	$C_{15}H_{24}$	204
16	1-Cyclohexene-1-methanol,4-(1-methylethenyl)-acetate	15.17	5.21	$C_{12}H_{18}O_2$	194

Table 11. Chemical composition of *Limnophila geoffrayi* essential oil (contd.)

No	Compound	RT(min)	Area %	Formula	MW
17	β-Famesene	15.84	1.69	$C_{15}H_{24}$	204
18	Alpha-Humulene	15.97	6.81	$C_{15}H_{24}$	204
19	β-Nerolidol	19.88	0.54	$C_{15}H_{26}O$	222
20	Caryophyllene oxide	20.60	0.90	$C_{15}H_{24}O$	220
21	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	22.81	0.26	$C_{15}H_{24}$	204
22	Phytol	35.72	1.04	$C_{20}H_{40}O$	296

Table 12. Chemical composition of *Kaempferia galanga* Linn. essential oil

No	Compound	RT(min)	Area %	Formula	MW
1	Borneol L	6.68	2.68	$C_{10}H_{18}O$	154
2	Eucarvone	10.72	1.27	$C_{10}H_{14}O$	150
3	p-Cymene	12.50	1.39	$C_{10}H_{14}$	134
4	α-Cyperene	14.07	1.31	$C_{15}H_{24}$	204
5	Ethyl cinnamate	16.51	65.28	$C_{11}H_{12}O_2$	176
6	Pentadecane	17.71	20.36	$C_{15}H_{32}$	212
7	Alpha-Amorphene	18.10	0.75	$C_{15}H_{24}$	204
8	Nerolidol	19.88	0.96	$C_{15}H_{26}O$	222
9	Ethyl ethoxycinnamate	26.85	5.20	$C_{12}H_{14}O_3$	206

APPENDIX B : Statistical analysis

Table 13. Analysis of Variance of Inhibition zones of essential oils on the aflatoxigenic strains

Source	df	SS	MS	F-Value	P-Value
Essential oils	55	83730.3	1522.37	4860.01	0.000
Aspergillus strains	1	16.1	16.08	51.33	0.000
Essential oils X *Aspergillus* strains	55	1298.9	23.62	75.39	0.000
Error	224	70.2	0.31		
Total	335	85115.5			

Table 14. Analysis of Variance of mycelial Growth, sporulation and aflatoxin B1 production inhibitory of *Aspergillus* strains at 300 ppm of essential oil

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Essential oil (A)	12	56011.2	4667.60	7920.65	0.000	73624.7	6135.39	317.66	0.000	98076	8172.99	812.99	0.000
Systems (B)	1	6562.4	6562.43	11136.07	0.000	6917.4	6971.89	360.95	0.000	5663	5662.54	563.27	0.000
Aspergillus strains (C)	1	46.5	46.53	78.96	0.000	711.5	711.47	36.84	0.000	74	74.04	7.36	0.008
AB	12	2343.3	195.28	331.37	0.000	6406.4	533.87	27.64	0.000	4555	379.55	37.76	0.000
AC	12	1394.5	116.21	197.20	0.000	5222.0	435.17	22.53	0.000	4100	371.64	33.98	0.000
BC	1	6.6	6.65	11.28	0.001	184.5	184.49	9.55	0.003	0.0	0.03	0.00	0.957
ABC	12	1054.0	87.88	149.12	0.000	1115.2	92.93	4.81	0.000	255	21.24	2.11	0.022
Error	104	61.3	0.59			2008.7	19.31			1046	10.05		
Total	155	67480.4				96244.2				113767			

Table 15. Analysis of Variance of the inhibitory effect of *Cuminum cymimum* essential oil on mycelail growth, sporulation, and aflatoxin production.

