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Current Opinion in Food Science

Impact of toxigenic fungi and mycotoxins in chickpea: a review

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Chickpea (*Cicer arietinum*) is one of the most cultivated pulses in terms of world production being India the largest producer. There is a high demand of this legume due to its nutritional value. Although is more popular in developing countries, it is becoming recognized throughout the world. Chickpea is often attacked by fungi during pre and post-harvest stages, significantly affecting its productivity, also some species can be potential mycotoxin producers that can lead to serious threats to human health. Since there is an increasing demand for high quality and innocuous foods, limits for mycotoxin contamination have been established. This review provides information about the occurrence of mycotoxigenic fungi and mycotoxins on chickpea seeds and/or based-products around the world. Some data about strategies to reduce the problem are also included.

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Current Opinion in Food Science 2018, **23**:32–37

This review comes from a themed issue on **Food mycology**

Edited by **Marta Taniwaki**

https://doi.org/10.1016/j.cofs.2018.05.003

Introduction

The chickpea (*Cicer arietinum*) also known as garbanzo is a legume of the family Fabaceae, subfamily Faboideae. It was one of the first grain legumes domesticated in the Old World, 7500-year-old remains have been found in the Middle East [1].

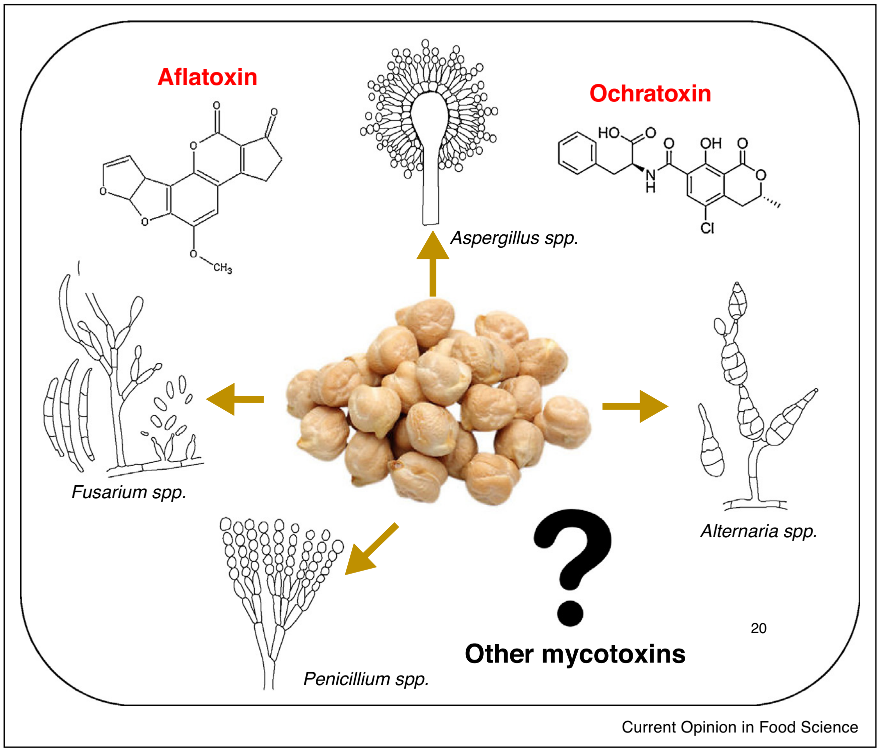
Globally, it is one of the most cultivated pulses in terms of world production with a total production of 14.2 million tonnes and an average yield of 0.96 tonnes ha^{-1} [2]. Currently, India represents the largest producer of chickpeas accounting for around 70% of the global production. India is followed by Australia, Pakistan, Myanmar, Ethiopia, Turkey, Iran, Mexico, Canada and Russia. Among the top exporting countries, Australia represents the biggest exporter of chickpeas accounting for more than one-third of the total global export volumes. Australia is followed by Russia, India, Mexico, Canada, the United States, Ethiopia, Argentina, Tanzania and Iran. At present, India represents the biggest importer of chickpeas accounting for around one-fifth of the total global import volumes. India is followed by Bangladesh, Egypt, the United States, Algeria, Pakistan, Spain, United Kingdom and Turkey [3].

