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REVIEW ARTICLE

Genetic Diversity of Fungi Producing Mycotoxins in Stored Crops

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Abstract

Mycotoxins are a variety of critical secondary metabolites for the defense, that produced by multiple types of fungi. These metabolites are toxins where metabolic pathways that produce these toxins are found in adjacent gene groups in the fungal genome when they have adequate environmental and dietary conditions. Mainly, they found in commodities stored by the wrong ways. Mycotoxins are the most potent known toxins that cause serious diseases with minimal concentrations. Genetic diversity was detected using polymorphic randomized amplification technique for DNA fragments between fungal isolates from different crops. This review article aims to review the current status of genetically diverse of mycotoxigenic fungi in various contaminated food. Several studies that have focused on the determination of prevalence and frequency of varies types of toxic fungi were reviewed. Also, the articles that study the toxicity of stored crops such as cereals and oilseeds were considered. The high contrast between findings of these works was presented in terms of the genetic diversity of fungal isolates produced toxins. *Aspergillus, Fusarium,* and *Penicillium* were observed among the most common fungus producing toxins. This study which derived from previous researches observed that Aflatoxin was the most toxin produced by most fungi. *Aspergillus* was the most genetically modified fungus, carrying the most genes responsible for producing the fungal toxins.

Keywords: Stored crops, Mycotoxins, Genetic diversity, *Aspergillus, Penicillium, Fusarium.*

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INTRODUCTION

Mycotoxins are secondary metabolic products (SM) produced by some fungi which are genetically capable of producing toxins when they have adequate environmental and nutritional conditions. The fungal toxins are the most potent known toxins that cause severe diseases with small concentrations of less than 10 ppm. This potency is due to the fungal toxins are heat resistant to the extent that they cannot be destroyed by conventional heat treatments used in manufacturing and cooking. The second reason is that they spread quickly from fungus colonies to food. Therefore, removing the fungal parts of food, as many people do, does not lead to the complete elimination of the fungal toxins produced in these foods and therefore the growth of fungus on these foods should be avoided [1, 2].

The most important toxins are produced by *Aspergillus, Fusarium, Penicillium*, and *Alternaria*. One fungus may produce more than one poison, and currently, there are more than 200 known types of fungal toxins that cause health risks to humans and animals. The most common toxins are Aflatoxins, Ochratoxins, Fumonisins, Trichothecene, Patulin, Rubratoxin, Citrinin and Zearalenone. The effect of these toxins does not appear quickly, but has a cumulative effect that appears after 10-20 years of eating contaminated food. The other problem is that it does not stimulate the immune system in the body to be detected and have no drug treatments to reduce the impact and thus constitute a health disaster in the world [3]. Aflatoxins, a group of about 20 metabolic compounds, are the most important fungal toxins. Aflatoxins B1, B2, G1 and G2 are usually found in foods and are present in a wide range of food commodities including cereals, nuts, spices, figs, and dried fruits [4].

The diagnosis of toxin-producing fungus was based on phenotypic characteristics and microscopic structure such as colony color, shape, pigmentation, as well as reproductive traits such as spores, type and shape, and size of produced stone bodies [5, 6]. However, these characteristics are unstable and can change under environmental conditions, as well as require considerable effort and time. The recently tended emergence of heterozygous strains within the same type drive the scientists to rely on the molecular diagnosis, which is based on Polymerase Chain Reaction (PCR) to give results and delicate accuracy in diagnosis [7]. The most important feature of the PCR-RAPD technique; it is a fast, low-cost and straightforward technique. The main disadvantages are the amplification process which either occurs or does not occur due to technical randomization. In addition, it reveals the presence of sovereignty and results in non-replicable [8-10]. The results of the PCR-RAPD analysis of a sample cannot be compared with the same conditions in two laboratories [11]. Ribosomal DNA is amplified to determine the taxonomic characteristics and relationship of evolution between fungi. The DNA sequence is often used to study taxonomic and developmental studies because they exist in living cells with important functions and therefore their evolution may be reflected in the evolution of the whole genome [12]. This review article aims to review the current status of genetically diverse of mycotoxigenic fungi in various contaminated food.

