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Prevention by Essential Oils of the Occurrence and Growth of *Aspergillus flavus* and Aflatoxin B1 Production in Food Systems: Review

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Abstract

Aspergillus flavus has been reported to be one of the most common fungal species in foods. Under conditions of high humidity and moderate temperature, this fungus may synthesize the mycotoxin Aflatoxin B1 (AFB1), which is reported to be hepatotoxic, teratogenic, mutagenic and immunosuppressive to human beings and livestock and it is classified as carcinogenic to humans (Group 1 by IARC). AFB1 affects cereals, oilseeds, nuts, spices, legumes, and dried fruits, while Aflatoxin M1 is a metabolite of AFB1 that can occur in milk and milk products. Current control is aimed at controlling fungal growth and AFB1 production in food by eco-friendly, biodegradable and safer alternatives, in contrast to synthetic chemicals that can be toxic to humans and cause adverse environmental effects. Recently, considerable attention has been directed towards natural compounds, such as essential oils (EOs) as a promising approach for controlling AFB1 production in food. The main reason for supporting the application of natural products is the consumer's preference for natural methods to preserve foods. The aim of the present review is to summarize knowledge of EOs and AFB1 production from the literature.

Keywords: *Aspergillus flavus*, aflatoxin B1 (AFB1), essential oils, food system

1. Introduction

Molds are ubiquitous micro-organisms with a high capacity to colonize different types of substrates and to proliferate under extreme environmental conditions [1]. They alter various types of foods namely cereals, nuts, oil seeds, legumes, spices, vegetables, fruits, etc. and some species produce mycotoxins. Of all mycotoxins, Aflatoxin B1 (AFB1) produced primarily by *Aspergillus flavus* and *A. parasiticus* is the most toxic form (hepatotoxic, teratogenic, mutagenic and carcinogenic) for humans and animals [2–5]. The health impact of this compound has justified the introduction of a consumer protection measure by the establishment of maximum levels in certain food categories. However, the existence of

these standards also has significant economic repercussions restricting trade from certain areas where the contamination is frequent and strongly decreasing the economic value of some productions in case of contamination. These losses are estimated by FAO (Food and Agriculture Organization of The United Nations) at about 1 billion tons a year. Therefore, it seems imperative to develop ways of combating contamination of food with these toxic compounds and/or to limit their harmful effects.

Pesticides and fungicides have been widely used to prevent the development of fungal agents. However, because of their own toxicity, their use is subjected to certain restrictions. Biological control is also a possible option. Thus, antiflatoxigenic bacterial and fungal strains were found effective in reducing the development of toxic strains of *A. flavus*. However, none of these strategies seems to be able to solve the problem of contamination of raw materials by aflatoxins, as evidenced by the numerous researches that show high levels of contamination, particularly in areas where the climate is favorable for the development of the toxigenic fungal species. At present, there is growing interest in identifying natural compounds able to limit the growth and/or the production of mycotoxins. Thus, the use of essential oils (EOs) of plants or spices could show their ability to limit food contamination. The aim of this review is to summarize the results from the literature on the effects of EOs against *A. flavus* growth and AFB1 production.

2. Aflatoxins

Aflatoxins are produced primarily by the common fungus *Aspergillus flavus* and the closely related species *A. parasiticus*, and they can be found in feedstuffs and foodstuffs throughout the world. These mycotoxins are a family of several molecules of which the most important are AFB1, B2, G1 and G2 (**Figure 1**). The letters refer to the fluorescence blue (B) or green (G) under UV and the numbers (1 or 2) refer to their relative chromatographic mobility. They are molecules with a polycyclic structure belonging to the furanocoumarin class.

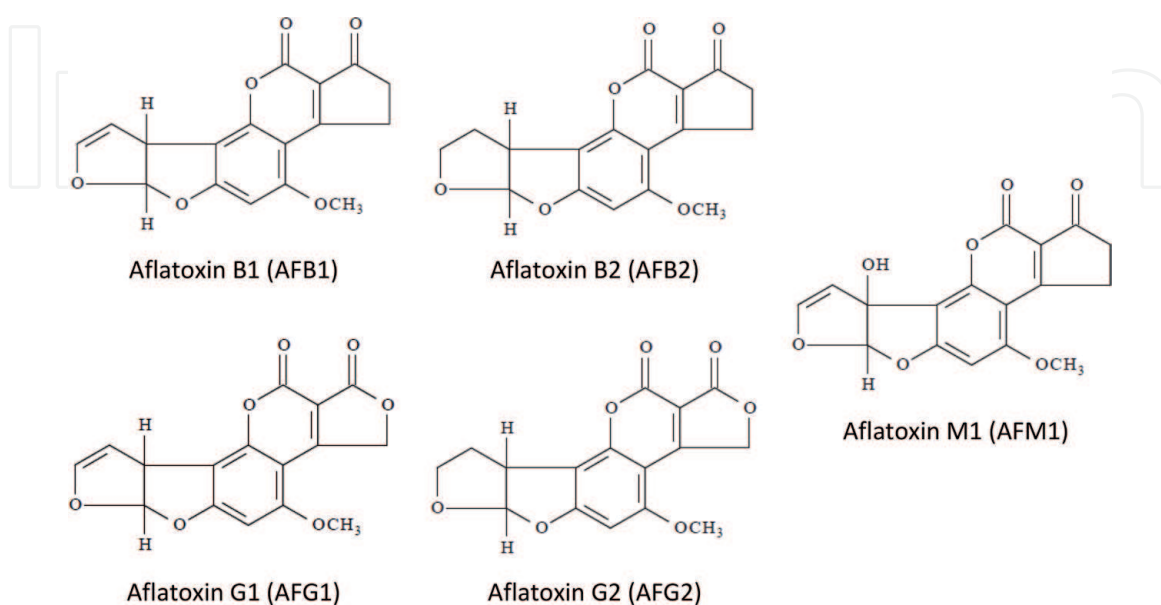


Figure 1.
Chemical structures of AFB1, B2, M1, G1 and G2.