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Concentration (A)	5	26524.1	5304.82	5494.85	0.000	66195.2	13239.0	1518.67	0.000	78291.1	15658.2	5510.76	0.000
Systems (B)	1	3160.1	3160.13	3273.33	0.000	134.8	134.8	156.46	0.000	21.1	21.1	7.43	0.009
Aspergillus strains (C)	1	485.7	485.68	503.08	0.033	5712.0	5712.0	655.24	0.000	10.4	10.4	3.67	0.061
AB	5	721.4	144.29	149.46	0.000	46.2	9.2	1.06	0.394	39.4	7.9	2.78	0.028
AC	5	431.4	86.29	89.38	0.000	2499.7	499.9	57.35	0.000	108.9	21.8	7.67	0.000
BC	1	0.0	0.01	0.01	0.905	4.4	4.4	0.50	0.483	16.1	16.1	5.65	0.021
ABC	5	108.7	21.74	22.52	0.000	181.2	36.2	4.16	0.003	28.6	5.7	2.01	0.094
Error	48	46.3	0.97			418.4	8.7			136.4	2.8		
Total	71	31477.9				75191.9				78652.0			

Table 16. Analysis of Variance of the inhibitory effect of *Foeniculum vulgare* essential oil on mycelail growth, sporulation, and aflatoxin production.

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Concentration (A)	5	7304.527	1460.90	2751.38	0.000	25398.1	5079.62	1150.68	0.000	81170.5	16234.1	3593.82	0.000
Systems (B)	1	170.20	170.20	320.55	0.000	425.3	425.35	96.35	0.000	835.7	835.7	185.01	0.000
Aspergillus strains (C)	1	204.36	204.36	384.87	0.000	396.7	396.68	89.86	0.000	179.6	179.6	39.75	0.000
AB	5	61.25	12.25	23.07	0.000	316.8	63.37	14.35	0.000	599.2	119.8	26.53	0.000
AC	5	89.89	17.98	33.86	0.000	161.2	32.24	7.30	0.000	178.4	35.7	7.90	0.000
BC	1	140.28	140.28	264.20	0.000	0.1	0.11	0.02	0.876	19.5	19.5	4.32	0.043
ABC	5	28.44	5.69	10.71	0.000	126.1	25.22	5.71	0.000	23.0	4.6	1.02	0.419
Error	48	25.49	0.53			211.9	4.41			216.8	4.5		
Total	71	8024.42				27036.3				83222.8			

Table 17. Analysis of Variance of the inhibitory effect of *Trachyspermum amni* essential oil on mycelail growth, sporulation, and aflatoxin production.

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Concentration (A)	5	28133.5	5626.69	8826.18	0.000	72692.6	14538.5	1293.96	0.000	78907.3	15781.5	2586.65	0.000
Systems (B)	1	2484.0	2483.95	3896.39	0.000	124.0	124.0	11.04	0.002	240.5	240.5	39.42	0.000
Aspergillus strains (C)	1	264.9	264.88	415.50	0.000	2049.1	2049.1	182.37	0.000	39.3	39.3	6.44	0.014
AB	5	533.0	106.61	167.23	0.000	171.0	34.2	3.04	0.018	295.0	59.0	9.67	0.000
AC	5	180.4	36.09	56.61	0.000	1084.3	216.9	19.30	0.000	249.3	49.9	8.17	0.000
BC	1	36.3	36.27	56.89	0.000	240.2	240.2	21.38	0.000	2.4	2.4	0.40	0.532
ABC	5	210.2	42.03	65.93	0.000	135.0	27.0	2.40	0.050	89.8	18.0	2.95	0.021
Error	48	30.6	0.64			539.3	11.2			292.9	6.1		
Total	71	31872.8				77035.4				80116.5			

Table 18. Analysis of Variance of the inhibitory effect of *Brassica juncea* L. essential oil on mycelail growth, sporulation, and aflatoxin production.

