Mycotoxins and mycotoxigenic fungi in spices and mixed spices: a review

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Abstract

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Mycotoxins are secondary metabolites produced by fungi mainly from the genus *Aspergillus, Penicillium, Fusarium*, and *Alternaria*. The occurrence of mycotoxin contamination has been reported in various types of food, including spices such as chilli, black pepper, white pepper, cumin, coriander seed, and the ground mix spices. Aflatoxins (AFs) and ochratoxin A (OTA) are the primary mycotoxins in spices produced by *Aspergillus* spp. and *Penicillium* spp. Among these spices, chilli is more susceptible to the invasion of these mycotoxigenic fungi, especially during prolonged storage and inappropriate storage conditions, resulting in mycotoxin production. The storage conditions, processing methods, and handling practices of spices may affect the growth of fungi and subsequent mycotoxin production. This paper reviewed mycotoxigenic fungi and mycotoxin contamination in spices, the impact on humans, and the regulatory limit adopted by many countries worldwide.

Keywords:

Spices, Aspergillus spp., Mycotoxins, Aflatoxins, Ochratoxin A, Food safety

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1. Introduction

Spices are dried commodities derived from different parts of plants such as roots, seeds, buds, leaves, flowers, and stigmas. Since ancient times, spices have been used in cuisines, medicine, preservatives, and colour. The word "spices" is derived from the Latin word "species", which means a specific kind (Raghavan, 2007). Spices are known for their popularity in their distinctive flavouring, aromatising properties, preservative, antimicrobial, and antioxidant effects (Pickova et al., 2020). Spices have a unique flavour profile used in small quantities to flavour or impart aroma to food. They are widely grown in tropical countries, mainly India and Southeast Asia. However, spices are susceptible to fungal invasion if the conditions are suitable for fungal growth.

Fungi are multicellular organisms made up of long filaments called hyphae. They are plant-like organisms but are not considered a member of the plant kingdom due to the lack of chlorophyll and filamentous structure (Adeyeye, 2016). Fungi are common spoilage organisms that can deteriorate stored crops and food products. The spoilage fungi can be classified into two groups; field and storage fungi. Field fungi consist of various genera such as *Alternaria*, *Fusarium*, and *Cladosporium*, which attack crops in the field (Hashem and Alamri, 2010). On the contrary, storage fungi can grow in stored crops if the

*Corresponding author. Email: norlia.mahror@usm.my temperature, humidity, and moisture level are relatively high to support their growth (Mandeel, 2005; Hashem and Alamri, 2010). According to Yogendrarajah *et al.* (2014), *Aspergillus* and *Penicillium* are the most common storage fungi contaminating stored crops. These fungal genera are known as mycotoxigenic fungi because they can produce harmful secondary metabolites known as mycotoxins.

Mycotoxins can occur naturally in foods and agricultural commodities, including spices. According to Cinar and Onbasi (2019), more than 400 types of mycotoxins have been identified. Nevertheless, the most important mycotoxins in foods are aflatoxin B₁, B₂, G₁, G₂ (AFB₁, AFB₂, AFG₁, and AFG₂), and ochratoxin A (OTA). AFB_1 and OTA are the most hazardous mycotoxins (Adam et al., 2017). Mycotoxins can cause illness or death to humans and animals, regardless of how they are ingested, i.e., through breathing, swallowing, or absorption in the skin. Hence, this review aimed to provide an overview and updated information on mycotoxin and mycotoxigenic fungal contamination in spices, focusing on chilli as the most affected commodity. In addition, the mycotoxin regulation limits and the impact of mycotoxin on humans are also discussed in this paper.

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2. Spice production worldwide

Spices are usually marketed as a whole, ground, or mixed ground spices. Cinnamon, clove, star anise, cardamom, cumin, fennel, coriander seeds, black pepper, white pepper, paprika, chilli, and turmeric are commonly used in Asian cuisines, as shown in Table 1. However, the attributes of spices will differ depending on the geographical area, soil conditions, and processing methods during postharvest.

Spices have been cultivated in many countries, mainly in India. According to FAOSTAT (2019), the worldwide production of spices was 2.8 million tonnes from 1.4 M ha of area harvested in 2019. Asia contributed to 86% of spice production by region, followed by Africa (12.4%) and America (1.5%). India is the primary producer of spices, with 1.4 M tonnes of spices produced in 2019 (FAOSTAT, 2019).

Chilli is the world's second most widely consumed spice after black pepper. In 2016, the global production region of chilli was 1,798,847 ha, with an annual crop of 3,918,159 tonnes (FAO, 2016). China, Mexico, and Turkey were the top three producers of chillies in 2016. The output statistics demonstrate the importance of the chilli crop, which is vital for developing economies.

3. Some important mycotoxins in food commodities

Mycotoxins are secondary metabolites produced by fungal species such as *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium*. Some researchers have reported the occurrence of mycotoxins in spices, mainly AFs and OTA. Besides that, other mycotoxins that might be present in food commodities are fumonisins (FBs), zearalenone (ZEN), deoxynivalenol (DON), trichothecenes (Selvaraj *et al.*, 2015; Pickova *et al.*, 2020).

3.1 Aflatoxins (AFs)

AFs are potent, mutagenic, carcinogenic, and immunosuppressants. They are highly toxic compared to other mycotoxins (Mahato et al., 2019). Among all AFs (AFB₁, AFB₂, AFG₁, and AFG₂), AFB₁ is the most toxigenic and has proven to be a human carcinogen (Group 1; IARC, 1993a). AF is one of the most important mycotoxins produced by fungi from the genus Aspergillus. Among all Aspergillus species, Aspergillus flavus, A. parasiticus, and A. nomius are the most frequent producers of AFs (Ali et al., 2015). The optimum temperature for the survival of A. flavus is 30° C, while the optimum temperature for AF production is between 25 to 30°C (Pitt and Hocking, 2009; Yogendrajajah et al., 2014). AFs commonly occur in peanuts, nuts, figs, corn, rice, spices, and dried fruits (Martinez-Miranda et al., 2019).