There are also other aflatoxins; there are in total more than a dozen (M_1 , P_1 , Q_1 , B_{2a} , G_{2a} , etc.). The most are products resulting from the metabolic metabolism of the four molecules produced by mold in food [6]. For example, after ingestion of contaminated feed, AFB1 is transformed into the liver of mammals by cytochrome P450 into several metabolites, mainly AFM1 excreted in milk, which is produced by hydroxylation of the tertiary carbon of the difuranocoumarin cycle. The hydroxyl group formed makes AFM1 more soluble in water and therefore rapidly excreted in mammalian milk, urine, bile and feces. The AFM1 owes its name to its presence in Milk [7]. Aflatoxins are stable molecules and very resistant to the various processes of food processing such as roasting, extrusion and cooking [8]. Indeed, the decomposition of aflatoxins takes place at very high temperatures which are difficult to comply with the manufacturing or processing processes of food. *A. flavus* and *A. parasiticus* are the main aflatoxin producers; *A. flavus* is responsible for the production of type B aflatoxins, while *A. parasiticus* produces both type B and G [9]. Aflatoxins can contaminate cereals, oil seeds, nuts, spices, legumes, dried fruits, milk and milk products, posing a high risk to public health [10–13]. Maize, peanuts and tree nuts (i.e. pistachios) are susceptible to aflatoxin contamination in the field, while other cereals, oilseeds and dried fruits are mostly contaminated at postharvest and during storage [14–16].

3. Methods of aflatoxin decontamination

Foodstuffs should not be hazardous to consumer health; as consequence, elimination of mycotoxin from products is a challenge for the food industry. Concerns have been directed towards aflatoxins because of their global threat and toxicity. Most of the factors obtained from studies on aflatoxins can be applied to other mycotoxins. Although prevention is the most effective intervention, chemical, physical and biological methods have been investigated to eliminate aflatoxins or reduce them (Figure 2). However, these techniques are not completely safe, are expensive and not well preferred by consumers.

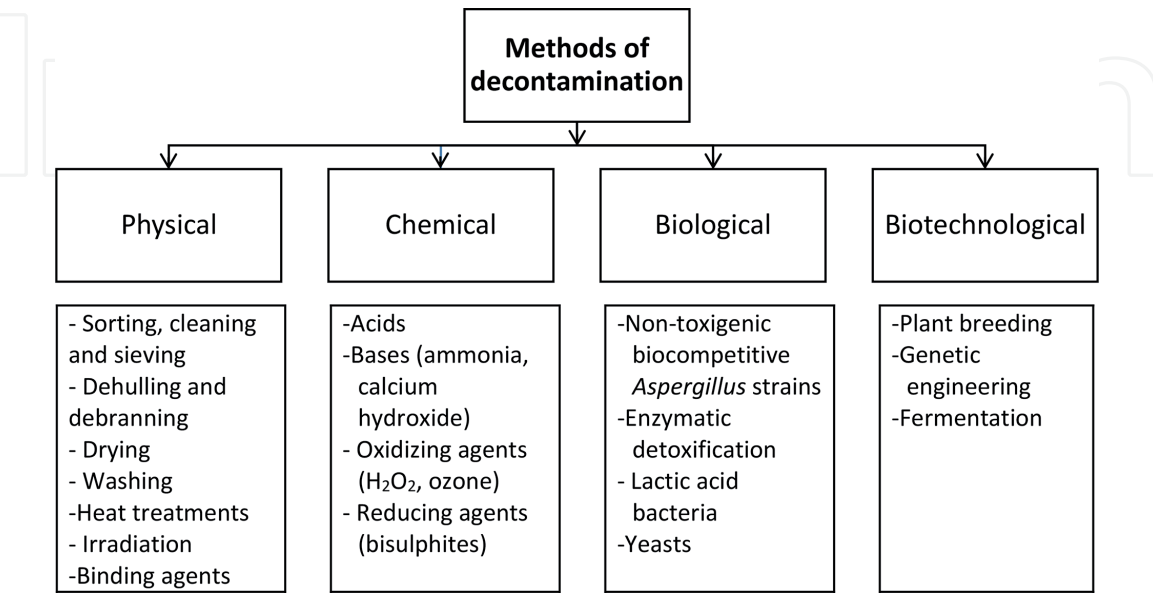


Figure 2.
Some commonly used physical, chemical and biological methods of aflatoxin detoxification.

4. Essential oils: an alternative strategy for control against aflatoxin contamination

The frequency of contamination of world crops by aflatoxins shows that the strategies currently used are insufficient to guarantee the security of the foods and that it is necessary to develop others, as a complement or substitution of those already existing. In this context, strategies based on the use of compounds naturally recognized as not harmful to the environment and to health, seem interesting. Indeed, plants produce different secondary metabolites (terpenoids, phenolic compounds, etc.) for their protection against external agents (mechanical, biological or climatic). These compounds could possibly be used as a means of combating fungal contamination and/or mycotoxins [17].

4.1 Essential oils

4.1.1 Overview

EOs are a mixture of volatile compounds (secondary metabolites) isolated from plants mainly by hydro-distillation. They are mostly consisting of mono- and sesquiterpenes but may also contain non-terpenoid hydrocarbons, phenylpropanoids, esters, lactones, phthalides, nitrogen or sulfurized structures and isothiocyanates. They are lipophilic compounds which are distinguished by their aromatic properties, hence their use as flavorings or perfumes [18]. In addition, certain compounds are also used for their many biological activities: bactericide, fungicide and antioxidant [19].

4.1.2 Mechanism of cellular action of essential oils

The mode of action of EOs has not been completely understood yet [20, 21]. In general, EOs actions are described in three phases. Firstly, EOs spreading on the cell wall of fungi changes the membrane permeability resulting in the loss of cellular components. Secondly, an acidification inside the cell that blocks the production of cellular energy (ATP) due to ion loss, the collapse of proton pumps, the reduction of membrane potential, and destruction of genetic materials that leads to the death of fungus. Furthermore, some reports have indicated that EOs can also coagulate the cytoplasm and damage lipids, proteins, cell walls and membranes that can lead to the leakage of macromolecules and the lysis [22–27].

Phenolic compounds are known to affect microbial cell permeability, allowing the loss of macromolecules from the interior. They could also interact with membrane proteins, causing a deformation in their structure and functionality [28].

