Send Orders for Reprints to reprints@benthamscience.ae

BENTHAM OPEN

CrossMark

The Open Food Science Journal

Content list available at: www.benthamopen.com/TOFSJ/

DOI: 10.2174/1874256401810010024, 2018, *10*, 24-32

REVIEW ARTICLE

Antifungal Activity and Detoxification of Aflatoxins by Plant Extracts: Potential for Food Applications

Bárbara Ponzilacqua, Carlos Humberto Corassin and Carlos Augusto Fernandes Oliveira*

Department of Food Engineering, School of Animal Science and Food Engineering, University of São Paulo, Av. Duque de Caxias Norte, 225, CEP 13635-900, Pirassununga, SP, Brazil

Received: August 22, 2018 Revised: September 26, 2018 Accepted: October 10, 2018

Abstract: Aflatoxins are secondary metabolites produced by fungi of the genus *Aspergillus*, which occur naturally in cereals like corn, beans and rice. Aflatoxin B_1 causes an extensive number of toxic effects in animals and humans. This mycotoxin is a stable term and can act in low concentrations due to their higher toxicity. Management to prevent commodities aflatoxin contamination is essential during the production, mainly in pre- and post-harvest steps. A number of essential oils and aqueous plant extracts have been reported to be fungal growth inhibitors and may provide an attractive alternative to prevent aflatoxin contamination in foods. Thus, the aim of this review is to highlight recent data on the *in vitro* antifungal activity of essential oils and aqueous extracts from plants and discuss the perspectives of their use in food products.

Keywords: *Aspergillus* spp., Aflatoxin B_1, Essential oils, Inhibition, Plant extracts, Essential oil, Antimicrobial.

1. INTRODUCTION

Aflatoxins are highly toxic secondary metabolites produced by fungi of the genus *Aspergillus*, mainly species *A. flavus*, *A. parasiticus* and *A. nomius*, which can develop naturally in food products such as beans, corn, rice and wheat, among others [1]. There are currently more than 20 different compounds known as aflatoxin, although the most important types from a public health perspective are the aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2) [2]. AFB_1 is the main fraction produced by the fungi and has the highest toxicity, mainly targeting the liver, while also exhibits teratogenic, mutagenic and carcinogenic effects in several animal models [3]. In 1987, the International Agency for Research on Cancer classified AFB_1 in Group 1 - human carcinogen [4].

The aflatoxins are chemically stable and heat-resistant, and may remain in the foods even after fungi have been removed by regular food treatment and preservation processes [5]. Considering aflatoxins thermic stability and high toxicity, management to prevent commodities contamination are essential throughout the production. In this context, plant extracts and essential oils have been studied as fungal growth inhibitors and regarded as safe alternatives in the prevention of mycotoxins in foods [6 - 10]. Some aqueous plant extracts have chemically active compounds that inhibit the biosynthesis of aflatoxins [11], which have increased the scientific attention on these issues. A number of recent publications have shown the efficiency of plant extracts against *Aspergillus* and/or aflatoxin production. The objective of this review is to highlight recent data on *in vitro* antifungal activity by essential oils and aqueous extracts from plants and discuss the perspectives for using these compounds in food products.

2. ANTIFUNGAL ACTIVITY OF PLANT EXTRACTS CONTAINING ESSENTIAL OILS

Throughout evolution, plants have developed adaptation mechanisms as a response to stress caused by abiotic or biotic factors [12]. Several essential plant oils are involved in the mechanisms of plant protection involving a range of antimicrobial effects *in vitro* [13 - 15] and *in vivo* [16, 17]. Essential oils have low risk in the development of

* Address correspondence to this author at the Department of Food Engineering, School of Animal Science and Food Engineering, University of São Paulo, Av. Duque de Caxias Norte, 225, CEP 13635-900, Pirassununga, SP, Brazil; Tel.: +5519-3565-4173; E-mail :carlosaf@usp.br

antimicrobial resistance and are nontoxic, being classified as GRAS (generally recognized as safe) by the United States Food and Drug Administration [18]. Essential oils damage the enzymatic system of fungi cells by reducing the synthesis of proteins and structural compounds. The mechanism action is denaturing enzymes responsible for spore germination and interfering with amino acids involved in germination [19]. Some compounds, such as monoterpenes and limonene, have been identified as potential inhibitors of pectin methylsterase, which is responsible for building the main components of the cell wall in fungi [20]. The greatest difficulty of studies on the antifungal activity of essential oils depends on the sufficient production of the raw materials. In this context, different processes have been used for extraction, such as classical methods including hydro-distillation [7], effleurage [21], organic solvent extraction [8] and maceration [11]. Laboratory-scale techniques like Clevenger distillation and innovative methods such as microwave and supercritical fluid also are being studied [22].

Studies attempting to understand the mechanisms of action and toxicological safety of the use of plants are still scarce, preventing more effective use of these compounds in agriculture, livestock production and the industry. Essential oils antimicrobial actions involve several chemical compounds found in plants, and this activity cannot be attributed to a single cell mechanism, but a set of them [23]. Vilela *et al*. [24] tested *Eucalyptus globulus* (common eucalyptus) essential oil and its main component alone (1,8-cineol) against *A. flavus* and *A. parasiticus.* They observed that 1,8-cineol has lower antifungal activity than the essential oil. These findings suggest that other components found in lower levels in the oil may be critical for promoting synergism and enhancing the effects [25].