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Concentration (A)	5	36251.0	7250.19	16231.78	0.000	74882.8	14976.6	3675.36	0.000	80122.6	16024.5	6432.68	0.000
Systems (B)	1	2410.5	2410.49	5326.63	0.000	389.2	389.2	95.51	0.000	422.0	422.0	169.38	0.000
Aspergillus strains (C)	1	46.1	46.08	103.16	0.000	1598.0	1598.0	392.16	0.000	99.6	99.6	40.00	0.000
AB	5	562.1	112.41	251.66	0.000	269.3	53.9	13.22	0.000	258.8	51.8	20.78	0.000
AC	5	190.3	38.05	85.19	0.000	864.5	98.9	24.28	0.000	175.3	35.1	14.08	0.000
BC	1	158.4	158.42	354.67	0.000	98.9	5.2	0.09	0.772	26.8	26.8	10.74	0.002
ABC	5	286.6	57.32	128.33	0.000	53.3	10.7	2.62	0.036	31.6	6.3	2.54	0.041
Error	48	21.4	0.45			195.6	4.1			119.6	2.5		
Total	71	39926.3				78351.6				81256.2			

Table 19. Analysis of Variance of the inhibitory effect of *Cinnamomum porrectum* L. essential oil on mycelail growth, sporulation, and aflatoxin production.

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Concentration (A)	5	88958.6	17791.7	7609.63	0.000	100033	20006.7	1083.58	0.000	10172.3	20344.5	1917.36	0.000
Systems (B)	1	849.4	849.4	363.30	0.000	2609	2608.8	141.30	0.000	1975	1975.1	186.14	0.000
Aspergillus strains (C)	1	25.3	25.3	10.83	0.002	18	17.6	0.95	0.334	38	38.3	3.61	0.064
AB	5	621.4	124.3	53.16	0.000	2611	522.2	28.28	0.000	1981	396.2	37.34	0.000
AC	5	74.2	14.8	6.35	0.000	45	8.9	0.48	0.787	216	43.2	4.07	0.004
BC	1	49.8	49.8	21.31	0.000	0	0.0	0.00	0.974	3	3.3	0.31	0.582
ABC	5	103.0	20.6	8.81	0.000	720	144.1	7.80	0.000	326	65.3	6.15	0.000
Error	48	112.2	2.3			886	18.5			509	10.6		
Total	71	90794.1				106922				106772			

Table 20. Analysis of Variance of the inhibitory effect of *Cinnamomum zeylanicum* essential oil on mycelail growth, sporulation, and aflatoxin production.

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Concentration (A)	5	80086.0	16017.2	40880.48	0.000	96627	19325.3	1048.56	0.000	95560	19112.1	2478.55	0.000
Systems (B)	1	6153.1	6153.1	15704.48	0.000	1149	1148.8	62.33	0.000	2270	2270.3	294.42	0.000
Aspergillus strains (C)	1	513.1	513.1	1309.49	0.000	4	3.6	0.19	0.662	4	4.4	0.56	0.456
AB	5	3125.9	625.2	1595.65	0.000	1203	240.5	13.05	0.000	2998	599.6	77.76	0.000
AC	5	914.0	182.8	466.53	0.000	364	72.9	3.95	0.004	86	17.1	2.22	0.067
BC	1	52.7	52.7	134.51	0.000	1	1.2	0.06	0.802	1	1.1	0.14	0.714
ABC	5	200.2	40.0	102.21	0.000	215	43.1	2.34	0.056	32	6.4	0.83	0.534
Error	48	18.8	0.4			885	18.4			370	7.7		
Total	71	91063.8				100447				101322			

Table 21. Analysis of Variance of the inhibitory effect of *Michelia alba* essential oil on mycelail growth, sporulation, and aflatoxin production.

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Concentration (A)	5	50854.7	10170.9	4310.99	0.000	80357.9	16071.6	262.13	0.000	44899.0	8979.80	373.44	0.000
Systems (B)	1	9616.5	9616.5	4076.00	0.000	788.0	788.0	12.85	0.001	1100.6	1100.59	45.77	0.000
Aspergillus strains (C)	1	11.4	11.4	4.85	0.033	14.6	14.6	0.24	0.628	1467.9	1467.92	61.05	0.000
AB	5	2318.8	463.8	196.56	0.000	693.2	138.6	2.26	0.063	929.5	185.90	7.73	0.000
AC	5	44.3	8.9	3.75	0.006	853.7	170.7	2.78	0.027	624.8	124.96	5.20	0.001
BC	1	42.5	42.5	18.00	0.000	5.2	5.2	0.09	0.772	15.4	15.40	0.64	0.427
ABC	5	103.5	20.7	8.77	0.000	631.3	126.3	2.06	0.087	93.4	18.67	0.78	0.572
Error	48	113.2	2.4			2942.9	61.3			1154.2	24.05		
Total	71	63104.9				86286.9				50284.8			