4.1.3 Use of essential oils as antifungal and antiaflatoxigenic agents

In view of their different biological properties, EOs have been tested as alternative strategy for combating mycotoxins, especially aflatoxins [29–34] (**Table 1**). EOs are molecules of natural origin, biodegradable, and are therefore considered as a possible alternative to synthetic pesticides [35]. Their use as food additives or flavorings has recently been authorized in the USA [36]. As their active components are highly volatile, they are mainly used as fumigants for products after harvest. A number of commercially available EOs can be used in crops produced according to the specifications such as E-Rase™ (jojoba EO, *Simmondsia californica*), Sporan™ (rosemary EO, *Rosmarinus officinalis*), Promax™ (thyme EO, *Thymus vulgaris*) [37],

Plant scientific name	Plant common name	Applied concentrations in culture medium	Inhibition of <i>A. flavus</i> growth	Inhibition of AFB1 production	Reference
<i>Ageratum conyzoides</i>	Mentrasito	0.10 µg/mL 0.04 µg/ mL	49.00% 30.00%	100.00% 65.00%	[1]
<i>Pimpinella anisum</i>	Anise	0.50 µg/g	100.00%	100.00%	[31]
<i>Ocimum sanctum</i>	Holy basil	0.10 µg/ mL 0.20 µg/ mL	72.50% 90.10%	88.40% 100.00%	[63]
<i>Ocimum sanctum</i>	Holy basil	0.10 µL/ mL 0.40 µL/ mL	72.25% 100.00%	82.43% 100.00%	[44]
<i>Piper betle</i>	Betel	0.40 µL/ mL 0.60 µL/ mL	26.70% 77.80%	74.50% 100.00%	[3]
<i>Callistemon lanceolatus</i>	Callistemon	0.546 mg/ mL 0.819 mg/ mL	79.60% 100.00%	100.00% 100.00%	[53]
<i>Cinnamomum jensenianum</i>	Cinnamon	4.00 µL/ mL 6.00 µL/ mL	63.00% 84.00%	84.00% 100.00%	[34]
<i>Amomum subulatum</i>	Cardamom	0.25 mg/ mL 0.50 mg/ mL	25.00% 74.00%	69.00% 100.00%	[64]
<i>Cicuta virosa</i>	Water hemlock	1.00 µL/ mL 4.00 µL/ mL	60.00% 65.70%	31.90% 100.00%	[51]
<i>Cymbopogon citratus</i>	Lemongrass	0.20 mg/ mL	3.00%	100.00%	[32]
<i>Coriandrum sativum</i>	Coriander	0.75 µL/ mL	66.50%	25.00%	[50]
<i>Cuminum cyminum</i>	Cumin	0.40 µL/ mL 0.50 µL/ mL	52.00% 91.00%	67.00% 100.00%	[31]
<i>Curcuma longa</i>	Turmeric	0.50% (v/v)	nd	99.00%	[59]
<i>Foeniculum vulgare</i>	Fennel	0.75 µL/ mL	54.40%	23.00%	[52]
<i>Mentha spicata</i>	Mint	0.60 µL/ mL 0.90 µl/ mL	66.40% 84.90%	50.20% 100.00%	[65]
<i>Cymbopogon martini</i>	Palmarosa-Indian geranium	0.30 µL/ mL 0.40 µL/ mL	50.00% 95.00%	80.00% 100.00%	[66]
<i>Citrus sinensis</i>	Orange	250.00 ppm 1000.00 ppm	18.20% 100.00%	68.32% 100.00%	[49]
<i>Citrus maxima</i>	Pomelo	250.00 ppm 1000.00 ppm	23.40% 100.00%	67.21% 100.00%	
<i>Rosmarinus officinalis</i>	Rosemary	0.45% (v/v)	0.00%	100.00%	[33]
<i>Thymus vulgaris</i>	Thyme	0.30 µL/ mL 0.70 µL/ mL	46.90% 100.00%	75.60% 100.00%	[67]
<i>Thymus daenensis</i>	Thyme	62.50 mg/L 500.00 mg/L	6.45% 100.00%	17.83% 100.00%	
<i>Satureja khozistanica</i>	Sater	62.50 mg/L 500.00 mg/L	12.38% 100%	18.53% 100.00%	[68]
<i>Satureja macrosiphonia</i>	Sater	62.50 mg/L 500.00 mg/L	3.07% 65.22%	10.22% 73.96%	
<i>Lippia rugosa</i>	Verbenacea	600.00 µg/mL 1000.00 µg/mL	5.00% 5.00%	61.00% 100.00%	[48]

Table 1.
Inhibition effect of some EOs on the growth of *A. flavus* and the production of AFB1.

Cinnamite™ and Valero™ (cinnamon EO, *Cinnamomum verum*) [19], Talent® (based on carvone, EO of cumin or dill) and eugenol-Tween® [38].

The antifungal activity of EOs of *T. vulgaris* and camphor (*Eucalyptus rostrata* L.) was tested on *A. flavus*. They completely inhibited mycelial growth of the fungus at 1000 and 2000 ppm (mg/kg), and aflatoxin production at 500 and 1000 ppm, respectively [39].

The EO of *Satureja hortensis* was assessed for antifungal activity against *A. flavus* *in vitro* on solid and liquid culture, and under storage conditions. The EO showed strong antifungal activity based on the inhibition zone and minimal inhibitory concentration (MIC) values on solid culture. The very low concentrations of EOs also reduced wet and dry mycelium weight of *A. flavus* in liquid culture [40]. The antifungal potential of EOs of leaves and seeds of *Aframomum daniellii*, *Aframomum melegueta* and *Aframomum latifolium* and those of husk of *A. latifolium* was evaluated against *A. flavus*. Results showed that the inhibition of tested EOs were between 28.06 and 100%, respectively for EOs of seeds of *A. melegueta* and EO of the husk of *A. latifolium* at 500 ppm. EO of leaves of *A. melegueta* and husk of *A. latifolium* exhibited the most effective inhibition [41]. EOs extracted from *Mentha arvensis* (0.5 mg/mL) caused total inhibition of *A. flavus* growth and of AFB₁ production [42]. *Cymbopogon martinii*, *Foeniculum vulgare* and *Trachyspermum ammi* EOs were tested against *A. flavus*. The MIC was recorded at 1 µL/mL for *T. ammi* EO and 4 µL/mL for *C. martinii* and *F. vulgare*. At these concentrations, the EOs completely inhibited the growth of *A. flavus* [43].

Ageratum conyzoides EO inhibited *A. flavus* growth and reduced AFB₁ production at 0.10 µg/mL. The ultra-structural changes in *A. flavus* cell under transmission electron microscopy (TEM) were observed on the endomembrane system, mainly the mitochondria. The plasma membrane cells lost its linear aspect. The fibrillar layers lost their building and failed to deposit on the cell wall. The mitochondria in treated cells suffered a disruption of the internal structure [1]. *Ocimum sanctum* EO was evaluated to inhibit *A. flavus* growth and AFB₁ production. The MIC of *O. sanctum* against *A. flavus* was found at 0.3 µL/mL while AFB₁ production was recorded at 0.2 µL/mL [44]. The efficacy of boldo (*Peumus boldus* Mol.), poleo (*Lippia turbinata* var. *integrifolia* (Griseb.)), clove (*Syzygium aromaticum* L.), anise (*Pimpinella anisum*), and thyme (*T. vulgaris*) EOs was tested against *A. flavus* and *A. parasiticus* growth and AFB₁ at different water activity (a_w) levels (0.98, 0.95, 0.93). Inhibition was estimated at 75, 83, and 100% at 0.98, 0.95, and 0.93 a_w , respectively [45].