Essential oils have two different modes of action: reduced fungal growth or aflatoxin biosynthesis and secretion. Therefore, inhibition of AFB_1 production may not be completely attributed to reduced fungal growth. Tian *et al*. [26] demonstrated that 5 µL/mL and 4 µL/mL of essential oils from *Cicuta virosa* L. var. *latisecta* were sufficient for antifungal and anti-aflatoxigenic activities of *A. flavus*, respectively. However, another study [27] reported that the oil extract from *Carum carvi* L. was only effective in reducing aflatoxin inhibition, with no effect on *A. parasiticus* growth. Essential oils prepared from flowers, herbs, seeds, leaves and roots have different inhibitory activities against the growth of toxigenic fungi (*A. flavus* and *A. parasitucus*), and aflatoxin production. Table **1** presents an overview of plants containing essential oils from the most commonly studied plant species that have antifungal effects *in vitro*. Inhibition reported in those studies varied from 48 to 100%, with a huge variation among the extract's concentrations used in the assays (0.25 to 3,000 µg/mL). The variation in the results may be partially attributable to different processes used for extraction in each experiment, leading to different concentrations of essential oils in the extracts. However, strong antifungal activities at lower extract concentrations (0.25 to 5.0 µL/mL) were reported for extracts from *Ageratum conyzoides* [28], *Carum carvi* [54], *Origanum vulgare* [17] and *Rosmarinus officinalis* [17];

Table 1. Plant extracts containing essential oils with *in vitro* antifungal activity against *A. flavus* and *A. parasiticus.*

Plant Species (Part Used)	*Aspergillus* Species	Conditions of the Assay[1]	Highest Fungal Inhibition (%)	Extract Concentration (µg/mL)[2]	Reference
Ageratum conyzoides (leaf)	*A. flavus*	Sabouraud agar, 25°C, 5 days	48	5.0 [3]	[28]
Carum carvi (leaf)	*A. flavus*	Potato dextrose agar, 21°C, 7 days	95	1.0	[27]
Chenopodium ambrosioides (leaf)	*A. flavus*	Czapek agar, 28°C, 7 days	100	100	[35]
Daucus carota (ripe fruit)	*A. flavus*	Malt extract infusion, 28°C, 72 hours	100	10	[15]
Daucus carota (green fruit)	*A. flavus*	Malt extract infusion, 28°C, 72 hours	100	25	[15]
Daucus carota (flower, leaf)	*A. flavus*	Malt extract infusion, 28°C, 72 hours	100	150	[15]
Foeniculum vulgare (leaf)	*A. flavus* *A. parasitucus*	Potato dextrose agar, 28°C, 7-14 days	100 100	3,000 2,000	[13]
Ocimum basilicum (leaf)	*A. flavus* *A. parasitucus*	Potato dextrose agar, 28°C, 7-14 days	100 100	3,000 3,000	[13]
Origanum vulgare (leaf, stem)	*A. flavus*	Sabouraud agar, 28°C, 7 days	98	0.25 [3]	[17]
Rosmarinus officinalis (leaf)	*A. flavus*	Sabouraud agar, 28°C, 7 days	98	1.0 [3]	[17]
Syzygium aromaticum (leaf, stem)	*A. flavus*	Potato dextrose agar, 25°C, 5 days	87	500	[31]
Thymus vulgaris (leaf, stem)	*A. flavus*	Potato dextrose agar, 25°C, 5 days	100	350	[31]

[1] Culture medium, temperature and time of incubation used in each assay.

[2] Values refer to the plant extract concentrations that resulted in the highest percentage of fungal growth inhibition.

[3] Values expressed in µL/mL.

A. conyzoides (baume, chick weed) is a popular medicinal plant in Brazil. Therapeutic properties have been attributed to volatile, terpenes and coumarins, and non-volatile phenolic acids and flavonoids. Nogueira *et al*. [28]

found nearly 48% inhibition of *A. flavus* growth in Sabouraud agar with 5.0 µL in a 6mm diameter of filter paper disk of the *A. conyzoides* extract. The component found in the highest concentration in the extract was precocene II (46.3%). Studies using *Rosmarinus officinalis* (rosemary) [29], which also has a large amount of precocene II, demonstrated important changes in the mitochondria and endomembrane system of fungal cells. The antifungal action of these plants is attributed to the presence of this substance, as well as its synergistic action with other compounds (*e.g.*, carvacrol, in the case of rosemary). *C. carvi* (caraway) is an important member of the Apiaceae family, which includes carrot and fennel. Studies demonstrated that *C. carvi* lacks inhibitory activity against *A. parasiticus* growth, although it can affect AFB_1 and AFG_1 production [27]. The main components of this plant are myristicin and dillapiole (two phenylpropanoids) which are responsible for the inhibition of aflatoxin production [29].