Table 22. Analysis of Variance of the inhibitory effect of *Myristica fragrans* essential oil on mycelail growth, sporulation, and aflatoxin production.

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Concentration (A)	5	56112.3	11222.5	7276.81	0.000	77305	15461.1	584.65	0.000	81529.5	16305.9	1027.06	0.000
Systems (B)	1	4446.2	4446.2	2883.01	0.000	14740	14740.4	557.40	0.000	4518.8	4518.8	284.63	0.000
Aspergillus strains (C)	1	27.6	27.6	17.91	0.000	0	0.2	0.01	0.927	414.7	414.7	26.12	0.000
AB	5	1255.4	251.1	162.80	0.000	11118	2223.5	84.08	0.000	5691.5	1138.3	71.70	0.000
AC	5	79.5	15.9	10.32	0.000	305	61.0	2.31	0.059	1323.2	264.6	16.67	0.000
BC	1	68.4	68.4	44.38	0.000	243	243.5	9.21	0.004	76.9	76.9	4.84	0.033
ABC	5	98.8	19.8	12.81	0.000	335	67.1	2.54	0.041	82.4	16.5	1.04	0.407
Error	48	74.0	1.5			1269	26.4			762.1	15.9		
Total	71	62162.4				105317				94399.1			

Table 23. Analysis of Variance of the inhibitory effect of *Cymbopogon citratus* essential oil on mycelail growth, sporulation, and aflatoxin production.

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Concentration (A)	5	86327.5	17265.5	44492.36	0.000	87390.6	17478.1	3643.81	0.000	88650.9	17730.2	8545.24	0.000
Systems (B)	1	104.2	104.2	268.42	0.000	1171.3	1171.3	244.19	0.000	238.3	238.3	114.87	0.000
Aspergillus strains (C)	1	99.9	99.9	257.37	0.000	4.9	4.9	1.02	0.317	3784.5	3784.5	1823.98	0.000
AB	5	133.6	26.7	68.85	0.000	914.6	182.9	38.13	0.000	178.2	35.6	17.18	0.000
AC	5	156.2	31.2	80.53	0.000	107.3	21.5	4.48	0.002	2027.1	405.4	195.40	0.000
BC	1	176.1	176.1	453.79	0.000	2.8	2.8	0.58	0.449	0.2	0.2	0.11	0.745
ABC	5	214.0	42.8	110.31	0.000	275.8	55.2	11.50	0.000	118.4	23.7	11.41	0.000
Error	48	18.6	0.4			230.2	4.8			99.6	2.1		
Total	71	87230.2				90097.6				95097.3			

Table 24. Analysis of Variance of the inhibitory effect of *Citrus hystrix* essential oil on mycelail growth, sporulation, and aflatoxin production.

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Concentration (A)	5	15666.1	3133.23	10561.44	0.000	27109.1	5421.83	1134.90	0.000	40316.2	8063.25	1828.34	0.000
Systems (B)	1	1777.1	1777.07	5990.14	0.000	320.0	320.05	66.99	0.000	1218.5	1218.53	276.30	0.000
Aspergillus strains (C)	1	243.8	243.84	821.92	0.000	315.8	315.84	66.11	0.000	0.0	0.03	0.01	0.938
AB	5	735.9	147.17	496.09	0.000	253.4	50.69	10.61	0.000	360.2	72.05	16.34	0.000
AC	5	242.2	48.44	163.28	0.000	282.5	56.50	11.83	0.000	337.9	67.59	15.33	0.000
BC	1	0.0	0.00	0.01	0.914	14.0	14.04	2.94	0.093	36.1	36.13	8.19	0.006
ABC	5	685.4	137.08	462.08	0.000	67.8	13.56	2.84	0.025	71.2	14.23	3.23	0.014
Error	48	14.2	0.30			229.3	4.78			211.7	4.41		
Total	71	19364.8				28592.1				42552.0			