The effects of EOs of *Ocimum gratissimum* and *Plectranthus glandulosus* leaves on growth and AFB₁ production by *A. flavus* were assessed at five levels (200, 400, 600, 800 and 1000 mg/L). Growth of *A. flavus* was completely inhibited by 800 mg/L of *O. gratissimum* EO and by 1000 mg/L of *P. glandulosus* EO. The AFB₁ production was inhibited by 1000 mg/L of both EOs of *O. gratissimum* and *P. glandulosus* [46]. *Cinnamomum camphora* and *Alpinia galanga* EOs inhibited *A. flavus* at 1000 ppm and the AFB₁ production at 500 ppm for *A. galanga* and 750 ppm for *C. camphora*. The combination of the two EOs was more effective than the individual ones. The mixture showed total inhibition of the mycelium growth at 750 ppm and AFB₁ production starting from 250 ppm [47]. *Lippia rugosa* EO was effective against fungal growth and production of AFB₁ from *A. flavus* was totally inhibited at 1000 mg/L. The inhibition was attributed to the presence of geraniol [48].

Both *A. flavus* growth and AFB₁ production were inhibited by EOs of *Citrus maxima* Burm, *Citrus sinensis* (L.) Osbeck, and their combination. DL-Limonene inhibited AFB₁ production at a concentration of 250 ppm that is lower than the individual EOs and the combination. It has been suggested that remaining constituents in the EO would mask the efficacy of DL-limonene and they may act in negatively.

Furthermore, the authors declared that there was no synergism between the EO constituents when the two EOs were mixed at the same concentration, attaining aflatoxin inhibition at 500 ppm [49].

Similar types of results were also found in the case of *Piper betle* var. *magahi* EO, where eugenol was more effective as inhibitor of fungal growth and aflatoxin production than the whole EO. It was suggested that the components of the EO acted synergistically in negative direction and diminished the activity of eugenol [3]. The effect of basil, fennel, coriander, caraway, peppermint and rosemary EOs on *A. flavus* growth and AFB1 production at 500, 750 and 1000 ppm was also studied. The complete inhibition of *A. flavus* growth was observed at 1000 ppm concentrations of basil, coriander, caraway and rosemary EOs. While, EOs of basil and coriander showed interesting inhibition of AFB1 at all concentrations [50].

Cicuta virosa L. EO inhibited the growth of *A. flavus* and AFB1 production at 4 $\mu\text{L/mL}$ of EO. The AFB1 was reduced to about half compared to the control at 2 $\mu\text{L/mL}$ [51]. Similar type of results was found with *Cinnamomum jensenianum* EO, which reduced AFB1 to about half compared to the control at 2 $\mu\text{L/mL}$. At this concentration of the EO, the plasma membrane of *A. flavus* became rough with continuous folding into the cytoplasm and festooned with small lomasomes. A decreased cytoplasmic matrix was also observed. They showed that some mitochondria suffered extensive disruption of the internal structure with a decrease in mitochondrial cristae. The cell ultrastructure damage was aggravated when the EO concentration was doubled. Major alterations were observed, including massive vacuolation of cytoplasm with vacuole fusion, appearance of numerous lomasomes with folding, and detachment of plasma membrane from the cell wall. The fibrillar layers gradually lost their integrity, becoming thinner, and eventually failing to deposit on the cell wall. The plasma membrane was also folded at many sites. The cytoplasmic matrix and some cytoplasmic organelles were absent. Moreover, the mitochondria suffered a severe disruption of the internal structure with complete lysis. The antifungal mode of action of EO was evaluated by quantification of the ergosterol production in cells. At concentration of 2 $\mu\text{L/mL}$, the ergosterol content in the plasma membrane of *A. flavus* was significantly reduced by the different concentrations of EO. A dose dependent decrease in ergosterol production was observed when isolates were grown in the presence of the EO. Therefore, this EO exerts its effect directly on the plasma membrane without any obvious damage to the cell wall. This emphasizes that the antimicrobial components of the EOs cross the cell membrane, interact with the enzymes and proteins of the membrane, thus producing a flux of protons towards the cell exterior which induces disruption to the fungal cell organization and, ultimately, their death [34].

The EO of *Anethum graveolens* was evaluated on *A. flavus*. Morphological changes in the cells of *A. flavus* and a reduction in the ergosterol quantity was caused by *A. graveolens* EO. An augmentation of mitochondrial membrane potential (MMP), and the suppression of the glucose-induced decrease in external pH were observed at concentration of 4 mL/mL . A decrease of the activities of ATPase and dehydrogenase in *A. flavus* cells were also observed. The authors attributed the dysfunctions of the mitochondria to the reactive oxygen species (ROS) accumulation in *A. flavus*. The addition of L-cysteine caused a reduction in cell viability, which indicates that ROS is an interesting mediator of the antifungal activity of *A. graveolens* EO [52].

The *Callistemon lanceolatus* (Sm.) Sweet EO inhibited AFB1 production at concentrations lower than its fungitoxic concentration [53]. As well, the EO of *Lantana indica* showed *in vitro* antifungal and antiaflatoxigenic activities against *A. flavus*. The antifungal activity of the *L. indica* EO, tested by disk diffusion test and by SMKY liquid culture, completely inhibited mycelia growth and AFB1 production

at 1.5 and 0.75 µg/mL, respectively [54]. The antifungal and antiaflatoxigenic activities of *Litsea cubeba* EO were tested on *A. flavus*. The EO showed a high activity against three toxigenic isolates of *A. flavus*. Under the scanning electron microscopy (SEM), the EO showed alterations of the hyphae and conidiophores structures. The results exhibited that *L. cubeba* EO could inhibit fungal growth and AFB₁ production [55]. *Caesulia axillaris* Roxb EO showed complete inhibition of the fungal growth at 1.0 µL/mL and AFB₁ at 0.8 µL/mL. Thus, the EO showed antiaflatoxigenic effects at concentration lower than their fungitoxic concentrations indicating two different mode of action for inhibition of fungal growth and AFB₁ production [56]. Jamrosa EO (*Cymbopogon khasans*) showed antifungal activity and inhibition of AFB₁ production at 0.4 µL/mL. The authors indicated that inhibition of AFB₁ production was secondary to inhibition of fungal growth [57]. The capacity of 14 EO components and their combinations to inhibit fungal growth and AFB₁ production revealed that thymol, eugenol, menthol, and their combinations were more effective for inhibition of fungal growth and AFB₁ was completely inhibited at 1.0 µL/mL. Geranyl acetate, linalool, β-asarone, 1, 8-cineol, and E-citral were moderately antifungal between 1.0 and 5.0 µL/mL [58].