3.2 Ochratoxin A (OTA)

OTA is known to be carcinogenic, nephrotoxic, and immunotoxic (Wang et al., 2016). The International Agency for Research on Cancer (IARC, 1993a) has classified OTA as the most probable carcinogen to humans (Group 2B). OTA is commonly produced by Aspergillus niger, Aspergillus ochraceus, Aspergillus carbonarius, and Penicillium verrucosum. Tropical commodities are typically infected with A. ochraceus and A. carbonarius, while P. verrucosum usually infects the commodities in the temperate region (Steyn, 1995; Richard, 2007; Turner et al., 2009). The ideal temperature for OTA production is 25-30°C. The tropical climates are favourable for the growth of OTAproducing fungi and support OTA production (Nguegwouo et al., 2016). According to Turner et al. (2009), OTA can usually be found in dried products, including spices.

3.3 Citrinin

Citrinin (CIT) was first isolated in 1931 from

English name	Family	Scientific name	Part of plant
Chilli pepper	Capsicum	Capsicum spp.	Fruits
Black pepper	Piperaceae	Piper nigrum	Berries
Cardamom seed	Zingiberaceae	Amomum spp.	Fruits
Cinnamon	Lauraceae	Cinnamomum spp.	Barks
Clove	Myrtaceae	Szygium aromaticum (L.)	Flower buds
Coriander seed	Apiaceae	Coriandrum sativum L.	Fruits
Cumin seed	Apiaceae	Cuminum cyminum L.	Seeds
Fennel seed	Apiaceae	Foeniculum vulgare Mill.	Fruits
Ginger	Zingiberaceae	Allium sativum L.	Rhizomes
Nutmeg	Myristicaceae	Myristica fragrans Houtt.	Seeds
Star anise	Illiaceae	Illicium verum	Fruits
Turmeric	Zingibearaceae	Curcuma longa L.	Rhizomes
White pepper	Piperaceae	Piper nigrum	Berries

Table 1. List of spices commonly used in the Asian cuisines

Penicillium citrinum isolates (Silva *et al.*, 2020). Other fungi that can produce CIT include *Penicillium expansum, Penicillium viridicatum, Aspergillus niveus, Aspergillus terreus* (Silva *et al.*, 2021), *Monascus purpureus*, and *Monascus ruber* (Pickova *et al.*, 2020). Citrinin is proven nephrotoxic, proven to target the kidney from a previous study of contaminated chicken fed by *P. citrinum* (Bhatnagar *et al.*, 2002). According to IARC, CIT is classified as a group 3 carcinogen, i.e., not carcinogenic to humans.

3.4 Fumonisin

Fumonisins are known as Fusarium mycotoxins, mainly produced by Fusarium verticillioides (formerly known as Fusarium monoliforme). To date, 28 fumonisins were identified and classified into four different groups (A, B, C, and P; Rheeder et al., 2002). The most occurring group is fumonisin B (FB), comprising FB₁, FB₂, and FB₃, with FB₁ being the most isolated fumonisin, mainly from corn and corn-based products (Alshannaq and Yu, 2017; Reddy et al., 2010). In 1970, F. verticillioides was associated with a field disease outbreak, leukoencephalomalacia (ELEM), in South Africa, as the species was the most isolated fungi in corn (Marasas, 2001). The disease can be described as liquefactive necrotic lesions in the white matter of the horses' brain (Marasas, 2001). Other studies have proven that this toxin targets the liver and kidneys of animals and cultured human cells (Seefelder et al., 2003; Fink-Gremmels, 2008; Afsah-Hejri et al., 2013). The assessment of fumonisins by IARC (1993b) showed their carcinogenic risk to humans and classified them as group 2B carcinogens, i.e., probably carcinogenic to humans.

The incidence of FBs in spices has piqued the interest of researchers to investigate them. In China, Kong *et al.* (2012) investigated the incidence of FB₁ and FB₂ in spices and herbs. The authors found mouldy samples (42.5%) were contaminated with FB₁ and FB₂, with the mean value of 129.0 and 165.9 μ g/kg. Meanwhile, normal samples (8.6%) were contaminated with 165.9 and 256.8 μ g/kg of FB₁ and FB₂. Waśkiewicz *et al.* (2013) screened herbs and spices from Poland markets and found FBs from the samples ranging from 5.29 to 62.78 μ g/kg for total FBs.

3.5 Trichothecene (TC)

Trichothecene (TC) is a huge group of TC toxins, and the first member of this group was identified in 1949 produced by *Trichothecium roseum* (Glenn, 2007). More than 150 TC variants were identified, but only a few commonly occur in agricultural products (Yazar and Omurtag, 2008; Alshannaq and Yu, 2017) and 40 TC variants were identified as *Fusarium* mycotoxins. Fusarium species generally invade plants in the field and produce TC (Bennet and Klich, 2003). Commonly species in commodities occurring Fusarium are Fusarium graminearum and Fusarium culmorum. Other species that could produce TCs include Cephalosporium, Cylindrocarpon, Dendrodochium, Myrothecium, Trichoderma, Trichothecium, and Stachybotrys species (Bottalico and Perrone, 2002; Alshannaq and Yu, 2017). TCs commonly produced by Fusarium species are deoxynivalenol (DON), T-2 toxin, diacetoxyscirpenol, and nivalenol (Afsah-Hejri et al., 2013; Glen, 2007). TCs commonly contaminate agricultural commodities such as wheat, barley, oats, rye, corn, and rice (Yazar and Omurtag, 2008). The incidence of Fusarium toxins in food commodities was reported elsewhere (Nathanail et al., 2015; Almeida et al., 2016; Bryla et al., 2018; Torović, 2018; Mahdjoubi et al., 2020).