Prevalence and Frequency of Fungi Producing Toxins

Cereals and products stored as oil crops accompany many microorganisms such as fungi, yeast, and bacteria. These microorganisms multiply when the conditions for their growth are suitable causing damage to stored materials. In turn, it causes a reduction in the quality and chemical changes in the product [13]. Fungi play a particularly dangerous role during storage operations compared to other microorganisms. The toxins produced by these fungi have significant economic effects in many agricultural crops, especially wheat, maize, field pistachios, nuts, cotton seeds, and tea. Twenty-five percent of the world's crop production is contaminated with fungal compounds [14].

In general, toxins reach the food of humans and animals through the contamination of food with fungi which produce these toxins (the process of the formation of toxins and their secretion depends on the type of fungi and the nature of the food and the availability of appropriate environmental conditions). The nutrient encourages the growth of the fungus either during the different stages of production or transportation or storage period. The most important species responsible for the secretion of more than two-thirds of Mycotoxins are *Aspergillus,*

Fusarium, and *Penicillium* [15]. The presence and spread of these toxic fungi were confirmed by isolating them from these agricultural products. Table (1) summarized the results of prevalence and frequency of mycotoxin fungi that isolated from different crops from previous studies.

Studies have elucidated the isolation of different types of fungi with different propagation percentages, where the most toxic fungi produced in grain and seed crops are *Aspergillus, Fusarium,* and *Penicillium,* which produce SM of high risk to human and animal health. Most of these

Table 1. Prevalence and frequency of Mycotoxigenic fungi isolated from different types of crop

Samples (crops)	Place of samples collection	Type of fungi	Prevalence %	Frequency %	Reference
Corn	Malaysia	*Aspergillus flavus*	87	99	
		Aspergillus niger	83	95	
		Fusarium verticillioides	47	51	
		Penicillium sp	5	3.1	
Zea maize	Saudi Arabia	*Aspergillus flavus*	53	11.4	[17]
Cereal	Morocco	*Aspergillus niger*	-	14.10	[18]
(wheat, rice, coffee)		*Aspergillus flavus*	-	11	
		Penicillium sp.	-	24.33	
		Fusarium sp.	-	1	
Grains (cereal)	Libya	*Aspergillus* sp.	11.7 - 45		[19]
Legumes		*Penicillium* sp.	6 - 71.17		
		Fusarium sp.			
		Rhizopus stolonifer			
		Mucor piriformis			
		Alternaria tunuissima			
		Rhizoctonia solani			
		Pythium ultimum			
		Phyllactinia rigida			
		Sccharomyces cerevisiae			
Wheat	Iraq	*Aspergillus flavus*		24.70	[20]
Zea mays		*Aspergillus niger*		33.2	
		Aspergillus ochraceus		4.41	
		Alternaria alternata		12.53	
		Fusarium oxysporum		9.97	
		Rizopus stolonifer		7.70	
		Curvilaria lunata		1.85	
coffee beans	Brazil	*A. niger*	83.3		[21]
		A.ochraceus	53.3		
		A.flavus Cladosporium	25.016.6		
		Penicillium	10.0		
Adlay seeds	Korea	*F. incarnatum*	11.67		[22]
		F. kyushuense	10.33		
		F. fujikuroi	8.67		
		F. concentricum	6.00		
		F. asiaticum	5.67		
		F. graminearum	1.67		
		F. miscanthi	0.67		
		*F. polyphi*alidicum	0.33		
		F. armeniacum	0.33		
		F. thapsinum	0.33		
Adlay seeds	Korea	*Fusarium* sp.	45.6		[22]
		Phoma	17.33		
		Alternaria	8.33		
		Cladosporium	7.00		
		Curvularia	1.00		
		Cochliobolus	0.67		
		Leptosphaerulina	33		

dangerous toxins are Aflatoxins.

In terms of relative dominance of species, *Aspergillus* was found to be the most frequent and widespread fungus [16-20, 23]. The reason is that this fungus can form large numbers of breeding units that are resistant to inappropriate environmental conditions, which form plankton in the air and thus reach many places. As well as their growth in wide ranges of heat and humidity conditions, as some species of *Aspergillus* grow at temperatures ranging from 5 to 45 °C.

According to the above-mentioned studies [16, 18, 19, 22], frequency and sovereignty indicators illustrate that some species in the environment have been confirmed and replicated, such as *Aspergillus, Fusarium,* and *Penicillium*, despite the different environmental conditions of each study. This evidence shows the extent of these species of fungi to tolerate the various environmental changes. Also, it shows their physiological activity, rapid growth, producing large numbers of reproductive units, and enzymatic and toxic activity compared to other species.