The inhibition of AFB₁ production by *A. flavus* was observed at 1.0 and 42.7 µL/mL, respectively, for the samples treated with the EO of *Curcuma longa* L. and curcumin at a concentration of 0.5%. The authors suggested that the antiaflatoxigenic activity might be attributed to phenolic compounds that inhibit lipid peroxidation. Thus, the antioxidant activity of *C. longa* could be important for the inhibition of AFB₁ production [59]. The *Boswellia carterii* Birdw EO inhibited *A. flavus* growth and AFB₁ production at 1.75 and 1.25 µL/mL, respectively. The EO caused reduction in ergosterol content of plasma membrane of *A. flavus* [60]. This observation showed that plasma membrane is an interesting site for the mechanism of EO supporting the findings of other authors [51]. The *Cuminum cyminum* L. EO was tested on *A. flavus* growth and AFB₁ production. The EO inhibited the fungal growth and AFB₁ production at 0.6 and 0.5 µL/mL, respectively. The EO totally reduced the ergosterol content at 0.6 µL/mL [31]. *T. vulgaris* L. EO was tested against *A. flavus* growth and AFB₁ production. Total inhibition of fungal growth was observed at 250 µg/mL. The *T. vulgaris* EO reduced ergosterol production by *A. flavus*. The morphological structure of *A. flavus* was analyzed by SEM, alterations in conidiophore characteristics were observed. Conidial head size varied between 71.3 and 20.5 µm at concentrations between 50 and 500 µg/mL. A decrease of cytoplasmic content and modifications of membrane integrity were observed. The results were proportional to ergosterol production, which decreased with each EO concentration. Such modifications induced by the EO might be related to the interference caused by its constituents in cell wall synthesis, which affects *A. flavus* growth and morphology. Complete inhibition of AFB₁ production was recorded at 150 µg/mL. *T. vulgaris* EO exhibited antiaflatoxigenic activity, as AFB₁ biosynthesis inhibition occurred at lower concentration (50 µg/mL) than that required for inhibition of ergosterol production (100 µg/mL) and for morphological alterations of hyphae, conidiophores and conidia (100 µg/mL) [61].

On the other hand, cinnamaldehyde was assessed on AFB₁ production of *A. flavus*. The results demonstrated that with cinnamaldehyde treatment, ROS formation reduction was associated with AFB₁ production inhibition, which indicate that AFB₁ inhibition induced by cinnamaldehyde is related to ROS reduction. Lipid peroxidation is the consequence of ROS formation and has been shown to be involved in AFB₁ biosynthesis [62].

Ben Miri et al. [74] reported that *Citrus limon* EO at 1.75 mg/mL and *Citrus sinensis* at 2 mg/mL could totally inhibit fungal growth as well as AFB₁ production.

Additionally, the EOs showed notable antioxidant activity and were non phytotoxic to wheat seeds.

5. *In vivo* assays of essential oils

Although *in vitro* assays of EOs is an important first step in determination of the strong activity of plants, *in vivo* confirmation of activity is important because food models may interact with the bioactive compounds, decreasing their efficacy. Generally, to obtain the same effect in food items as those found *in vitro* experiences, higher concentrations of EOs must be applied. Thyme, summer savory and clove EOs was tested in tomato paste and all inhibited the mycelia growth of *A. flavus*. The thyme and summer savory EOs, exhibited the strongest inhibition at concentrations of 350 and 500 ppm, respectively. Taste panel evaluations were carried out in a tomato ketchup base, and the percent of inhibition of each EO in tomato paste was lower than in culture medium. Panelists accepted the results of taste panel with concentration of 500 ppm of thyme EO [69]. Under storage conditions, lemon fruits were completely prevented of *A. flavus* by *S. hortensis* at concentrations of 25, 12.5 and 6.25 $\mu\text{L/mL}$ [40].

The effect of hemlock (*Cicuta virosa* L. var. *latisecta* Celak) EO on inhibition of decay development in cherry tomatoes was assessed by exposing them to EO vapor at 200 $\mu\text{L/mL}$. Results showed that the *C. virosa* var. *latisecta* EO has potential to control food spoilage [51]. *Mentha viridis* EO can reduce *A. flavus* and aflatoxin production in stored corn. The authors showed that this of 300 μL of the EO in 100 g of corn is enough to control the fungal growth and the aflatoxin synthesis [70].

EOs of *Origanum majorana* L., *Coriandrum sativum* L., *Hedychium spicatum* Ham. ex Smith, *Commiphora myrrha* (Nees) Engl., and *Cananga odorata* Hook.f. and Thomson, were tested *in vivo* at different concentration against *A. flavus* in chickpea seed. During the investigations in food system all EOs exhibited above 50% protection of chickpea seed from *A. flavus* contamination [71]. The holy basil *O. sanctum* EO reduced the number of *A. flavus* up to 62.94, 67.87 and 74.01% when fumigated at concentrations of 0.3, 0.5 and 1.0 $\mu\text{L/mL}$, respectively, on *Rauvolfia serpentina* medicinal plant during storage [44]. *Boswellia carterii* Birdw EO showed protection of the fumigated black pepper fruits up to 65.38% from *A. flavus* contamination after 6 months of storage [60]. The efficacy of the combined application of chitosan (CH) and Locust Bean Gum (LBG) in combination with bergamot and bitter orange EOs was evaluated to inhibit *A. flavus* on artificially infected dates. In fruit decay assays coatings based on CH incorporating *Citrus*, EOs were capable to reduce fungal decay between 52 and 62%. Furthermore, the complete absence of off-flavors and off-odors demonstrated the potential of CH coatings carrying bergamot and bitter orange EOs at sub-inhibitory concentrations to control postharvest growth of *A. flavus* in dates [72].

6. Limitations of the use of EOs in food systems

In spite of the great potential of EOs against fungal growth and mycotoxin production, their large scale utilization is limited because of volatile nature, organoleptic effect in food systems and susceptibility to oxidation under light, heat, oxygen and moisture. To develop stability, control the release and enhance the efficacy of EOs in food systems, it is necessary for the current research to develop some structural barriers to enclose these bioactive compounds. In this regards, encapsulation

of EOs by different physical, physico-chemical and mechanical methods with the assistance of carrier matrices is a trending area of research.