3.6 Zearalenone (ZEA)

Zearalenone (ZEA, formerly known as F-2 toxin) is a non-steroid oestrogen mycotoxin (Zinedine et al., 2007). ZEA has a molecular structure similar to natural oestrogens, allowing them to bind to oestrogenic receptor sites, resulting in increased oestrogenicity. Exposure to this toxin is associated with lower levels of progesterone and serum testosterone in the circulation, resulting in infertility and lower pregnancy rates in cows, pigs, and rats (Yang et al., 2007; Rai et al., 2019; Mahato et al., 2021). ZEA is produced by some *Fusarium sp.*, mainly Fusarium graminearum and Fusarium semitectum (Mahato et al., 2021). Other ZEA producers include Fusarium culmorum, Fusarium verticillioides, Fusarium cerealis, Fusarium crookwellense, Fusarium pseudograminearum, and Fusarium equiseti. (European Commission, 2000; Glenn, 2007; Afsah-Hejri et al., 2013). Fungi-producing ZEA is mainly found in plants grown in temperate and warmer climates and is usually found in corn, wheat, oats, barley, and sorghum (Mally et al., 2016). The incidence of ZEA in these commodities have been reported worldwide (Ayalew et al., 2006; Almeida-Fereira et al., 2013; Nathanail et al., 2015; Piacentini et al., 2018; Golge and Kabak, 2020). ZEA has also been reported in spices (Patel et al., 1996; Schollenberger et al., 2005). IARC classified ZEA as a group 3 carcinogen, i.e., not classifiable as carcinogenic to humans. It has less severe toxicity, but due to its high oestrogenic activity, long-term exposure to it may pose a health concern.

4. Regulatory limits

Implementing prevention and control measures and establishing regulatory limits are vital to avoiding mycotoxin exposure to consumers. The primary goal is

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to establish a maximum level of mycotoxins in foods to enable fair trade while preserving consumers' health. The regulatory limits for mycotoxins have been established in over a hundred countries (van Egmond, 2013). Currently, the regulation limit of mycotoxins in spices ranges from 5–30 μ g/kg depending on each country (FAO 2004; van Egmond *et al.*, 2007; Reddy *et al.*, 2010). Although each country has its mycotoxin regulations, standardised regulations are yet to be implemented (Iha and Trucksess, 2019). For example, certain countries did not prescribe AFs specifically in spices; therefore, spices fall under "other types of food." Moreover, OTA regulations are only available in coffee and cereal-based foods for infants in most countries.

According to the European Union (EU) standard, the AFB₁ maximum limit in spices like chilli, peppers, nutmeg, ginger, turmeric, and a mixture of spices is 5 μ g/kg, while the sum of AFB₁, AFB₂, AFG₁, and AFG₂ in spices is 10 μ g/kg (European Commission, 2010). Meanwhile, the maximum regulated limit of OTA in spices is 15 μ g/kg (European Commission, 2012).

5. Fungal and mycotoxin contamination in spices

Spices are prone to fungal contamination, mainly by mycotoxigenic species during pre- and post-harvest. In addition, most of these products are sold without being properly processed. In most cases, they are packed upon being dried and ground and sold to the public (Kneifel and Berger, 1994; Costa *et al.*, 2019). The entire spice production must be carefully managed to minimise fungal infection and mycotoxin contamination.

Spoilage fungi present in spices can alter the organoleptic properties of the spice products, lowering their commercial value (Garcia *et al.*, 2018). Apart from that, high fungal contamination can lead to mycotoxin

contamination, affecting consumer wellbeing since mycotoxins are heat-stable and difficult to be eliminated (Alshannaq and Yu, 2017; Kabak and Dobson, 2017). The occurrence of fungi in spices, mainly *A. flavus* and *A. niger*, has been extensively studied worldwide (Table 2). The incidence of mycotoxins (AFs and OTA) in spices produced by these species is summarised in Table 3.

5.1 Chilli

Chillies are the fruit of the capsicum plant belonging to the family Solanaceae, which originates from America. Several types of chillies are available in the market, such as fresh chillies, dried chillies, chilli pastes, chilli powder, and chilli flakes. Chilli can be consumed right after being harvested; however, most chilli production is devoted to processed dried chilli, often used to make seasonings such as spice powder mix. According to Khadka et al. (2017), most fungi are likely a pathogen or contaminant instead of native to the plant. Thus, controlling water activity (a_w), humidity, temperature, and hygiene conditions during the drying, storage, packing, and distribution stages is critical to ensure a low level of mycoflora in chilli after processing. After the drying process, chilli can be contaminated with xerophilic fungi such as Aspergillus and Penicillium. These could be due to the xerophilic properties of these fungi, where they gain a competitive advantage over other fungal pathogens in low aw conditions (Sanzani et al., 2016). According to Costa et al. (2019), chilli products are hygroscopic. Efficient packaging systems, such as modified atmosphere systems, can avoid moisture reabsorption during the post-processing stage to reduce the risk of mycotoxin contamination.

High levels of fungi have been recorded in food products that contain chilli (Khan et al., 2014; Singh and

Table 2. Isolation frequency of mycotoxigenic fungi in spices								
Spice complex	Isolation frequency (%)		References					
Spice samples	A. flavus	A. niger	- Kelefences					
Black pepper	28	52	Garcia et al. (2018)					
Chilli	31	4	Wikandari et al. (2020)					
Red Chilli	96	62	Mandeel (2005)					
Red pepper	10	26	Saidi et al., (2021)					
Cinnamon	12	10	Kong et al. (2014)					
Cumin	ND	15.2	Kong et al. (2014)					
Cumin	40	60	Hashem and Alamri (2010)					
Clove	ND	25	Hashem and Alamri (2010)					
Coriander	12	36	Saidi et al., (2021)					
Coriander	15	12	Kulshrestha et al. (2014)					
Fennel	12	32	Garcia et al. (2018)					
Fennel	19	7	Kulshrestha et al. (2014)					
Star anise	ND	ND	Kong et al. (2014)					
White pepper	32	40	Garcia et al. (2018)					
Mixed spices	57	ND	Makhlouf et al. (2019)					