Plants in the family Lamiaceae have been drawing huge attention from the scientific community [30, 31]. They have two types of phenols, carvacrol and thymol. Studies have demonstrated that these substances are able to inhibit the growth of a wide range of microorganisms, including fungi and bacteria [15, 32]. These two classes of compounds affect the integrity of the cell membrane in microorganisms, thus changing the pH homeostasis and the inorganic ion balance [33]. Carvacrol is found in the highest percentage in *Origanum* spp., ranging from 50 to 86% depending on the species [34]. This plant has been widely employed for a long time as a therapeutic agent and as an antioxidant in the food industry. However, its antimicrobial and anti-aflatoxin characteristics are still issues that should be studied due to conflicting results described in different studies [14].

There is evidence that other plant extracts also inhibit *Aspergillus* spp. growth, although at much higher concentrations. *Chenopodium ambrosioides* (Mexican tea, wormseed) is used in the treatment of respiratory and digestive problems in Central and South America. It originated in Mexico and belongs to the Amaranthaceae family. Kumar *et al*. [35] demonstrated that the essential oil from its leaves (100 µg/mL) caused the lysis of mycelia and spores, thereby reducing aflatoxin production of two aflatoxigenic strains of *A. flavus*, Navjot 4NSt and Saktiman 3NSt (procured from Mycotoxin Laboratory, University of Bhagalpur, India). *Foeniculum vulgare* (fennel) has been widely known and used as a medicinal plant for many years. The roots of this plant contain approximately 90% dillapiol, which may be responsible for the inhibitory action of AFG_1 production [36]. However, it has been reported that high concentrations (2,000-3,000 µg/mL) of *F. vulgare* extracts are required for growth inhibition of *A. flavus* and *A. parasiticus* [13]. Alinezhad *et al*. [37] reported 99% inhibition of AFB_1 and AFG_1 production. The authors attributed the effects to the most prevalent components in the oil, 1,8-cineole, limonene, trans-anethole, and fenchone, whose antifungal activities are well described in the literature [20,24,36]. The essential oil from *Ocimum basilicum* (basil) at a concentration of 3,000 µg/mL had fungistatic and fungicidal activity against *A. parasiticus* and *A. flavus* strains [13]. These effects are attributed to the two substances found in the highest concentration in the oil, estragole (50%) and ocimene (11.2%), which are known for their antifungal activity [13]. Soković *et al*. [15] carried out studies with the flowers, stems, leaves, roots and seeds of *Daucus carota* (wild carrot) and their potential inhibition effect on fungal and bacterial growth. Results showed that fungi are more sensitive than bacteria to the essential oils of this species of plant. The analysis of the main components of the oil showed two important terpenes in high concentrations (α-pinene and sabinene), to which the antifungal activity of the oil may be attributed.

3. EFFECT OF PLANT EXTRACTS CONTAINING ESSENTIAL OILS ON AFLATOXIN BIOSYNTHESIS

The results from several *in vitro* studies on plant extracts containing essential oils with inhibitory activity against aflatoxin production by *Aspergillus* species are presented in Table **2**. Similar to the antifungal activity of plant extracts, the concentrations of the extract reported for total inhibitory effect against aflatoxin production varied markedly among different studies, from 0.1 to 2.5 µL/mL, or 100 to 50,000 µg/mL.

Table 2. Plant extracts containing essential oils with total (100%) *in vitro* inhibitory activity against the aflatoxin production by *Aspergillus* species.

Plant Species (Part Used)	*Aspergillus* Species	Conditions of the Assay[1]	Extract Concentration (µg/mL)[2]	Reference
Ageratum conyzoides (leaf)	*A. flavus*	YES broth, 25°C, 5 days	0.1 [3]	[28]
Chenopodium ambrosioides (leaf)	*A. flavus*	SMKY broth, 28°C, 10 days	100	[35]
Cinnamomum tamala (leaf)	*A. flavus*	SMKY broth, 27°C, 10 days	0.75 [3]	[39]
Cuminum cyminum (seed)	*A. flavus*	SMKY broth, 27°C, 10 days	0.5 [3]	[32]
Curcuma longa (leaf)	*A. flavus*	YES broth, 27°C, 7 days	5,000	[44]
Lippia alba (leaf)	*A. flavus*	SMKY broth, 28°C, 10 days	0.6 [3]	[38]

(Table 2) contd.....

Plant Species (Part Used)	*Aspergillus* Species	Conditions of the Assay[1]	Extract Concentration (μg/mL)[2]	Reference
Mentha spicata (leaf, stem)	*A. flavus*	SMKY broth, 27°C, 7 days	0.9 [3]	[41]
Ocimum basilicum (leaf)	*A. parasiticus*	Palm kernel broth, 28°C, 7 days	50,000	[56]
Origanum majorana (leaf)	*A. flavus*	SMKY broth, 27°C, 10 days	2.5 [3]	[16]
Rosmarinus officinalis (leaf)	*A. flavus*	YES broth, 28°C, 7 days	450	[29]
Trachyspermum ammi (fruit)	*A. flavus*	SMKY broth, 27°C, 10 days	0.75 [3]	[45]

[1] Culture medium, temperature and time of incubation used in each assay.

[2] Values refer to the plant extract concentrations that resulted in total (100%) inhibition of aflatoxin production.

[3] Values expressed in μL/mL.