Thus, although the use of EOs can be an interesting strategy, it faces several constraints:

Phytotoxicity: The alteration of the integrity of the cell membrane following exposure to EO (responsible for their anti-fungal effect) could also affect plants and induce phytotoxicity at slightly higher than those used to control fungi [19]. It should also be noted that the most effective EOs are generally the most phytotoxic [21].

Toxicity to mammals: exposure to EOs or their components may be toxic to mammals. For instance, EO with a high content of furanocoumarins may cause dermal irritation and burns during exposure to light due to photosensitization. The linalool, present in the EO of thyme and lavender is also toxic to human dermal cells [73].

Rapid volatilization: Compounds of EO are highly volatile and may also be oxidatively degraded following exposure to light or temperature rise. This loss of activity would therefore require their reintroduction continuously to maintain the protective effect [35].

Alteration of organoleptic qualities: EOs consists of substances aromatic and are often used in cosmetics industry for their pleasant scent. Their application to foods could change their organoleptic qualities altering the taste [54]. To mitigate this effect, strategies such as encapsulation of EOs have been designed to strengthen the prospective use of EOs in the food industry.

The present review has focused on the antifungal and antiaflatoxigenic activity of essential oils. Additionally, other plant extracts possess potential antifungal activities against *A. flavus* and AFB1 production, but they are generally similar to those observed with the EOs. However, it would seem interesting to further explore the potential of these extracts and their bioactive compounds as they may have several advantages over EOs, such as less sensorial impact on the food.

7. Conclusion

In view of the potential of EOs as inhibitory of *A. flavus* growth and AFB1 production and their efficacy in food system in controlling fungal contamination, the EOs may be recommended for the formulation of plant based preservatives for enhancement of shelf life and safety of foodstuffs during post-harvest processing because consumers are looking for food with natural characteristics.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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References

- [1] Nogueira JHC, Gonçalves E, Galleti SR, Facanali R, Marques MOM, Felício JD. *Ageratum conyzoides* essential oil as aflatoxin suppressor of *Aspergillus flavus*. International Journal of Food Microbiology. 2010;**137**:55-60
- [2] Frisvad CJ, Skouboe P, Samson AR. Taxonomic comparison of three different groups of aflatoxin producers and new efficient producer of aflatoxin B₁, sterigmatocystine and 3-Omethylsterigmatocystine *Aspergillus rumbelli* sp. nov. Systematic and Applied Microbiology. 2005;**28**:442-453
- [3] Prakash B, Shukla R, Singh P, Kumar A, Mishra PK, Dubey NK. Efficacy of chemically characterized *Piper betle* L. essential oil against fungal and aflatoxin contamination of some edible commodities and its antioxidant activity. International Journal of Food Microbiology. 2010;**142**:114-119
- [4] Prakash B, Shukla R, Singh P, Mishra PK, Dubey NK, Kharwar RN. Efficacy of chemically characterized *Ocimum gratissimum* L. essential oil as an antioxidant and a safe plant based antimicrobial against fungal and aflatoxin B₁ contamination of spices. Food Research International. 2011;**44**:385-390
- [5] Reddy KRN, Raghavender CR, Salleh B, Reddy CS, Reddy BN. Potential of aflatoxin B₁ production by *Aspergillus flavus* strains on commercially important food grains. International Journal of Food Science and Technology. 2011;**46**:161-165
- [6] IARC Monograph. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. International Agency for Research on Cancer. 2002;**82**:1-556
- [7] Becker-Algeri TA, Castagnaro D, de Bortoli K, de Souza C, Drunkler DA, Badiale-Furlong E. Mycotoxins in bovine milk and dairy products: A review. Journal of Food Science. 2016;**81**(3):R544-R552
- [8] Marin S, Ramos AJ, Cano-Sancho G, Sanchis V. Mycotoxins: Occurrence, toxicology, and exposure assessment. Food and Chemical Toxicology. 2013;**60**:218-237
- [9] Varga J, Baranyi N, Chandrasekaran M, Vágvölgyi C. Mycotoxin producers in the *Aspergillus* genus: An update. Acta Biologica Szegediensis. 2015;**59**:151-167
- [10] Yassin MA, El-Samawaty AMA, Moslem M, Bahkali A, Abd-Elsalam K. Fungal biota and occurrence of Aflatoxigenic *Aspergillus* in postharvest corn grains. Fresenius Environmental Bulletin. 2011;**20**:903, 1018-909, 4619
- [11] Moghadam MM, Hokmabadi H. Study on the effect of pistachio testa on the reduction of *Aspergillus flavus* growth and aflatoxin B-1 production in kernels of different pistachio cultivars. Australian Journal of Crop Science. 2010;**4**:744, 1835-749, 2693
- [12] Liang XQ, Wang T, Zhang EH, Chen XP, Li L. Identification of seed proteins associated with resistance to pre-harvested aflatoxin contamination in peanut (*Arachis hypogaea* L). BMC Plant Biology. 2010;**10**:1471-2229
- [13] Cornea CP, Ciuca M, Voaides C, Gagi V, Pop A. Incidence of fungal contamination in a Romanian bakery: A molecular approach. Romanian Biotechnological Letters. 2011;**16**:5863, 1224-5871, 5984
- [14] Sanchez-Hervas M, Gil JV, Bisbal F, Ramon D, Martinez-Culebras PV. Mycobiota and mycotoxin producing fungi from cocoa beans. International Journal of Food Microbiology. 2008;**125**:336, 0168-340, 1605