ND: Not detected

Country	Spices	Positive samples (%)	Amount of mycotoxins in samples (µg/kg)	No. of samples exceeding the EU limit (%)	Reference		
Malaysia	Dried chilli	AFs: 65.00 OTA: 81.25	AFs: 0.20-79.70 OTA: 0.20-101.20	NA	Jalili and Jinap (2012)		
Indonesia	Dried chilli	NA	AFB ₁ : 39.30–139.50 AFB ₂ : 2.60–33.30 OTA: 23.70–84.60	NA	Wikandari <i>et al.</i> (2020)		
Italy	Peppers, chilli, cinnamon, cloves, coriander, mixed spices	AFs: 15.40 AFB ₁ : 8.50 OTA: 23.80	AFs: 0.96 AFB ₁ : 0.31 OTA: 6.18	AFs: NA OTA: 2.30	Prelle <i>et al.,</i> (2014)		
Malaysia	Chilli, peppers, cumin	AFB ₁ : 93.33	AFB ₁ : 1.20-3.26	NA	Reddy <i>et al.</i> (2011)		
Pakistan	Chilli powder, whole chilli, chilli sauce and crushed chilli	AFs: 56.40 OTA: 40.40	AFs: 12.50-15.16 OTA: 16.68-120.90	AFs: 26.30 OTA: 32.70	Iqbal <i>et al.</i> (2017)		
Thailand	Chilli powder	AFB ₁ : 100.00	AFB ₁ : 5.32 -11.14	AFB ₁ : 0.00	Thanaboripat <i>et al.</i> (2016)		
Qatar	Black pepper	AFs: 41.7	AFs: 1.85-70.33	NA	Hammami <i>et</i> <i>al.</i> , (2014)		
Malaysia	Dried chilli, fennel, cumin, black pepper, white pepper, coriander and mixed spices	AF: 85 OTA: 79	AFs: 0.01-9.34 OTA: 0.14-20.40	NA	Ali <i>et al.</i> , (2015)		
Benin	Pepper, chilli, cumin, cinnamon, anise	AFB ₁ : 84	AFB ₁ : 0.46-84.84	40.00	Akpo- Djènontin <i>et al.</i> , (2018)		
Turkey	Red chilli powder, black pepper, cumin	AFB ₁ : 43.75 OTA:26.56	AFB ₁ : 0.13-35.77 OTA: 0.63-98.2	AF: 13.60 OTA: 13.60	Ozbey and Kabak, (2012)		
Hungary	Black pepper, mixed spices, chilli	31.25	AFB ₁ : 0.16-8.10 OTA:2.1	AFB ₁ : 20.00 OTA: NA	Fazekas <i>et al.</i> , (2005)		
India	Black pepper	AFB ₁ : 45.24 AFs: 76.00 OTA: 78.57	AFB ₁ : NA AFs: 4.80-26.40 OTA: 4.60-13.80	NA	Jeswal and Kumar (2015)		
India	Red chilli	AFB ₁ : 56.36 AFs: 85.45 OTA: 72.73	AFB ₁ : NA AFs: 4.30-33.60 OTA: 3.50-8.60	NA	Jeswal and Kumar (2015)		
Ethiopia	Pepper	AFs: 42.00	AFs: 3.10-19.20	AFs: 98.00	Tsehaynesh <i>et</i> <i>al.</i> (2021)		
Korea	Chilli powder, chilli paste	AFs: 13.60	AFs: 0.08-4.66	AFs: 0.00	Cho <i>et al</i> . (2008)		
Turkey	Chilli powder	AFs: 63.60 OTA: 54.50	AFs: 0.20-35.77 OTA: 0.78-98.20	AFs: 4.50 OTA: 13.60	Ozbey and Kabak (2012)		

Table 3. Worldwide occurrence of mycotoxin in spices

NA: Not available, AFs: Aflatoxins, OTA: Ochratoxin A

Cotty, 2017; Gambacorta *et al.*, 2018). According to Costa *et al.* (2019), antifungal substances and low a_w in spices are the key obstacles to microbial growth in these products. However, fungal growth cannot be fully inhibited in chillies due to the fungal metabolic plasticity that helps to adapt to adverse conditions throughout chilli production. Previous researchers reported a significant level of fungal contamination in chillies primarily by *Aspergillus, Penicillium*, and *Fusarium* species and some soil-borne fungi such as *Cladosporium* and *Mucor*. Moreover, Mandeel (2005) revealed that of the 17 spices tested, chillies had the highest fungal contamination from *A. flavus* and *A. niger*.

Singh and Cotty (2017) studied the occurrence of

AFs in chilli from various markets in Nigeria and the United States of America (USA). The authors discovered that 93% of Nigerian chilli were contaminated with AFB₁, while the chilli samples from the USA recorded 64% contamination. Nigerian chilli had comparatively higher AFB₁ concentrations, with the most contaminated sample having 156 µg/kg AFB₁. Approximately 38% of USA chillies were contaminated with $> 5 \ \mu g/kg \ AFB_1$. On the other hand, Pakistani chillies have lost their international market share due to mycotoxin contamination despite having exceptional sensorial qualities. Another study by Khan et al. (2014) also reported a high level of AFs in Pakistani chilli, with most samples exceeding the EU regulation limit.

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Reddy *et al.* (2001) investigated AFB₁ contamination in fresh chillies and chilli powders from various markets in Andra Pradesh, India. They discovered that AFB₁ was detected in 59% of the chilli samples, and fresh chilli recorded the highest amount of AFB₁ (969 μ g/kg). The physical damage to fresh chillies could be the reason for the high AF level of the fresh chilli since most of the samples were discoloured with noticeable fungal infestation.

Previous studies have stated that chilli sauce, chilli flakes, chilli powder, and dried chillies were contaminated with OTA (Ozbey and Kabak, 2012; Prelle et al., 2014; Iqbal et al., 2017; Wikandari et al., 2020). Mycotoxin contamination levels are directly influenced by geography, climate conditions, and crop management practices. Almela et al. (2007) identified 115 fungal strains, with 85 of them being A. carbonarius, A. niger, and A. ochraceus in the assessment of OTA in chilli samples grown in Spain, Zimbabwe, Brazil, and Peru. Furthermore, they also observed the climatic conditions of the sample's origin, and the cultural, and technological practices of chilli crops. They revealed that Peru has the highest OTA contamination in chilli, which may be associated with high humidity levels in chilligrowing regions.