A. conyzoides extracts showed the greatest inhibition capacity on aflatoxin production by *A. flavus*, at concentrations above 0.1 μg/mL [28]. The essential oils from *Lippia alba*, a species belonging to Verbenaceae family, caused complete inhibition *in vitro* of the AFB_1 production at the concentration tested, 0.6 to 1.0 μg/mL [38]. *Cinnamomum tamala* was effective against aflatoxin production by *A. flavus* at very low concentration, 0.75 μg/mL [39]. In this study, the aflatoxigenic activity was attributed to the compound with the highest concentration in the plant, eugenol (45.6%). The leaves from *C. zeylanicum*, another plant also belonging to the family Lauraceae, and widely used as condiment and flavoring of cooked foods, had similar antifungal activity [40]. The authors [40] also noted that high levels of eugenol were present in the leaf extracts (87.3%) and that 2.0 μL of the extract resulted in 100% inhibition of the fungal activity.

Cuminum cyminum (cumin) is one of the most known spices in the world, commonly found in oriental food preparations. Kedia *et al*. [32] investigated the applicability of these essential oils as an antifungal agent in foods and found that 0.5 μg/mL inhibited AFB_1 production. The same research group also demonstrated the potential of *Mentha spicata* (spearmint), a native species from Africa belonging to the family Lamiaceae. Although 13 compounds were identified in the essential oil composition of spearmint, the major components were carvone (59%) and the limonene (26%), and the minimum concentration of the extract to inhibit AFB_1 production was 0.9 μg/mL [41].

Curcuma longa, also known as turmeric, is a plant from the southeast of Asia. Food industries use this spice as a seasoning and coloring agent. It is also an essential ingredient of industrialized products, such as mustard. The synergistic and cumulative action of its main component (turmerone) promotes antimicrobial, antifungal, anti-inflammatory, and antioxidant activity [42, 43]. Ferreira *et al*. [44] confirmed the complete inhibitory activity of plant extracts from *C. longa* on production AFB_1 and AFB_2 adding 0.5% (v/v) of the oil on YES medium was 96% and 98.6%, respectively. It has been assumed that the inhibition mechanism is based on cell lipid oxygenation and peroxidation caused by phenolic compounds. Investigations of the essential oil extracted from the fruits of *Trachyspermum ammi* (ajowan), an important member of the Apiaceae family, suggested a strong inhibition effect at 0.75 μL/mL on AFB_1 production, and it had no mammalian toxicity when tested *in vivo* in mice [45]. These data highlight the potential of essential oil from *T. ammi* as a food preservative with anti-aflatoxigenic activity.

Studies carried out by Reddy *et al*. [11] have shown that aqueous extracts also have chemically active properties against aflatoxin production. These authors elucidated the effect of some dried plant extracts mixed with rice seeds on the production of AFB_1, and found that extracts from *Allium sativum* (garlic), *Ocimum sanctum* (basil), *Curcuma longa* (turmeric), and *Syzigium aromaticum* (clove) at 5.0 g/kg concentration effectively inhibited aflatoxin production by 75%, 85.7%, 72.2% and 100%, respectively. The use of aqueous extracts is an attractive alternative compared with essential oils which need specific equipment to be extracted. In summary, the potential application of plants extracts has been attracting greater attention in the scientific community because they are biodegradable, environmental friendly, biologically safe, renewable and potentially low-cost [46].

4. PERSPECTIVES FOR USING ESSENTIAL OILS AND PLANT AQUEOUS EXTRACTS IN FOOD PRODUCTS

In the last 25 years, several studies have attempted to evaluate the efficacy of natural compounds from different species of plants, regional or not, against agricultural pests. Food products such as corn, rice, nuts, beans, wheat, peanuts and chickpeas are the most important agricultural commodities that are affected by fungal contamination and oxidative deterioration during processing, transportation and storage [12]. There is scant information on methods for application of the plant extracts to effectively prevent the fungal development in foods. However, fumigation is one of the best methods to protect commodities against contamination during storage. Plant extracts fumigation has already been considered an eco-friendly alternative to prevent fungal growth and the production of mycotoxins [47]. The vapor phases of purified essential oils or plant extracts preserve their bioactivity, hence making them highly effective

fumigants for protecting stored products [12].

The application of essential oils as antimicrobial and antioxidant agents in food and food packaging has no safety restrictions because they are considered Generally Recognized as Safe (GRAS) by the Food and Drug Administration [48]. Although the majority of studies evaluating the antifungal activity of plant extracts have been conducted *in vitro* or *in vivo* under laboratory conditions, a few experiments described the efficacy on some grains, fruits and vegetables. Reddy *et al*. [11] observed that fungal development and aflatoxin production were completely inhibited after the application of aqueous extracts from *S. aromaticum* on healthy seeds of a rice cultivar for five days. In another study, wheat and chickpea were stored for one year at 10-46°C and relative humidity of 30-90%. The samples were inoculated with *A. flavus* and then fumigated with essential oils from cumin seed. The percent protection from fungal development was 65.8% for wheat and 75.0% for chickpea [32]. Chickpea was also used in an *in vivo* experiment to evaluate the efficacy of fumigant essential oils from *Cananga odorata*, *Hedychium spicatum*, *O. majorana*, *Coriandrum sativum* and *Commiphora myrrha* [16]. The authors observed that incubation for six months at 28°C and 70% relative humidity resulted in 77.4%, 72.0%, 67.9%, 65.5% and 55.4% protection values, respectively.