At present there is a high demand for world production, exports and imports of chickpeas due to the crop's nutritional value low in fat and sodium, cholesterol free and being an excellent source of both soluble and insoluble fibre, complex carbohydrates, vitamins, folate, and minerals, especially calcium, phosphorous, iron, and magnesium [4–6]. Also, chickpea-based food consumption is supported by trends towards more healthful and varied snacking, as well as growing demand for gluten-free products. Chickpea-based food consumption is more popular in developing countries, but is increasingly becoming recognized as an excellent part of a healthy diet throughout the world [7]. Chickpeas are either consumed fresh or processed into different products. There are a variety of traditional chickpea preparation and processing methods that include soaking, decortication, grinding, sprouting, fermentation, boiling, mashing, roasting, parching, frying, and steaming treatments [8].

Chickpea is usually cultivated in regions where climate variability, drought and limited use of fertilizers significantly reduce productivity. However, being a leguminous crop, it exhibits the important characteristic of fixing atmospheric nitrogen (N) through its symbiosis with rhizobia, enabling cultivation in many N poor soils with acceptable yields [7].

Chickpea is often attacked by fungi during both pre and post-harvest (during transport and/or in storage), significantly affecting its productivity. Seeds and infected harvest debris are the main sources of primary infections, and the level of seed damage depends on environmental conditions such as high relative humidity, dew, and

Figure 1



Main fungal genera and mycotoxins present in chickpeas. (This figure summarizes the main concepts discussed.)

temperatures above 25 °C. The fungal species that infect chickpeas can be potential mycotoxin producers (Figure 1). The main species mycotoxin producers are included in the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria*. Mycotoxins can be acutely or chronically toxic, or both, depending on the kind of toxin, the dose, the health, the age and nutritional status of the exposed individual or animal, and the possible synergistic effects between mycotoxins [9,10].

Nowadays, there is an increasing world consumer demand for high quality and innocuous foods with the lowest possible level of contaminants such as mycotoxins. As a result, the food industry in the developed world demands raw ingredients of the best quality and that conform to statutory limits for mycotoxins. Because the mycotoxins are unavoidable, it is relevant the trazability of mycotoxins through the food and feed chains. This paper will review on mycotoxigenic fungi, mycotoxins, and efficacy of botanicals and physical decontamination strategies for mitigating risks associated with exposure to mycotoxin-contaminated chickpeas.

Fungal occurrence in chickpea and by products

Although it is known that chickpea is susceptible to more than 25 very well documented fungal pathogens, Ascochyta blight caused by *Ascochyta rabiei* [Pass.] Labrousse is among the most serious diseases affecting this crop worldwide. This disease causes severe damage affecting all the aerial parts of the plant causing necrosis, tissue collapse, and also affects the quantity and quality of grains and seeds with losses that can reach 100% when conditions that favour this pathology are presented [11]. But some fungi considered weak parasites are also important contaminants, due to the nutritional richness of the chickpea seed and the texture of the seed coat that can cause deterioration of the seed during storage. The most prevalent fungi in chickpeas worldwide are species belonging to *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria* spp., and *Rhizopus*, among others (see Table 1). These fungal genera are present in the field and, in addition their presence is favoured by the mechanical damage produced during harvest, inadequate transport, problems during processing, and so on. Because contaminated seeds can often result in poor germination and poor seedling vigour, resulting in an un-healthy crop there are many reports in the literature about fungal occurrence in chickpea seeds. Most of the studies have been done in the major chickpea producing and consuming countries such as India, Pakistan, and Turkey. Also, most of the surveys have been focus on chickpea seeds rather than in processed chickpea such as chickpea flour (Table 1).

An interesting study about the effect of storage on chickpeas was done by Ahmad and Singh [12]. The changes in

Table 1

Fungal contamination in chickpeas and by products.