5.2 Cinnamon

Cinnamon is the tree's inner bark of the genus *Cinnamomum*, derived from the Greek word *kinamon* (Raghavan, 2007). Although more than 250 varieties of cinnamon were identified (Rao and Gan, 2014), four types are most commonly used in various cuisines, including Chinese cinnamon (*Cinnamomum cassia*), Sri Lanka cinnamon (*C. zeylanicum*), Indonesian cinnamon (*C. burmannii*), and Vietnamese cinnamon (*C. loureirii*; Nabavi *et al.*, 2015). Cinnamon mainly contains volatile compounds such as cinnamaldehyde (65%–80%) and eugenol (5%–10%) in the plant's bark (Rao and Gan, 2014). These two compounds are responsible for their spicy sweetness and act as effective compounds against bacteria and moulds (Davidson and Naidu, 2000; Tajkarimi *et al.*, 2010).

Cinnamon has been used for cooking since ancient times. It is used in several cuisines as an aromatic condiment and flavouring additive. It also has great importance in medicine due to its antioxidant and antimicrobial properties (Nabavi *et al.*, 2015). In the market, cinnamon can be found as whole or ground spices. The whole and ground cinnamon are widely used in Asian and Latin American countries, while the European and Mediterranean countries prefer the ground form. Several studies had reported a low incidence of fungi in cinnamon samples. In Indonesia, cinnamon recorded low fungal counts, with the most occurring species of *Eurotium chevalieri* (Nurtjahja *et al.*, 2019). *A. flavus* and *A. carbonarius* were also recorded at low levels. Similarly, a study in Tanzania found that cinnamon was among the less contaminated samples (Temu, 2016). A similar study in Saudi Arabia found *A. flavus* and *A. niger* in cinnamon samples at 33% and 75% (Hashem and Alamri, 2010). Meanwhile, some significant species isolated from cinnamon in Bahrain were *A. niger* (25%) and *Rhizopus stolonifer* (56%) (Mandeel, 2005).

A study in Benin by Akpo-Djènontin reported a mixture of spices, including cinnamon, contaminated with 8.86 μ g/kg of AFB1 (Akpo-Djènontin, 2018). Cho *et al.* (2007) found no detectable AF from cinnamon samples in Korea. Similarly, Ozbey and Kabak (2002) and Riordan and Wilkinson (2008) demonstrated an undetectable limit of AFs in cinnamon samples from Turkey and Ireland.

5.3 Clove

Cloves are dried flower buds of Syzygium aromaticum in the family of Myrtaceae (Raghavan, 2007). The name "clove" is derived from Latin clavus, Spanish clavo, French clou, and German nelke, which means "nail" due to its resemblance to a nail's shape (Raghavan, 2007). The flower buds are picked before opening and sun-dried. After drying, the colour of the cloves turns dark brown with a slightly light brown content. Cloves have an intensely bitter and woody flavour and release a spicy-sweet aroma. The volatile oil of clove buds ranges from 5% to 20%, with eugenol, eugenyl acetate, and β -caryophyllene as the main components. The volatile oil has a significant amount of eugenol, which exhibits biological and antimicrobial properties (Leela and Sapna, 2008). In a recent study, Hlebová et al. (2021) revealed that clove oil has the highest inhibitory effect against all Aspergillus species tested, i.e., A. flavus, A. fumigatus, A. terreus, and A. niger (Hlebová et al., 2021). Eugenol volatile oils may inhibit the growth of microorganisms such as A. niger, Saccharomyces cerevisiae. Mvcoderma sp., Lactobacillus acidophilus, and Bacillus cereus to varying degrees (Meena and Sethi, 1994; Leela and Sapna, 2008).

Cloves function best in a whole form because the flavour will disappear once the cloves are ground. Sweet, fruity, caramelised, chocolatey, and meaty flavours all pair well with clove. It is used in various dishes such as salad dressings, desserts, and fruitcakes in the USA. Cloves are used in the whole form or ground in garam masala, biryani, meat dishes, and pickles by Sri Lankans and North Indians.

Several studies have shown little to no occurrence of fungi in cloves. Nurtjahja *et al.* (2019) examined the clove samples from the markets in Indonesia and found cloves among the less contaminated samples of all the studied samples. Similarly, Hashem and Alamri (2010) and Elshafie *et al.* (2002), who studied clove samples in Saudi Arabia and the Sultanate of Oman, also reported less incidence of fungi. Temu (2016) revealed that cloves showed no fungal colonies after multiple culturing. Volatile oils could be the main reason for low fungal counts in cloves (Hashem and Alamri, 2010; Guyenot *et al.*, 2003; Eilsen and Rios, 2000). Aiko and Mehta (2016) also reported no occurrence of fungi from clove samples in India.

5.4 Star anise

Star anise or Illicium verum is a tree containing a star -shaped fruit used as a spice. The fruits have eight carpels with a seed inside each carpel. Star anise turns to reddish-brown colour after it is sun-dried. The plant is indigenous to China and Vietnam. Nowadays, star anise is cultivated in many countries such as India, Japan, Korea, Laos, and the Philippines. Star anise has almost the same taste as fennel. It has a sweetish and spiciness flavour due to its volatile oil, such as anethole, to 85–90% of total volatile contributing oils (Chempakam and Balaji, 2008). According to the authors. anethole may also contribute the to antimicrobial properties of star anise.

The sweetness and spiciness of star anise are enhanced when it is cooked. Star anise symbolises Chinese cuisines. It is usually blended with other spices such as clove, fennel, cassia, and Sichuan pepper (George, 2004). The mixture is called the Chinese fivespice, and it is commonly used for batters, meat marinade, stews, and soups (George, 2004; Raghavan, 2007). Apart from China, star anise is also used as a fivespice powder mix in Vietnam, mainly to make beef soups. In Malaysia, star anise is used in various cuisines, such as soup and curry.