Tian *et al*. [49] evaluated the essential oil from *Anethum graveolens* (dill) as a potential food preservative. The study analyzed wound-inoculated cherry tomatoes with *A. flavus* and healthy tomatoes as *in vivo* models. The essential oil was sprayed and the samples were incubated for 9 days. As a result, fungal development was completely inhibited at 120 µg/mL of essential oil for the contaminated tomatoes and 100 µg/mL for healthy tomatoes. Feng and Zeng [50] demonstrated the efficacy of the *Cassia fistula* (golden shower) essential oil *in vivo* experiments. As an alternative to synthetic chemicals to control the *Alternaria alternata* postharvest contamination, this essential oil demonstrated results with concentrations of 300-500 µg/mL. Anžlovar *et al*. [51] showed another example of using essential oil with fumigation technique. Essential oil of thyme was tested for the protection of wheat grain from fungal colonizers (*Alternaria alternata, Alternaria infectoria, Aspergillus flavus, Epicoccum nigrum* and *Fusarium poae*). The fungitoxicity potential was good against all the strains tested. The highest tolerance for the essential oil was *A. flavus*. These results differ from those with the fumigation of *Boswellia carterii* essential oil on *Piper nigrum* fruits (black pepper) [47]. The authors found an increased protection against *A. flavus* during a 6-month period of storage [47].

According to write of authors [52], the major obstacle to the use of essential oils in foodstuffs is the reproducibility of their activity caused by the variations in the bioactive components and their strong aroma which may restrict their applications. Despite this, some studies have demonstrated the efficacy of using plants incorporated without relevant negative effects in foods, as alternative additives with antioxidant and antimicrobial activities [53]. Azizkhani and Tooryan [54] tested rosemary and mint extracts incorporated in beef sausage during storage at 4°C, and observed that both extracts were efficient against major spoiling microorganisms including yeasts and molds. Vilela *et al*. [55] demonstrated that application of essential oils from *R. officinalis* and *Laurus nobilis* (laurel) in the packaging of "Maronesa" beef was efficient in reducing spoilage caused by bacteria and fungi. The same study concluded that the addition of this oil also contributed to the fresh beef color, an important point for consumers. These results suggest the potential for further development of methods using essential oils and aqueous plant extracts as antifungal food preservatives.

CONCLUSION

The development of new reliable techniques to eliminate the aflatoxin contamination of foods and commodities is a very important task for the food industry, as well as for food production. In this context, considerable experimental research has demonstrated that essential oils and aqueous plant extracts inhibit the fungal development and/or the biosynthesis of aflatoxins, hence demonstrating a potential for their use in food products. However, there is limited information available on the use of essential oils or aqueous plant extracts directly on food commodities to prevent *Aspergillus* growth or aflatoxin production. Therefore, more studies are necessary to evaluate the potential application of plant extracts under field conditions, particularly on stored cereals and their manufactured products. As a first step, the procedures for preparation of plant extracts and/or essential oils need standardization. Additionally, further studies are necessary to identify the main active compounds of plant extracts and understand their mechanisms of action, as well to determine the safety levels for their use by the food or feed industry. Some practical aspects of using essential oils and aqueous plant extracts in food products also need to be investigated, especially their potential effects on sensory characteristics of foods, and their shelf life for the maintenance of antifungal properties under different environmental conditions.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

REFERENCES

[1] Bhat R, Rai RV, Karim AA. Mycotoxins in food and feed: Present status and future concerns. Compr Rev Food Sci Food Saf 2010; 9: 57-81. [http://dx.doi.org/10.1111/j.1541-4337.2009.00094.x]

[2] Mycotoxins: Risks in plant, animal, and human systems. Council Agricul. Sci. Tech. 2003.

[3] Oliveira CA, Corassin CH. Aflatoxins In: Mycotoxins and their Implications in Food Safety (pp 6--19) Unitec House, 2 Albert Place, London N3 1QB, UK: Future. 2014.

[4] International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans. World Health Organization. International monography. Some traditional herbal medicines, some mycotoxins, naphthalene and Styrene 2002.

[5] Oliveira CAF, Bovo F, Corassin CH, Jager AV, Reddy KR. Recent trends in microbiological decontamination of aflatoxins in foodstuffs. In: Aflatoxins - Recent Advances and Future Prospects InTech. 2013.

[6] El-Habib R. Antifungal activity of some essential oils on *Aspergillus flavus* growth and aflatoxin production. J Food Agric Environ 2005; 1010: 274-9.