The Concentration of Mycotoxins in Crops

SM products of fungi are biologically active compounds. They are non-antigenic toxins and most of them are toxic to humans, animals, plants, and microorganisms [24]. Mycotoxic fungi divided into three groups; field fungus, storage fungus, advanced decomposition fungi. Mycotoxins, in turn, classified according to their secretion time. Direct pollution for the Mycotoxins secreted during the stages of production and circulation of food. Whereas, the indirect pollution that results of contamination of food by feeding humans on animal products produced from animals that have been fed on contaminated food with fungal toxins and this type is the most

Table 2. The concentration of Mycotoxins in different types of crops

Crops	Name of toxin	Concentration of toxins (ppb)	Estimation technique	Reference
Corn, Rice, Nut	AFB1	100	HPLCHPTLC	[26]
	OTA	10-100		
Corn	Fumonisin	261-288	ELISA	[16]
	AFB1	3-49		
Zea maize	AFB1	10	HPLC	[17]
	AFB2	6		
Wheat	DON	82.5	LC/MS/MS	[27]
	ZEN	36.7		
	T-2	77.5		
	AFB1	2.04		
	AFB2	2.07		
Cereal (grains)	FB1	17.3	LC/MS/MS	[28]
	FB2	14.6		
	DON	41.5		
	NIV	50.2		
	ZEN	6.1		
Yellow rice	CitreoviridinAFT1	5.9	LC/MS/MS	[29]
Nuts, Dried fruits	Aflatoxins	4-14.5	LC	[30]
Peanut	Aflatoxins	30-851.9	TLC	[31]
	Zearalenone	35.1-129.4		
Peanut	Met-cycladextrin	20	TLC	[32]
Nuts	Aflatoxins (B1-B2-G1-G2)	70-140	IACHPLC	[33]
Peanut	Aflatoxins (B1-B2-G1-G2)	5-103.8	HPLC	[34]
Peanut	Aflatoxin B1	6.83	ELISA	[35]
Nuts	Aflatoxins	1-113	TLC	[36]
Wheat, Zea mays	OTA	35	TLC	[20]
Adlay seeds	FUM	4.52-9.9	ELISA	[22]
	ZEN	161.85-398.94		

dangerous [25]. Table 2 shows the concentration of toxins (by ppb) according to the different crops samples.

Fungi varied in the production of fungal toxins and the variation in proportions was attributed to the genetic ability of different fungal isolates as shown in many studies [22, 28, 32, 34].

The findings of the chemical analysis of fungal isolates from many crop types using different techniques showed various types of fungal toxins which produced in different proportions [29, 31, 33, 35]. The difference in toxin production is due to the ability to produce toxins according to the genetic diversity of the fungal isolates. Aflatoxin is the most frequently reported Mycotoxin [26, 30, 37]. Aflatoxin is produced in the poor stored agricultural products especially in the tropics and sub-tropical regions where the appropriate climatic conditions as high temperature and humidity. These conditions allow the growth of a broad spectrum of fungal species on the water agricultural thoroughbred especially species producing these toxins [38].

The concentration of toxins may also be attributed to the techniques used to estimate the quantity of toxin such as the HPLC / HPTLC techniques that don't estimate toxin values. Also, some works have shown a conclusion that, their tests cannot be appropriate to estimate the amount of all toxins [26]. Alternatively, the number of genes may be related to the amount of toxin, and the method of estimate the toxin can affect the amount of poison [39]. Previous study also showed that the rate of production of toxins on oilseeds crops (exceeding 300 ppb) was more than the production it on grain [22].

Although different conditions and areas of studies have been conducted, most have confirmed that *Aspergillus* strains can proliferate rapidly on nutrients such as peanuts and some other species with high moisture content [40]. Fungi do not grow evenly on all nutrients. Different species of the same type of food differ in their susceptibility to fungi and the production of Mycotoxins [41, 42]. The increase in the production of Mycotoxins in oilseeds is expected to be due to the internal structure of seeds, and moisture content within it. In addition to the conditions of humidity and temperature in the storage field [43].