The study of fungal and mycotoxin occurrence in star anise is scarce. From the previous screenings of fungi, several researchers found no occurrence of fungi in star anise samples (Kong *et al.*, 2014; Aiko and Mehta, 2016). This can be explained by the presence of anethole, the major essential oil in star anise that inhibits fungal growth. Moreover, studies have shown the antifungal activity of star anise against *A. flavus* and *A. parasiticus* (Freire *et al.*, 2011; Aly *et al.*, 2016).

5.5 Coriander seeds

Coriander that is used as a spice is referred to as coriander seeds. It is the spherical seeds of *Coriandrum sativum* from the family Apiaceae. Coriander originated from Asia and is now cultivated in other countries such as India, Argentina, Mexico, Morocco, and the USA (Raghavan, 2007). It has a yellowish-brown colour and is available whole or ground. Coriander is added to various cuisines as a flavouring. Coriander seeds contain 0.2%–2% of volatile oils, mainly *d*-linalool, which contribute to the fragrance. Coriander is most commonly used as a ground spice. It is the main ingredient of blended powdered spices like curry and korma. Coriander seeds will produce a strong aroma when it is dry-fried.

Saidi *et al.* (2021) studied coriander samples from military caterings and found *Aspergillus* spp. (36%) as the most occurring genera, primarily *A. niger* (36%) and *A. flavus* (12%), commonly known as mycotoxinproducing fungi. Similarly, Jeswal and Kumar (2015) investigated 30 coriander samples from local markets in Bihar, India, and isolated 3 most dominant fungal species; *A. niger* (10.6%), *A. flavus* (8.0%), and *P. verrucosum* (6.3%). In contrast, a recent study by Nurtjahja *et al.* (2019) revealed a low occurrence of toxigenic *Aspergillus* spp. from coriander samples purchased from traditional markets in Medan, Indonesia.

The incidence of mycotoxins, mainly AFs and OTA, in coriander seeds has been extensively reported in various countries. Jeswal and Kumar (2015) reported the incidence of AFB₁ and OTA in 50% and 30% of coriander samples. Darra *et al.* (2019) reported 75.3 μ g/kg of total AFs and no occurrence of OTA in coriander samples. In addition, a study in Tunisia reported a high incidence of OTA in 50% of coriander samples ranging from 74 to 576 μ g/kg (Zaied *et al.*, 2010).

5.6 Pepper

Pepper is a dried small berry called peppercorn from the flowering vine *Piper nigrum* in the family Piperaceae. The word pepper is derived from the Sanskrit term *pippali*, which means Indian long pepper, a close relative of black pepper. There are many types of pepper, including black and white pepper. The difference between black and white pepper is they were picked at different stages of growth and the way it was processed (Raghavan, 2007).

Black peppercorn is harvested from green, dried, unripe berries from the pepper plant. Black pepper is prepared by boiling the harvested berries for a short time (Farkas and Mohácsi-Farkas, 2014). After boiling, the berries are dried until they become blackened or 7IEW

brownish with a hard wrinkled surface (Farkas and Mohácsi-Farkas, 2014). White peppercorn is near ripe berries. Unlike the black pepper, white pepper is made up of only the inside seed of peppercorn, not the whole fruit. The outer pericarp of white peppercorns is prepared by soaking the berries in water to soften the skin, and the pericarp, along with the pulpy coating, is removed by rubbing. Then, it is bleached, rinsed, and dried. White pepper can also be made from black peppercorns by removing the outer shells manually by a process known as decortication (Farkas and Mohácsi-Farkas, 2014). This kind of white pepper will partly resemble the taste of black pepper.

Pepper fruits are pungent and aromatic. Black and white pepper have two basic components contributing to their pungency and aroma. The pungency of black and white pepper is due to a non-volatile chemical compound called *piperine*. The pungent aroma of black pepper is due to its volatile oil, monoterpenes, which consists of sabinene, α -pinene, β -pinene, limonene, and 1,8-cineole (Raghavan, 2007). Similarly, white pepper also contains piperine which contributes to pungency. Nevertheless, white pepper has less aroma than black pepper because it lacks essential oil, primarily present in the pericarp (Raghavan, 2007). Globally, peppers are the most widely used table spices. Black pepper is often known as "black gold" and "king of spices." Peppers are used to alter the taste of food on the table, added during cooking, or used as marinades. In Southeast Asia, including Malaysia, peppers are generally used in soups, curries, and to marinate meat and poultry.

Several studies have reported a high occurrence of fungi in black pepper samples. Nurtjahja et al. (2019) reported a high incidence of fungi, mainly Aspergillus spp., from black and white pepper in Indonesia. Garcia et al. (2018) reported that black pepper was among the most contaminated samples by toxigenic Aspergillus spp., i.e., A. flavus, A. parasiticus, A. nomius, A. ochraceus, A. niger, and A. carbonarius in Brazil. Temu (2016) reported black pepper contaminated with AFproducing fungi, A. flavus, A. parasiticus, and A. nomius in Tanzania. In contrast, white pepper was among the less contaminated samples, with only two genera, Aspergillus and Rhizopus spp. Black pepper samples from Bahrain was contaminated with 1.12×10^3 CFU/g of fungi, mostly by the A. flavus species (78%; Mandeel, 2005).

Some studies reported on the presence of mycotoxins in black pepper. In Korea, Cho *et al.* (2007) reported the presence of mycotoxin below the detection limit in black pepper. Fewer mycotoxins detected in black pepper samples compared to other countries could be due to the

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climate in Korea with low temperature and humidity, which is not ideal for fungal growth and mycotoxin production. In contrast, Hammami *et al.* (2014) reported a high level of AFs in black pepper at 84.09 μ g/kg in Qatar. Meanwhile, a study in Turkey found that 30.4% of black pepper samples were detected for AFs, mainly AFB1 and AFB2, ranging from 0.13 to 0.42 μ g/kg and 0.04 to 0.05 μ g/kg. In comparison, OTA was detected in 17.4% of the samples at concentrations of 0.87 to 3.48 μ g/kg (Ozbey and Kabak, 2012). Similarly, in Cameroon, OTA was detected in 10% of black pepper samples at 1.53 μ g/kg and 40% of white pepper samples at 3.30 μ g/kg.