Country	Type of sample	Isolated fungi[a]	Reference
Argentina	Chickpea seeds	*Ascochyta rabiei*, *Fusarium oxysporum*, *Alternaria* spp., *Colletotrichum* sp., *Macrophomina phaseolina*, *Rhizoctonia* sp., *A. niger*, *Aspergillus* spp., *Penicillium* spp., *Rhizopus* sp., *Nigrospora* sp. and Yeasts.	[24]
Bangladesh	Stored chickpea seeds	*Alternaria alternata*, *A. flavus*, *A. niger*, *A. fumigatus*, *A. nidulans*, *Curvularia lunata*, *Penicillium* spp., *Rhizopus* spp.	[25]
India	Harvested chickpea seeds	*Alternaria* spp., *Fusarium* spp., *Cladosporium* sp., *Curvularia* spp., *Mucor* spp., *Penicillium* spp., *A. flavus*.	[12]
	Stored chickpea seeds in jute bags	*Penicillium* spp., *A. flavus*, *A. niger*, *A. nidulans*, *A. ochraceus*, *Fusarium* spp., *A. terreus*, *Rhizopus nigricans*, *Alternaria* spp.	
	Stored chickpea seeds in metal bins	*Penicillium* spp., *A. flavus*, *Fusarium* spp., *Rhizopus nigricans*.	
	Chickpea seeds	*A. niger*, *Rhizopus* spp., *F. oxysporum*, *A. alternata*, *A. flavus*, *Penicillium* spp., *Chaetomium* spp., *A. fumigatus*.	[26]
	Chickpea seeds	*A. alternata*, *A. flavus*, *A. niger*, *A. fumigatus*, *C. lunata*, *Fusarium verticillioides*.	[27]
	Chickpea seeds	*F. oxysporum*, *Rhyzopus stolonifer*, *Phoma herbarum* and *A. flavus*.	[28]
	Farmer stored chickpea seeds	*A. flavus*, *A. niger*, *A. ochraceous*, *A. terreus*, *A. fumigatus*, *A. nidulans*, *F. oxysporum*, *A. sydowi*, *Cladosporium macrocarpum*, *F. semitectum*	[29]
	10 varieties of chickpeas seeds	*A. niger*, *A. flavus*, *F. oxysporum*, *Rhyzopus* sp.	[30]
	Farmer stored chickpea seeds	*Aspergillus* spp., *Alternaria* spp., *Fusarium* spp. and *Penicillium* spp.	[31]
Libia	Chickpea	*A. flavus*, *A. niger*, *A. fumigatus*, *A. parasiticus*, *A. ficuum*, *A. terreus*, *A. heteromorphus*, *P. chrysogenum*, *P. cyclopium*, *M. hiemalis*, *A. alternata*, *F. oxysporum*, *F. solani*, *F. equiseti*, *R. stolonifer*, *C. cladosporioides*, *Talaromyces* sp.	[32]
Pakistan	Chickpea flour	*A. flavus*, *Rhizopus oryzae*, *A. niger*, *Penicillium digitatum*, *Botrytis cinerea*, *C. cladosporoides*, *F. oxysporum*, *M. petrinsularis*, *M. recemosus*, *Rhizopus arrhizus*, *Penicillum* sp., *Mucor* sp., *A. oryzea*	[14•]
	Black chickpea	*A. niger*, *A. fumigatus*, *Rhizopus* sp., *A. terreus*, *A. flavus*.	[33]
	White chickpea	*Rhizopus* sp., *A. fumigatus*, *A. niger*.	
Saudi Arabia	Salted chickpea	*A. niger*, *A. flavus*, *Emericella* spp.	[34]
Turkey	Roasted chickpeas	*Aspergillus* spp., *Penicillium* spp., *Mucor* spp., *Fusarium* spp.	[35]

[a]Decreasing order of fungi isolation.

the mycobiota population, moisture level and aflatoxin increase in chickpea seeds during 12 months' storage in jute bags and metal bins was evaluated in India. It was shown that fungal contamination in chickpea seeds at harvest time was very high. As the seeds were kept under storage a shift in the spectrum of fungal population was observed: *Alternaria*, *Cladosporium*, *Botrytis* and *Fusarium* species, which are carried within seeds from the field gradually disappear and were replaced by *A. flavus*, *Aspergillus terreus*, *Aspergillus nidulans*, *Penicillium*, *Absidia*, *Chaetomium* and *Rhizopus* species. The moisture of harvest chickpea seeds was 13%, an increase in moisture content was observed during the storage, especially during the raining season, being the moisture higher in jute bags in comparison with the metal bin. During mycobiota study of stored chickpea seeds approximately 569 isolates of *A. flavus* and 61 isolates of *Aspergillus parasiticus* were isolated, among them over 84% of *A. parasiticus* isolates were toxicogenic, while 64% of *A. flavus* were able to produce aflatoxin.