On the other hand, some studies have shown high concentration rates for the production of fungus growing on cereals indicating that the toxicity of Mycotoxins due to the storage of such types of crops at very high humidity limits [43-45]. At these levels of moisture are considered dangerous. Thus, the safe storage limits for the grain will depend on the initial or primary content of the moisture.

Genetic Diversity

Since the initial reports of DNA amplification using PCR, the number of different applications of this technique has increased dramatically. One of the first applications of the PCR in 1990 was described by White and his co-worker and dealt with the amplification and direct sequencing of ribosomal DNA (rDNA) to establish taxonomic and formative relationships between fungi [46]. The emergence of PCR has allowed the development of reliable molecular markers for the detection and differentiation of fungi, both at the species and strain level. Extensive applications have been found in the science of mycology including classification, plant composition, and diagnosis. PCR detection of pathogenic fungi has been reported for numerous vital genes such as *Phytophtora* sp., *Fusarium* sp., and *Colletotrichum* sp [47]. DNA-based PCR techniques are specific, sensitive and fast compared to many other detection methods. There is a wealth of methodologies for detecting microorganisms, including traditional quantification of fruiting structures, disease symptom record, and biochemical and microbiological methods. Recently, PCR techniques have gained remarkable popularity in diagnosis, due to sensitivity, quality, and ease of implementation [48].

Table 3 showed the different genes responsible for the production of Mycotoxins from different fungal isolates from different crop types. This genetic diversity or genetic variation may be due to the effects of climate, various environmental factors, storage conditions, pollution, the effect of certain chemicals and the biological composition of the seed, or the PCR pattern that may be affected by several factors [20, 51]

Genetic variation may also be attributed to the method of breeding fungi or the way of coexistence with fungi and other organisms [20, 52]. Some studies have indicated that there is a lack of toxic genes in some isolates [20]. This could

Table 3. Genes responsible for producing mycotoxins by different fungal isolates from varies crop types

Type of fungi	Crops	Genes responsible for toxin production	Reference
Aspergillus flavus	-	avfA	[49]
Aspergillus flavus	-	omtB	
Aspergillus flavus	Soybeen	omtB oflR Ver-1 omtA	[39]
Aspergillus niger *Aspergillus flavus* *Aspergillus fumigatus* Aspergillus carbonarius	Corn, Rice, Nut	Nor-1 ontA Pks	[26]
Aspergillus tobingensis *Fusarium sporotrichioides* *Penicillium expansum*	Wheat	caM Tir13 IDH	[27]
Fusarium graminearuium MTCC 2089 *Fusarium graminearuium* ITCC 1805 *Fusarium graminearuium* MTCC 1893 *Fusarium graminearuium* MTCC 1894 *Fusarium sporotrichioides* MTCC 2081	Rice, Finger millet	rDNA-tri5-tri6	[50]
Fusarium solani ITCC 3359 *Fusarium culmorum* ITCC 149		rDNA	
Fusarium moniliform MTCC 156 *Fusarium moniliform* ITCC 3362 *Fusarium moniliform* NCIM 1099 *Fusarium proliferatum* MTCC 286 *Fusarium proliferatum* NCIM 1101		rDNA-fum1-Fum13	
Colletotrichum sp.	Legume crops	ITS Actin Chitin GPDH B-tubulin Histone	[48]
Aspergillus flavus *Aspergillus niger* *Aspergillus ochraceus* *Alternaria alternata* *Fusarium oxysporum* *Rizopus stolonifer* *Curvilaria lunata*	Wheat, Zea mays	PKS	[20]

be due to the inability of the isolate to produce the fungal toxins, the environmental conditions (such as the storage medium), or to inhibition of the PCR reaction by cell wall components during the process of reaction [53]. However, the use of ways based on PCR-targeted methods for DNA is an excellent choice and quick option to diagnose fungi because they are highly specialized, sensitive and better than other techniques.

CONCLUSION

Genetic diversity was observed between the mycotoxigenic fungi, and various genes are responsible especially avfA, omtA, and omtB for the production of fungal toxins. One of the most toxic fungi is *Aspergillus,* and Aflatoxin was most common Mycotoxin produced by this fungus. We recommend for studies that determine the

role of each Mycotoxic gene/loci responsible for Mycotoxin production.

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INTEREST CONFLICTION

The authors have declared that no conflict of interest exists.

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