5.7 Fennel

Fennel (*Foeniculum vulgare* Mill.) is a member of the Apiaceae family. Fennel seed is oval-shaped with a pale green to yellowish-brown colour. The flavour of fennel resembles the star anise, which comes from its chemical compound, anethole (Shamina Azeez, 2008). Fennel exists in various types, and the most commonly used are sweet fennel (*F. vulgare* var.) and bitter fennel (*F. vulgare* Mill). Fennel seeds comprise 1% to 6% volatile oil, which distinguishes the bitter and sweet fennel. Bitter fennel contains 1% to 6% volatile oil, with anethole as the major compound, comprising 50% of the oil. Meanwhile, the sweet fennel has 50% to 80% anethole.

In India, Jeswal and Kumar (2015) demonstrated moderately contaminated fennel samples with *A. flavus* (12.3%), *A. niger* (5.3%), and *A. ochraceus* (5.0%). Kulshrestha *et al.* (2014) also revealed that the fennel samples in India were mostly contaminated by *A. flavus* (18.51%) and *Fusarium oxysparum* (14.81%). Simultaneously, *A. flavus* and *A. fumigatus* were also detected at 7.40%.

Consequently, Garcia *et al.* (2018) reported only 12.5% (1 out of 8) of *A. niger* strains isolated from fennel could produce OTA. Ali *et al.* (2015) reported the presence of AFs from 0.01 to 5.29 μ g/kg and OTA was from undetectable levels to 1.26 μ g/kg in fennel samples.

5.8 Cumin

Cumin is a dried, ripe fruit from the flowering plant *Cuminum cyminum* in the family Apiaceae. The name cumin is from the word *sughandan*, which means pleasant smell. Cumin is a long oval-shaped, greenish or light-brown seed. It has a spicy, earthy, and bitter taste. Cumin is native to India, Iran, Egypt, the eastern Mediterranean, China, Indonesia, and Syria (Raghavan, 2007). Cumin is sold as ground or whole spices. The main volatile components in cumin are cuminaldehyde, β -pinene, terpinene, and ρ -cymene. The presence of

cuminaldehyde contributes to its flavour and aroma (Amin, 2001). Whole and ground cumin is used in a wide range of cuisine. In India, cumin is used to make pickles or blended spices called *sombar padi* (Amin, 2001; Raghavan, 2007).

The study by Mandeel (2005) in Bahrain demonstrated contaminated cumin samples with 4.6×10^2 CFU/g fungi, with *A. niger* the most isolated species (36%). In addition, Hashem and Alamri (2010) reported *A. niger* (60%) as the most frequently occurring *Aspergillus* species, followed by *A. flavus* (40%) and *A. fumigat*us (20%). These three species are known as OTA - and AF-producing fungi. Temu (2016) reported that cumin samples were among the less contaminated spices in Tanzania.

Zinedine et al. (2007) demonstrated that 57% of cumin samples purchased from popular markets in Morocco exhibited very low occurrence of AFB1 and total AFs, with the mean values of 0.03 μ g/kg and 0.05 µg/kg. Additionally, Bircan (2005) observed no AF contamination in cumin samples in Turkey at the detection limit of 0.2 µg/kg. Riordan and Wilkison (2007) also reported no AFs in cumin samples from the Irish market. Reddy et al. (2011) reported that 67% of positive samples of cumin for AFB1 ranged from 1.89 to 4.64 µg/kg and a mean level of 3.26 µg/kg. This level did not exceed the EU regulation (5 μ g/kg). However, a study by Mwangi et al. (2014) reported a very high incidence of AF (98 µg/kg) in cumin samples from the Kenyan market, which exceeded the EU regulatory limits.

5.9 Mixed spices

Mixed spice is a blend of different types of spice. Commercial blend spices are convenient and easy to use and can be found easily in the market. Some foods require a lot of spices; therefore, mixed spices are useful to reduce the preparation time. Mixed spices can create a consistent flavour, colour, and texture of the cuisines. They usually consist of spices, salt, sugar, monosodium glutamate (MSG), anti-caking agents, and a lot more. The other ingredients were added to enhance the overall flavour and improve the quality of the mixed spices. Some popular mixed spices in the markets are curry and korma mix.

Curry powder is a mixture of many spices. The main spices in curry powder include coriander seeds, cumin, turmeric, and ginger (Ravindran and Kallupurackal, 2001). Other minor ingredients are fenugreek, celery, black pepper, red pepper, cinnamon, nutmeg, cloves, caraway, fennel, cardamom, and salt. The ingredients differ depending on the desired outcome (Sharma and Sharma, 2004). Curry powders are widely used in cooking worldwide, especially in South Asian cuisines (Ravindran and Kallupurackal, 2001). Besides curry powder, korma mix is an instant powder of various spices. It consists of coriander seeds, cumin, fennel, white pepper, star anise, cinnamon, dhal, dry ginger, cardamom, clove, and black pepper. Korma powder is widely used in Malaysia to prepare many delectable cuisines.

A study in Qatar revealed no occurrence of fungi in curry samples (Hammami et al., 2014). Likely, Temu (2016) reported that curry was among the less contaminated samples. Hammami et al. (2014) emphasised that some spices in the curry mixture exhibit antifungal properties. This could be explained by the minimal contamination of the curry samples. In contrast, Makhlouf et al. (2019) found high fungal contamination in spice mixtures. Corroborating their findings, the authors explained that the mixture of various spices in products contributed these to higher fungal contamination as each ingredient used in the mixed spices might be exposed to a different source of contamination.

Ali *et al.* (2015) reported the incidence of total AFs, AFB₁, and OTA in curry and korma mix ranging 0.03 to 3.04 μ g/kg, 0.03 to 2.26 μ g/kg, and 0.14 to 9.59 μ g/kg, respectively. Another study revealed that the amount of AFB₁ in mixed spices purchased from different cities in Malaysia was above the EU legislation limit (5 μ g/kg), ranging from 4.7 to 14.36 μ g/kg (Alsharif *et al.*, 2019).