[7] Sandosskumar R, Karthikeyan M, Mathiyazhagan S, Mhankumar M, Chandrasekar G, Velazhahan R. Inhibition of *Aspergillus flavus* growth and detoxification of aflatoxin B_1 by the medicinal plant zimmu (*Allium sativum* L. × *Allium* cepa L.). World J Microbiol Biotechnol 2007; 23: 1007-14. [http://dx.doi.org/10.1007/s11274-006-9327-x]

[8] Patil RP, Nimbalkar MS, Jadhav UU, Dawkar VV, Govindwar SP. Antiaflatoxigenic and antioxidant activity of an essential oil from *Ageratum conyzoides* L. J Sci Food Agric 2010; 90(4): 608-14. [PMID: 20355088]

[9] Iram W, Anjum T, Iqbal M, Ghaffar A, Abbas M. Mass spectrometric identification and toxicity assessment of degraded products of aflatoxin B_1 and B_2 by *Corymbia citriodora* aqueous extracts. Sci Rep 2015; 5: 14672. [http://dx.doi.org/10.1038/srep14672] [PMID: 26423838]

[10] Youssef MM, Pham Q, Achar PN, Sreenivasa MY. Antifungal activity of essential oils on *Aspergillus parasiticus* isolated from peanuts. J Plant Prot Res 2016; 56: 139-42. [http://dx.doi.org/10.1515/jppr-2016-0021]

[11] Reddy KRN, Reddy CS, Muralidharan K. Potential of botanicals and biocontrol agents on growth and aflatoxin production by *Aspergillus flavus* infecting rice grains. Food Control 2009; 20: 173-8. [http://dx.doi.org/10.1016/j.foodcont.2008.03.009]

[12] da Cruz Cabral L, Fernández Pinto V, Patriarca A. Application of plant derived compounds to control fungal spoilage and mycotoxin production in foods. Int J Food Microbiol 2013; 166(1): 1-14. [http://dx.doi.org/10.1016/j.ijfoodmicro.2013.05.026] [PMID: 23816820]

[13] Soliman KM, Badeaa RI. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem Toxicol 2002; 40(11): 1669-75. [http://dx.doi.org/10.1016/S0278-6915(02)00120-5] [PMID: 12176092]

[14] Carmo ES, de Oliveira Lima E, de Souza EL. The potential of *Origanum vulgare* L. (Lamiaceae) essential oil in inhibiting the growth of some food-related *Aspergillus* species. Braz J Microbiol 2008; 39(2): 362-7. [http://dx.doi.org/10.1590/S1517-83822008000200030] [PMID: 24031231]

[15] Soković M, Stojković D, Glamočlija J, Ciric A, Ristic M, Grubisic D. Susceptibility of pathogenic bacteria and fungi to essential oils of wild *Daucus carota.* Pharm Biol 2009; 47: 38-43. [http://dx.doi.org/10.1080/13880200802400535]

[16] Prakash B, Singh P, Kedia A, Dubey NK. Assessment of some essential oils as food preservatives based on antifungal, antiaflatoxin,

antioxidant activities and *in vivo* efficacy in food system. Food Res Int 2012; 49: 201-8.
[http://dx.doi.org/10.1016/j.foodres.2012.08.020]

[17] de Sousa LL, de Andrade SCA, Athayde AJAA, *et al.* Efficacy of *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils in combination to control postharvest pathogenic *Aspergilli* and autochthonous mycoflora in *Vitis labrusca* L. (table grapes). Int J Food Microbiol 2013; 165(3): 312-8.
[http://dx.doi.org/10.1016/j.ijfoodmicro.2013.06.001] [PMID: 23810954]

[18] Cardile V, Russo A, Formisano C, *et al.* Essential oils of *Salvia bracteata* and *Salvia rubifolia* from Lebanon: Chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. J Ethnopharmacol 2009; 126(2): 265-72.
[http://dx.doi.org/10.1016/j.jep.2009.08.034] [PMID: 19715748]

[19] Nychas GJE. Natural antimicrobials from plants.New Methods of Food Preservation. Glasgow: Blackie Academic and Professional 1995; pp. 59-89.
[http://dx.doi.org/10.1007/978-1-4615-2105-1_4]

[20] Marei GIK, Abdel Rasoul MA, Abdelgaleil SAM. Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi. Pestic Biochem Physiol 2012; 103: 56-61.
[http://dx.doi.org/10.1016/j.pestbp.2012.03.004]

[21] Salomé-Abarca LF, Soto-Hernández RM, Cruz-Huerta N, González-Hernández VA. Chemical composition of scented extracts obtained from *Calendula officinalis* by three extraction methods. Bot Sci 2015; 93: 633-8.
[http://dx.doi.org/10.17129/botsci.143]

[22] Dima C, Dima S. Essential oils in foods: extraction, stabilization, and toxicity. Curr Opin Food Sci 2015; 5: 29-35.
[http://dx.doi.org/10.1016/j.cofs.2015.07.003]

[23] Kitic D, Stojanovic G, Palic R, Randjelovic V. Chemical composition and microbial activity of the essential oil of *Calamintha nepeta* (L.) Savi ssp. *nepeta* var. *subisodonda* (Borb.) Hayek from Serbia. J Essent Oil Res 2005; 17: 701-3.
[http://dx.doi.org/10.1080/10412905.2005.9699036]

[24] Vilela GR, de Almeida GS, D'Arce MABR, *et al.* Activity of essential oil and its major compound, 1,8-cineole, from *Eucalyptus globulus* Labill., against the storage fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. J Stored Prod Res 2009; 45: 108-11.
[http://dx.doi.org/10.1016/j.jspr.2008.10.006]

[25] Burt S. Essential oils: their antibacterial properties and potential applications in foods: A review. Int J Food Microbiol 2004; 94(3): 223-53.
[http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.022] [PMID: 15246235]