- [15] Bankole SA, Adenusi AA, Lawal OS, Adesanya OO. Occurrence of aflatoxin B1 in food products derivable from 'egusi' melon seeds consumed in southwestern Nigeria. *Food Control*. 2010;**21**:974, 0956-976, 7135
- [16] Rustom IYS. Aflatoxin in food and feed: Occurrence, legislation and inactivation by physical methods. *Food Chemistry*. 1997;**59**:57, 0308-67, 8146
- [17] Isman MB, Machial CM. Chapter 2 pesticides based on plant essential oils: From traditional practice to commercialization. *Advances in Phytomedicine*. 2006;**3**:29-44
- [18] Chizzola R. Regular monoterpenes and sesquiterpenes (essential oils). In: Ramawat KG, Mérillon JM, editors. *Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes*. Heidelberg: Springer; 2013:2973-3008
- [19] Isman MB. Plant essential oils for pest and disease management. *Crop Protection*. 2000;**19**:603-608
- [20] Burt S. Essential oils: Their antibacterial properties and potential applications in foods: A review. *International Journal of Food Microbiology*. 2004;**94**:223-253
- [21] Carson CF, Mee BJ, Riley TV. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. *Antimicrobial Agents and Chemotherapy*. 2002;**46**:1914-1920
- [22] Gustafson JE, Liew YC, Chew S, et al. Effects of tea tree oil on *Escherichia coli*. *Letters in Applied Microbiology*. 1998;**26**:194-198
- [23] Cox SD, Mann CM, Markham JL, et al. The mode of antimicrobial action of essential oil of *Melaleuca alternifolia* (tea tree oil). *Journal of Applied Microbiology*. 2000;**88**:170-175
- [24] Lambert RJW, Skandamis PN, Coote P, Nychas GJE. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology*. 2001;**91**:453-462
- [25] Saad NY, Muller CD, Lobstein A. Major bioactivities and mechanism of action of essential oils and their components. *Flavour and Fragrance Journal*. 2013;**28**:269-279
- [26] Turgis M, Han J, Caillet S, Lacroix M. Antimicrobial activity of mustard essential oil against *Escherichia coli* O157:H7 and *Salmonella typhi*. *Food Control*. 2009;**20**:1073-1079
- [27] Di Pasqua R, Hoskins N, Betts G, Mauriello G. Changes in membrane fatty acids composition of microbial cells induced by addition of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. *Journal of Agricultural and Food Chemistry*. 2006;**54**:2745-2749
- [28] Fung DY, Taylor S, Kahan J. Effects of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on growth and aflatoxin production of *Aspergillus flavus*. *Journal of Food Safety*. 1977;**1**:39-51
- [29] Abd El-Aziz ARM, Mahmoud MA, Al-Othman MR, Al-Gahtani MF. Use of selected essential oils to control aflatoxin contaminated stored cashew and detection of aflatoxin biosynthesis gene. *The Scientific World Journal*. 2015;**2015**:958192
- [30] Bluma R, Amaiden MR, Daghero J, Etcheverry M. Control of *Aspergillus* section *Flavi* growth and aflatoxin accumulation by plant essential oils. *Journal of Applied Microbiology*. 2008;**105**:203-214

- [31] 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. International Journal of Food Microbiology. 2014;**168-169**:1-7
- [32] Paranagama PA, Abeysekera KHT, Abeywickrama K, Nugaliyadde L. Fungicidal and anti-aflatoxigenic effects of the essential oil of *Cymbopogon citratus* (DC.) Stapf. (lemongrass) against *Aspergillus flavus* link. Isolated from stored rice. Letters in Applied Microbiology. 2003;**37**:86-90
- [33] Rasooli I, Fakoor MH, Yadegarinia D, Gachkar L, Allameh A, Rezaei MB. Antimycotoxigenic characteristics of *Rosmarinus officinalis* and *Trachyspermum copticum* L. essential oils. International Journal of Food Microbiology. 2008;**122**:135-139
- [34] Tian J, Huang B, Luo X, Zeng H, Ban X, He J, et al. The control of *Aspergillus flavus* with *Cinnamomum jensenianum* Hand.-Mazz essential oil and its potential use as a foodpreservative. Food Chemistry. 2012;**130**:520-527
- [35] Kedia A, Jha DK, Dubey NK. Plant essential oils as natural fungicides against stored product fungi. In: Mendez-Vilas A editor. The Battle Against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs. Badajoz: Formatex Research Center; 2015:208-214
- [36] Food and Drug Administration. US Code of Federal Regulations. 2013; Title 21, Part 182, Section 182.20, 129-130
- [37] Dayan FE, Cantrell CL, Duke SO. Natural products in crop protection. Bioorganic and Medicinal Chemistry. 2009;**17**:4022-4034
- [38] Prakash B, Kedia A, Mishra PK, Dubey NK. Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities—potentials and challenges. Food Control. 2015;**47**:381-391
- [39] Salem EA, Shalaby K. Effect of plant essential oils and gamma irradiation on growth and aflatoxin production by *Aspergillus flavus* isolated from wheat grains. Arab Journal of Nuclear Science and Applications. 2016;**94**(1):123-130
- [40] Dikbas N, Kotan R, Dadasoglu F, Sahin F. Control of *Aspergillus flavus* with essential oil and methanol extract of *Satureja hortensis*. International Journal of Food Microbiology. 2008;**124**:179-182
- [41] Kwazou LN, Dongmo JMP, Ngoune TL, Sameza LM, Dongmo NB, Zollo AHP, et al. Propriétés antifongiques des huiles essentielles de quelques plantes du genre *Aframomum* du Cameroun contre *Aspergillus flavus*. Cameroon Journal of Experimental Biology. 2009;**5**(1):44-51
- [42] Kumar R, Dubey NK, Tiwari OP, Tripathi YB, Sinha KK. Evaluation of some essential oils as botanical fungitoxicants for the protection of stored food commodities from fungal infestation. Journal of Scientific and Food Agriculture. 2007;**87**:1737-1742
- [43] Gameda 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 Pacific Journal of Tropical Biomedicine. 2014;**4**(1):S373-S381
- [44] Kumar A, Dubey NK, Srivastava S. Antifungal evaluation of *Ocimum sanctum* essential oil against fungal deterioration of raw materials of *Rauwolfia serpentina* during storage. Industrial Crops and Products. 2013;**45**:30-35
- [45] Passone MA, Girardi NS, Ferrand CA, Etcheverry M. *In vitro* evaluation

of five essential oils as botanical fungitoxicants for the protection of stored peanuts from *Aspergillus flavus* and *A. parasiticus* contamination. International Biodeterioration and Biodegradation. 2012;**70**:82-88

[46] Tatsadjieu NL, Etoa FX Mbofung CMF, Ngassoum MB. Effect of *Plectranthus glandulosus* and *Ocimum gratissimum* essential oils on growth of *Aspergillus flavus* and aflatoxin B₁ production. Tropicultura. 2008;**26**(2):78-83

[47] Srivastava B, Singh P, Shukla R, Dubey NK. A novel combination of the essential oils of *Cinnamomum camphora* and *Alpinia galanga* in checking aflatoxin B₁ production by a toxigenic strain of *Aspergillus flavus*. World Journal of Microbiology and Biotechnology. 2008;**24**:693-697

[48] Tatsadjieu NL, Dongmo PMJ, Ngassoum MB, Etoa FX, Mbofung CMF. Investigations on the essential oil of *Lippia rugosa* from Cameroon for its potential use as antifungal agent against *Aspergillus flavus* link ex. fries. Food Control. 2009;**20**:161-166