6. Conditions of fungal growth

Spices are susceptible to fungal contamination despite the low a_w of the commodities. Contamination can occur depending on the place of cultivation, harvest, and processing (Nguegwouo et al., 2018). Spices are mainly cultivated in tropical countries with high temperatures and humidity, suitable for fungal contamination and mycotoxin production (Vyhnánek et al., 2018). During preharvest, pest and insect infestation in the spices contribute to fungal contamination (Cinar and Onbasi, 2019). Fungi can contaminate spices from the farm to the market. Once the spices are infected in the field, the fungi will continue to grow during storage in favourable conditions (Reddy et al., 2010). In addition, the processing of spices is one of the primary sources of contamination. For example, since sun drying is cost-efficient, spices are usually sun-dried to reduce the moisture content and extend the shelf life (Thanushree et al., 2019). However, inadequate drying could result in fungal growth during storage, leading to the deterioration in the quality and safety of the spices

Aspergillus spp. is generally known as storage fungi that can contaminate various food and feedstuffs during prolonged storage. Aspergillus species are xerophilic fungi and able to survive at very low aw and produce mycotoxins. Several researchers have reported the occurrence of toxigenic fungi in spices. A. flavus and A. niger are the most predominant toxigenic fungi usually found in spices. Table 2 shows the isolation frequency of mycotoxigenic fungi in some common spices.

Penicillium is another storage fungi that can produce mycotoxins, especially in countries with tropical climates (Pardo *et al.*, 2006). *Penicillium* has a wide range of species and habitats, and some of the species are known as mycotoxin producers (Pitt and Hocking, 2009). It can grow in various environments such as soil, air, interior spaces, and many food products (Visagie *et al.*, 2014). However, the conditions that promote fungal growth do not confirm the presence of mycotoxins (Sanchis and Magan, 2004; Pardo *et al.*, 2006). According to the authors, the fungi can grow abundantly in certain conditions without producing mycotoxins.

7. Impact of mycotoxin contamination

Mycotoxicoses can be classified as acute or chronic, just like any toxicological syndrome. Acute toxicity is defined by a rapid onset and an obvious toxic response. In contrast, chronic toxicity is defined by a low-dose exposure over a long period, likely to result in cancers and other typically irreversible effects (Zain, 2011). Aflatoxicosis is a type of toxic hepatitis that causes jaundice, and in more severe cases, may lead to death (Lewis et al., 2005). Chronic dietary exposure is usually the source of the most significant risk of AF in humans. Human hepatocellular carcinomas have been linked to dietary AF exposure, which may be exacerbated by the hepatitis B virus. Hepatocellular carcinomas are responsible for fatalities in Sub-Saharan Africa and China. The disease is linked to several risk factors, such as high daily consumption of AFs (1.4 µg) and high cases of hepatitis B (Wild et al., 1992; Zain, 2011). In Gambian children, consumption of AF-contaminated food was linked to lower levels of secretory immunoglobulin A (IgA; Turner et al., 2003). AFs contamination has also caused functional changes in particular lymphocyte subsets and changes in differential subset distributions in Ghanaian adults, demonstrating that AFs may impair individual cellular immunity, lowering resistance to infections (Jiang et al., 2005).

8. Management and control strategies of mycotoxin contamination in spices

Globally, mycotoxin contamination in spices is widespread, primarily in tropical areas. Proper pre and postharvest practices are the best mitigation strategy to prevent the increased level of mycotoxins (Moses et al., 2014: and Truckses, 2019). Besides, Iha the implementation of good agricultural practices (GAP), good storage practices (GSP), and good manufacturing practices (GMP) can mitigate the production of mycotoxin (Kamle et al., 2019). Preharvest practices such as irrigation, pest control, fungicides and fertilisers are useful to control mycotoxin production (Bhatnagar et al., 2002; Iha and Trucksess, 2019). Nevertheless, irrigation application during preharvest might be challenging as it is costly and difficult to adapt to a new technique in agricultural practices. The use of fungicides can also retard the growth of AF-producing fungi such as A. flavus (Santos et al., 2010). The authors revealed that the most effective fungicides against A. flavus strains and AF production were tebuconazole 25% and mancozeb 80%.

Postharvest control measures are fundamental to ensure the safety and quality of commodities. Various methods are implemented to degrade and remove mycotoxins from contaminated plants (Jallow et al., 2021). For instance, postharvest practices such as sorting and removing the damaged and mouldy fruits are important to prevent increased mycotoxin production (Iha and Trucksess, 2019). In addition, prompt drying of spices after cleaning is crucial as moisture is an important factor in fungi proliferation (Iha and Trucksess, 2019). Besides, physical, chemical, and biological methods are also tested and implemented. Chemical methods such as acids, bases, and oxidising and reducing agents are useful to degrade and detoxify mycotoxins (Jallow et al., 2021). Weng et al. (1994) revealed that the use of ammonia caused high degradation rates of AF. This can be explained by the fact that AFs are not stable under alkaline conditions and will reduce the toxins into less toxic substances (Moerck et al., 1980; Jallow et al., 2021). Oxidising agents such as ozone treatment and irradiation (sunlight, gamma, and ultraviolet [uv]) have the potential to control mycotoxins (Iha and Trucksess, 2019) and maintain the quality of agricultural commodities (Mahato et al., 2019). Moreover, atoxigenic fungi and non-aflatoxin forming strains of A. flavus have been used as a biological control to protect the crops from mycotoxin contamination (Dorner et al., 2003; Udomkun et al., 2017; Mahato et al., 2019).

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9. Conclusion

In conclusion, spices are vulnerable to fungal infection, leading to mycotoxin contamination if the conditions are favourable for fungal growth. However, the level of mycotoxins in spices varies depending on the types of spices, processing methods, storage conditions, and others. AFs and OTA are the main mycotoxins reported in spices, especially chilli products. The high consumption of chilli-based products may expose consumers to the harmful effect of mycotoxins. Hence, implementing preventive and control measures is crucial to control the growth of mycotoxigenic fungi and prevent mycotoxin contamination in spices.

Conflict of interest

The authors declare no conflict of interest.

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