[26] Tian J, Ban X, Zeng H, He J, Huang B, Wang Y. Chemical composition and antifungal activity of essential oil from *Cicuta virosa* L. var. *latisecta* Celak. Int J Food Microbiol 2011; 145(2-3): 464-70.
[http://dx.doi.org/10.1016/j.ijfoodmicro.2011.01.023] [PMID: 21320730]

[27] Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Rezaee M-B, *et al.* Chemical composition and antiaflatoxigenic activity of *Carum carvi* L., *Thymus vulgaris* and *Citrus aurantifolia* essential oils. Food Control 2009; 20: 1018-24.
[http://dx.doi.org/10.1016/j.foodcont.2008.12.007]

[28] Nogueira JHC, Gonçalez E, Galleti SR, Facanali R, Marques MOM, Felício JD. *Ageratum conyzoides* essential oil as aflatoxin suppressor of *Aspergillus flavus.* Int J Food Microbiol 2010; 137(1): 55-60.
[http://dx.doi.org/10.1016/j.ijfoodmicro.2009.10.017] [PMID: 19906457]

[29] Rasooli I, Fakoor MH, Yadegarinia D, Gachkar L, Allameh A, Rezaei MB. Antimycotoxigenic characteristics of *Rosmarinus officinalis* and *Trachyspermum copticum* L. essential oils. Int J Food Microbiol 2008; 122(1-2): 135-9.
[http://dx.doi.org/10.1016/j.ijfoodmicro.2007.11.048] [PMID: 18190993]

[30] Pinto E, Pina-Vaz C, Salgueiro L, *et al.* Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida, Aspergillus* and dermatophyte species. J Med Microbiol 2006; 55(Pt 10): 1367-73.
[http://dx.doi.org/10.1099/jmm.0.46443-0] [PMID: 17005785]

[31] Omidbeygi M, Barzegar M, Hamidi Z, Naghdibadi H. Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. Food Control 2007; 18: 1518-23.
[http://dx.doi.org/10.1016/j.foodcont.2006.12.003]

[32] Kedia A, Prakash B, Mishra PK, Dubey NK. Antifungal and antiaflatoxigenic properties of *Cuminum cyminum* (L.) seed essential oil and its efficacy as a preservative in stored commodities. Int J Food Microbiol 2014; 168-169: 1-7.
[http://dx.doi.org/10.1016/j.ijfoodmicro.2013.10.008] [PMID: 24211773]

[33] Lambert RJ, Skandamis PN, Coote PJ, Nychas GJ. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol 2001; 91(3): 453-62.
[http://dx.doi.org/10.1046/j.1365-2672.2001.01428.x] [PMID: 11556910]

[34] Kulisic T, Radonic A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem 2004; 85: 633-40.
[http://dx.doi.org/10.1016/j.foodchem.2003.07.024]

[35] Kumar R, Mishra AK, Dubey NK, Tripathi YB. Evaluation of *Chenopodium ambrosioides* oil as a potential source of antifungal, antiaflatoxigenic and antioxidant activity. Int J Food Microbiol 2007; 115(2): 159-64.
[http://dx.doi.org/10.1016/j.ijfoodmicro.2006.10.017] [PMID: 17174000]

[36] Razzaghi-Abyaneh M, Yoshinari T, Shams-Ghahfarokhi M, Rezaee M-B, Nagasawa H, Sakuda S. Dillapiol and Apiol as specific inhibitors of the biosynthesis of aflatoxin G_1 in *Aspergillus parasiticus.* Biosci Biotechnol Biochem 2007; 71(9): 2329-32.
[http://dx.doi.org/10.1271/bbb.70264] [PMID: 17827697]

[37] Alinezhad S, Kamalzadeh A, Shams-Ghahfarokhi M, *et al.* Search for novel antifungals from 49 indigenous medicinal plants: *Foeniculum vulgare* and *Platycladus orientalis* as strong inhibitors of aflatoxin production by *Aspergillus parasiticus.* Ann Microbiol 2011; 61: 673-81.
[http://dx.doi.org/10.1007/s13213-010-0194-1]

[38] Shukla R, Kumar A, Singh P, Dubey NK. Efficacy of *Lippia alba* (Mill.) N.E. Brown essential oil and its monoterpene aldehyde constituents against fungi isolated from some edible legume seeds and aflatoxin B_1 production. Int J Food Microbiol 2009; 135(2): 165-70.
[http://dx.doi.org/10.1016/j.ijfoodmicro.2009.08.002] [PMID: 19726096]

[39] Srivastava B, Sagar A, Dubey NK. Evaluation of *Cinnamomum tamala* oil and its phenylpropanoid eugenol for their antifungal and antiaflatoxigenic activity. Food Anal Methods 2011; 4: 347-56.
[http://dx.doi.org/10.1007/s12161-010-9177-x]

[40] Singh G, Maurya S, DeLampasona MP, Catalan CAN. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food Chem Toxicol 2007; 45(9): 1650-61.
[http://dx.doi.org/10.1016/j.fct.2007.02.031] [PMID: 17408833]