There is in the literature only one study done on fungal occurrence in chickpea flour. Gram flour, also referred to as chickpea flour, garbanzo flour or besan, is used all over the world but as a staple food in Indian, Pakistani and Bangladeshi cuisine. Gram flour is also commonly used as a paste with water or yoghurt to make a popular facial exfoliant in the Indian subcontinent and also it is popular in Pakistan as an ingredient in a number of different recipes including bonda, pakoras, papdums, onion bhajis and Bikaneri Bhujia [13]. As chickpea flour, is one of the alternatives of wheat flour, and because chickpea is the most important crop of Indo-Pak region, Mushtaq *et al.* [14•] studied the natural occurrence of aflatoxins, the mycological profile and the molecular characterization of aflatoxicogenic strains in chickpea flour sold in the markets in the Rawalpindi district, Pakistan. Ten chickpea flour samples were collected from different retailers and shops. A total of 13 different fungal species were isolated, being *A. flavus* the predominant one, followed by *Rhizopus oryzae*.

Mycotoxin occurrence in chickpeas and by products

It is important to note that many fungal genera/species commonly isolated from chickpea seeds and chickpea-by products are potential mycotoxin-producers of aflatoxins, ochratoxin A (OTA), and trichothecenes, among others, so there would be a potential risk of contamination of chickpea with those metabolites. Such contamination may cause significant economic losses due to the potential impact on human and animal health, and the rejection or reduction of prices during commercialization in foreign markets. There are international regulations that restrict imports of raw materials contaminated mainly with aflatoxins, ochratoxin A, deoxynivalenol and trichothecenes type A [15]. Among aflatoxins, aflatoxin B_1 (AFB_1) is the most important, and International Agency for Research on Cancer (IARC) classifies it as a group I human carcinogen [16]. Natural contamination with aflatoxins and ochratoxin A has been reported in chickpeas and chickpea-based products in India, Turkey, Pakistan and Iran (Table 2). Although there are a limited number of studies, aflatoxin levels reported seems to be fairly high in stored chickpea seeds in comparison with those levels found in processed chickpeas (flour, roasted). Ahmad and Singh [12] studied aflatoxin increase in chickpea seeds during 12 months storage in jute bags and metal bins, in India, the toxin was not detected in seeds after 3 and 6 months of storage in jute bags and metal bins, respectively. Maximum amounts of aflatoxin were detected in seeds in jute bags (205 μg/kg) after 7 months of storage. Mushtaq *et al.* [14•] investigated the aflatoxins occurrence in 10 chickpea flour samples, and they found out that only 20% of them were contaminated with aflatoxin B_1 (mean level, 3.63 μg/kg), while AFB_2, AFG_1, and AFG_2 were not detected. In a further study Mushtaq *et al.* [13] observed aflatoxin level in gram flour. Those studies showed high level of AFB_1 in gram flour.

Ochratoxin A together with aflatoxins, fumonisins, deoxynivalenol and zearalenone is considered to be of major importance for human and animal health. Ochratoxin (OTA) is produced by both *Aspergillus* and *Penicillium* species in a range of foods, including grapes, wines, dried vine fruit, cereals in cool temperature climates, coffee, cocoa and chocolate, among others [17••]. OTA has been shown to be toxic and carcinogenic in animals, being kidney the main target organ. Also, OTA is a potent renal carcinogen in several animal species, and other adverse effects include immunotoxicity, inhibition of macromolecular synthesis, increased lipid peroxidation, and inhibition of mitochondrial respiration. Moreover, this toxin has been suspected as a cause of various human nephropathies and chronic interstitial nephropathy [18]. The IARC has classified OTA as a Group 2B possible human carcinogen [16]. OTA have been detected in chickpeas in Iran at low incidence levels [19,20]. The concentration detected were higher in some samples that those recommended by the European Commission for unprocessed cereals [21].

Postharvest control of toxigenic fungi in chickpeas

As chickpea seeds are often infested with potential toxigenic fungi and their associated toxins during storage and processing, reducing food quality and safety, there is an urgent need to evaluated strategies to protect food items like chickpeas from fungi, and toxins. One approach of controlling the postharvest losses caused by fungi is the use of synthetic chemicals. However, food industries are looking for safer alternatives because of their adverse effects on consumers' health and environment. In this way Indian researchers proposed the use of essential oils (EOs) which are biodegradable (which generally makes them less toxic to the environment) and have shown antimicrobial and antioxidant potential [22•]. The efficacy of essential oil components, viz. *Z*-citral, *E*-citral, methyl cinnamate and fenchone individually and in combination, against food contaminating fungi, aflatoxin B_1 production and as antioxidant. As a result, oil components inhibited an aflatoxigenic strain of *A. flavus* and 13 additional food-infesting fungi, also aflatoxin production was inhibited. Moreover, in order to recommend oil components and their combination as a plant-based preservative, the authors tested there *in vivo* practical applicability

Table 2

Mycotoxin presence in chickpea samples.