[49] Singh P, Shukla R, Prakash B, Kumar A, Singh S, Mishra KP, et al. Chemical profile, antifungal, antiaflatoxigenic and antioxidant activity of *Citrus maxima* Burm. And *Citrus sinensis* (L.) Osbeck essential oils and their cyclic monoterpene, DL-limonene. Food and Chemical Toxicology. 2010;**48**:1734-1740

[50] Deabes MM, Neveen H, El-Soud A, Lamia T, El-Kassem A. *In Vitro* inhibition of growth and Aflatoxin B₁ production of *Aspergillus flavus* strain (ATCC 16872) by various medicinal plant essential oils. Macedonian Journal of Medical Sciences. 2011;**4**(4):345-350

[51] 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. International Journal of Food Microbiology. 2011;**145**:464-470

[52] Tian J, Ban X, Zeng H, He J, Chen Y, Wang Y. The mechanism of antifungal action of essential oil from dill (*Anethum graveolens* L.) on *Aspergillus flavus*. PLoS One. 2012;**7**(1):e30147

[53] Shukla R, Singh P, Prakash B, Dubey NK. Antifungal, aflatoxin inhibition and antioxidant activity of *Callistemon lanceolatus* (Sm.) sweet essential oil and its major component 1,8-cineole against fungal isolates from chickpea seeds. Food Control. 2012;**25**:27-33

[54] Kumar A, Shukla R, Singh P, Anuradha, Dubey NK. Efficacy of extract and essential oil of *Lantana indica* Roxb. Against food contaminating moulds and aflatoxin B₁ production. International Journal of Food Science and Technology. 2010;**45**:179-185

[55] Li Y, Kong W, Li M, Liu H, Zhao X, Yang S, et al. Litsea cubeba essential oil as the potential natural fumigant: Inhibition of *Aspergillus flavus* and AFB₁ production in licorice. Industrial Crops and Products. 2016;**80**:186-193

[56] Mishra PK, Shukla R, Singh P, Prakash B, Dubey NK. Antifungal and antiaflatoxigenic efficacy of *Caesulia axillaris* Roxb. Essential oil against fungi deteriorating some herbal raw materials, and its antioxidant activity. Industrial Crops and Products. 2012;**36**:74-80

[57] Mishra PK, Shukla R, Singh P, Prakash B, Kedia A, Dubey NK. Antifungal, anti-aflatoxigenic, and antioxidant efficacy of *Jamrosia* essential oil for preservation of herbal raw materials. International Biodeterioration and Biodegradation. 2012;**74**:11-16

[58] Mishra PK, Singh P, Prakash B, Kedia A, Dubey NK. Assessing essential oil components as plant-based

preservatives against fungi that deteriorate herbal raw materials. International Biodeterioration and Biodegradation. 2013;**80**:16-21

[59] Ferreira FD, Kimmelmeier C, Arrotéia CC, da Costa LC, Mallmann CA, Janeiro V, et al. Inhibitory effect of the essential oil of *Curcuma longa* L. and curcumin on aflatoxin production by *Aspergillus flavus* link. Food Chemistry. 2013;**136**:789-793

[60] 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. Food Science and Technology. 2014;**56**:240

[61] Kohiyama CY, Ribeiro MMY, Mossini SAG, Bando E, Bomfim NDS, Nerilo SB, et al. Antifungal properties and inhibitory effects upon aflatoxin production of *Thymus vulgaris* L. by *Aspergillus flavus* link. Food Chemistry. 2015;**173**:1006-1010

[62] Sun Q, Wang L, Lu Z, Liu Y. *In vitro* anti-aflatoxigenic effect and mode of action of cinnamaldehyde against aflatoxin B₁. International Biodeterioration and Biodegradation. 2015;**104**:419-425

[63] Kumar A, Shukla R, Singh P, Dubey NK. Chemical composition, antifungal and antiaflatoxigenic activities of *Ocimum sanctum* L. essential oil and its safety assessment as plant based antimicrobial. Food and Chemical Toxicology. 2010;**48**:539-543

[64] Singh P, Srivastava B, Kumar A, Kumar R, Dubey NK, Gupta R. Assessment of *Pelargonium graveolens* oil as plant-based antimicrobial and aflatoxin suppressor in food preservation. Journal of the Science of Food and Agriculture. 2008;**88**:2421-2425

[65] Kedia A, Prakash B, Mishra PK, Chanotiya CS, Dubey NK. Antifungal, antiaflatoxigenic, and insecticidal efficacy of spearmint (*Mentha spicata* L.) essential oil. International Biodeterioration and Biodegradation. 2014;**89**:29-36

[66] Mishra PK, Kedia A, Dubey NK. Chemically characterized *Cymbopogon martinii* (Roxb.) Wats. Essential oil for shelf life enhancer of herbal raw materials based on antifungal, antiaflatoxigenic, antioxidant activity, and favorable safety profile. Plant Biosystems. 2015;**3504**:1-10

[67] Kumar A, Shukla R, Singh P, Prasad CS, Dubey NK. Assessment of *Thymus vulgaris* L. essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities. Innovative Food Science and Emerging Technologies. 2008;**9**:575-580

[68] Gorran A, Farzaneh M, Shivazad M, Rezaeian M, Ghassempour A. Aflatoxin B₁-reduction of *Aspergillus flavus* by three medicinal plants (Lamiaceae). Food Control. 2013;**31**:218-223

[69] 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**:1518-1523

[70] Gibriel YAY, Hamza AS, Gibriel AY, Mohsen SM. *In vivo* effect of mint (*Mentha viridis*) essential oil on growth and aflatoxin production by *Aspergillus flavus* isolated from stored corn. Journal of Food Safety. 2011;**3**:445-451

[71] 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 Research International. 2012;**49**:201-208

[72] Aloui H, Khwaldia K, Licciardello F, Mazzaglia A, Muratore G, Hamdi M, et al. Efficacy of the combined application of chitosan and locust bean gum with different citrus essential oils to control postharvest spoilage caused by *Aspergillus flavus* in dates. International Journal of Food Microbiology. 2014;**170**:21-28

[73] Prashar A, Locke IC, Evans CS. Cytotoxicity of lavender oil and its major components to human skin cells. Cell Proliferation. 2004;**37**:221-229

[74] Ben Miri Y, Ariño A, Djenane D. Study of antifungal, anti-aflatoxigenic, antioxidant activity and phytotoxicity of Algerian *Citrus limon* var. Eureka and *Citrus sinensis* var. Valencia essential oils. Journal of Essential Oil-Bearing Plants. 2018;**21**(2):345-361