[41] Kedia A, Prakash B, Mishra PK, Chanotiya CS, Dubey NK. Antifungal, antiaflatoxigenic, and insecticidal efficacy of spearmint (*Mentha spicata* L.) essential oil. Int Biodet Biodegr 2014; 89: 29-36.
[http://dx.doi.org/10.1016/j.ibiod.2013.10.027]

[42] Apisariyakul A, Vanittanakom N, Buddhasukh D. Antifungal activity of turmeric oil extracted from *Curcuma longa* (Zingiberaceae). J Ethnopharmacol 1995; 49(3): 163-9.
[http://dx.doi.org/10.1016/0378-8741(95)01320-2] [PMID: 8824742]

[43] Khattak S, Saeed-ur-Rehman , Ullah Shah H, Ahmad W, Ahmad M. Biological effects of indigenous medicinal plants Curcuma longa and Alpinia galanga. Fitoterapia 2005; 76(2): 254-7.
[http://dx.doi.org/10.1016/j.fitote.2004.12.012] [PMID: 15810156]

[44] Ferreira FD, Kemmelmeier C, Arrotéia CC, *et al.* Inhibitory effect of the essential oil of Curcuma longa L. and curcumin on aflatoxin production by Aspergillus flavus Link. Food Chem 2013; 136(2): 789-93.
[http://dx.doi.org/10.1016/j.foodchem.2012.08.003] [PMID: 23122128]

[45] Gemeda N, Woldeamanuel Y, Asrat D, Debella A. Effect of essential oils on *Aspergillus* spore germination, growth and mycotoxin production: A potential source of botanical food preservative. Asian Pac J Trop Biomed 2014; 4(Suppl. 1): S373-81.
[http://dx.doi.org/10.12980/APJTB.4.2014C857] [PMID: 25183114]

[46] Vijayanandraj S, Brinda R, Kannan K, *et al.* Detoxification of aflatoxin B_1 by an aqueous extract from leaves of *Adhatoda vasica* Nees. Microbiol Res 2014; 169(4): 294-300.
[http://dx.doi.org/10.1016/j.micres.2013.07.008] [PMID: 23928380]

[47] Prakash B, Mishra PK, Kedia A, Dubey NK. Antifungal, antiaflatoxin and antioxidant potential of chemically characterized *Boswellia carterii* Birdw essential oil and its *in vivo* practical applicability in preservation of *Piper nigrum* L. fruits. Lebensm Wiss Technol 2014; 56: 240-7.
[http://dx.doi.org/10.1016/j.lwt.2013.12.023]

[48] Food and Drug Administration. Code of Federal Regulations (CFR). Title 21: Food and drugs. Chapter I - food and drug administration, department of health and human services, subchapter B - food for human consumption (continued), Part 182 - substances generally recognized as safe (GRAS), subpart a - general provisions, subpart 182.20 - essential oils, oleoresins, and natural extractives. Office of the Federal Register, Washington 2016.

[49] Tian J, Ban X, Zeng H, Huang B, Jingsheng H, Wang Y. *In vitro* and *in vivo* activity of essential oil from dill (*Anethum graveolens* L.) against fungal spoilage of cherry tomatoes. Food Control 2011; 22: 1992-9.
[http://dx.doi.org/10.1016/j.foodcont.2011.05.018]

[50] Feng W, Zheng X. Essential oils to control *Alternaria alternata in vitro* and *in vivo.* Food Control 2007; 18: 1126-30.
[http://dx.doi.org/10.1016/j.foodcont.2006.05.017]

[51] Anžlovar S, Likar M, Koce JD. Antifungal potential of thyme essential oil as a preservative for storage of wheat seeds. Acta Bot Croat 2017; 76: 64-71.
[http://dx.doi.org/10.1515/botcro-2016-0044]

[52] Ribeiro-Santos R, Andrade M, Melo N. Use of essential oils in active food packing: Recent advances and future trends. Trends Food Sci Technol 2017; 61: 132-40.
[http://dx.doi.org/10.1016/j.tifs.2016.11.021]

[53] Zabka M, Pavela R, Slezakova L. Antifungal effect of *Pimenta dioica* essential oil against dangerous pathogenic and toxinogenic fungi. Ind Crops Prod 2009; 30: 250-3.
[http://dx.doi.org/10.1016/j.indcrop.2009.04.002]

[54] Azizkhani M, Tooryan F. Antioxidant and antimicrobial activities of rosemary extract, mint extract and a misture of tocopherols in beef sausage during storage at 4°C. J Food Saf 2015; 35: 128-36.
[http://dx.doi.org/10.1111/jfs.12166]

[55] Vilela J, Martins D, Monteiro-Silva F, Gonzalez-Aguilar G, Almeida JMMM, Saraiva C. Antimicrobial effect of essential oils of *Laurus nobilis* L. and *Rosmarinus officinallis* L. on shelf-life of minced "Maronesa" beef stored under different packaging conditions. Food Packag Shelf Life 2016; 8: 71-80.
[http://dx.doi.org/10.1016/j.fpsl.2016.04.002]

[56] Atanda OO, Akpan I, Oluwafemi F. The potential of some spice essential oils in the control of *A. parasiticus* CFR 223 and aflatoxin production. Food Control 2007; 18: 601-7.
[http://dx.doi.org/10.1016/j.foodcont.2006.02.007]