Country	Type of sample	Mycotoxin	Incidence %	Range (μg/kg)	Average (μg/kg)	Reference
India	Stored chickpea seeds in jute bins	Aflatoxin		5.5–205		[12]
	Stored chickpea seeds in metal bins	Aflatoxin		23.5–130		
	3 varieties of chickpeas	AFB1	100	62.2–250	167.4	[36•]
Iran	Chickpea from retail stores	OTA	18.7	2.1–12.5	5.9	[20]
	Chickpea from outlets	OTA	13.3	0.39–0.20	0.29	[19]
Pakistan	Chickpea from local retail shops	AFB1	10	2.5 μg/kg	2.5	[37]
	Gram flour/chickpea flour	Aflatoxins	60	AFB1 0.32–1.02 AFB2 0.12 AFG1 0.59 AFG2 0.35 AFT 0.80–2.1		[13]
	Chickpea flour from local retailers	Aflatoxins	20	AFB1 3.03–4.24 AFB2 ND AFG1 ND AFG2 ND	3.63	[14•]
Turkey	Roasted chickpeas	AFB1	10	1.7	1.7	[35]

ND, not detected.

as fumigant in chickpea food system in plastic containers and concluded that all EOs showed remarkable protection (>50%) of chickpea seeds up to 6 months storage without affecting their viability (absolute germination of treated chickpea seeds) [23]. Later on, Prakash *et al.* [22•] carried out other study in order to analyze the antifungal, anti-aflatoxin, and antioxidant efficacy of some EOs on several fungal species. EOs obtained from *Carum carvi* and *Cymbopogon citratus* exhibited higher efficacy, and all the tested EOs significantly inhibited mycelia growth and aflatoxin B_1 production in a dose-dependent manner. Regarding EOs phytotoxicity, 100% germination of chickpea seed was observed indicating that EOs did not have significant effect on seed viability. Based on their virtues in protecting chickpea seed samples from fungal contamination during storage conditions and non-phytotoxic EOs could be an alternatives of synthetic preservatives.

Regarding other alternatives to reduce mycotoxin contamination in chickpeas, Rahimi *et al.* [20] showed that thermal treatment could reduce OTA content. As long as the time of cooking increased, degradation of OTA occurred, being significant the reduction after 5 hours of cooking.

Conclusions and future perspectives

- The present review showed the worldwide occurrence of mycotoxin producer fungi and mycotoxins in chickpea and chickpea-based products.
- *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria* and *Rhizopus* are the main fungal species isolated from chickpea seeds and chickpea-based products.
- Fungal population on chickpea changes under storage condition: *Alternaria*, *Cladosporium*, *Botrytis* and *Fusarium* species, are carried within seeds from the field, and are replaced by *A. flavus*, *A. terreus*, *A. nidulans*, *Penicillium*, *Absidia*, *Chaetomium* and *Rhizopus* species.
- Aflatoxin and OTA are the main mycotoxins detected in chickpea.
- Aflatoxin levels seem to be high in stored chickpea seeds in comparison with those levels found in processed chickpeas.
- Chickpeas thermal treatment reduced OTA content, as long as the time of cooking increased degradation of this toxin occurred.
- Essential oils have been proposed as an alternative of synthetic preservatives for protecting chickpea seeds from fungal contamination during storage conditions. Although, further large-scale research the food system is required.
- Aflatoxin B_1 presence in chickpeas is a major public health concern and studies are needed for minimizing or eliminating them. The prevention of contamination with toxicogenic fungi during harvest, processing and storage is the best way to control aflatoxin accumulation.
- At present there is limited data about OTA and aflatoxins accumulation on chickpeas. More studies are needed in order to know the real exposure after chickpea consumption.
- There is need for proper storage chickpea seeds (control of time, temperature, and humidity) to minimize the fungal infestation and mycotoxin accumulation during storage.
- The co-occurrence of mycotoxins in chickpeas need to be study due the possible synergist effects.

Conflict of interest statement

Nothing declared.

Acknowledgements

Cendoya E, and Nichea MJ are fellows of CONICET, and Ramirez ML, Chulze SN, and Zachetti VGL are members of the Research Career of CONICET.

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