Table 25. Analysis of Variance of the inhibitory effect of *Limnophila geoffrayi* essential oil on mycelail growth, sporulation, and aflatoxin

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Concentration (A)	5	34193.4	6838.68	5677.87	0.000	102131	20426.2	1833.75	0.000	89080.8	17816.2	1243.06	0.000
Systems (B)	1	454.0	454.01	376.94	0.000	1067	1067.2	95.81	0.000	15.0	15.0	1.05	0.311
Aspergillus strains (C)	1	790.7	790.69	656.48	0.000	51	51.3	4.61	0.037	93.2	93.2	6.50	0.014
AB	5	194.2	38.84	32.24	0.000	1626	325.2	29.19	0.000	292.1	58.4	4.08	0.004
AC	5	201.9	40.38	33.52	0.000	399	79.8	7.17	0.000	491.7	98.3	6.86	0.000
BC	1	154.3	154.29	128.10	0.000	58	58.0	5.20	0.027	62.5	62.5	4.36	0.042
ABC	5	66.2	13.25	11.00	0.000	485	97.0	8.71	0.000	99.6	19.9	1.39	0.245
Error	48	57.8	1.20			535	11.1			688.0	14.3		
Total	71	36112.5				106352				90822.9			

Table 26. Analysis of Variance of the inhibitory effect of *Kaempferia galanga* essential oil on mycelail growth, sporulation, and aflatoxin

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Concentration (A)	5	93950	18790.0	19868.97	0.000	978.39	19567.8	1477.00	0.000	97239	19447.9	2695.79	0.000
Systems (B)	1	3819	3819.4	4038.70	0.000	1334	1333.9	100.68	0.000	1988	1987.7	275.52	0.000
Aspergillus strains (C)	1	145	145.1	153.40	0.000	2	1.6	0.12	0.731	2	1.5	0.21	0.647
AB	5	4650	930.1	983.49	0.000	1445	289.0	21.82	0.000	2304	460.7	63.86	0.000
AC	5	226	45.3	47.87	0.000	20.2	40.4	3.05	0.018	35	7.0	0.97	0.445
BC	1	158	158.4	167.52	0.000	2	1.6	0.12	0.731	65	64.8	8.98	0.004
ABC	5	166	33.1	35.05	0.000	45	9.0	0.68	0.639	263	52.5	7.28	0.000
Error	48	45	0.9			636	13.2			346	7.2		
Total	71	103161				101504				102241			

Table 27. Analysis of Variance of IC50 of essential oil against *Aspergillus* strains

Source	dF	Mycelial growth inhibition				Sporulation Inhibition				Aflatoxin B1 inhibition			
		SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value
Aspergillus strains (A)	1	4624	4624	4624	0.000	4624	4624	4624	0.000	4624	4624	4624	0.000
Systems (B)	1	309358	309358	309358.44	0.000	309358	309358	309358.44	0.000	309358	309358	309358.44	0.000
Essential oil (C)	11	2727300	247936	247936.33	0.000	2727300	247936	247936.33	0.000	2727300	247936	247936.33	0.000
AB	1	1332	1332	1332.25	0.000	1332	1332	1332.25	0.000	1332	1332	1332.25	0.000
AC	11	196770	17888	17888.20	0.000	196770	17888	17888.20	0.000	196770	17888	17888.20	0.000
BC	11	342040	31095	31094.55	0.000	342040	31095	31094.55	0.000	342040	31095	31094.55	0.000
ABC	11	29254	2659	2659.43	0.000	29254	2659	2659.43	0.000	29254	2659	2659.43	0.000
Error	96	96	1			96	1			96	1		
Total	143	3610774				3610774				3610774			

AUTHOR'S BIOGRAPHY

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