A SHIFT FROM USING LC-MS-BASED SYSTEMS IN THE DETERMINATION OF MULTIPLE *FUSARIUM*-PRODUCED TOXINS IN GRAIN: A REVIEW

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Abstract. Many regulations have been imposed, especially on the most threatening toxins produced by various pathogens of grain crops. Among these are *Fusarium*-produced toxins which may accumulate in high quantities when the pathogen infects the crop. To ensure compliance with the regulations on the safety of grain products, analytical instruments for detection and quantification of mycotoxins, such as liquid chromatography-mass spectrometry (LC-MS) and others, must have high sensitivity and high-resolution power to trace toxin contaminants. However, a deviation from LC-MS-based detection systems has become the norm. There is also an increasing quest and development of methods for the simultaneous detection of multiple toxins. Moreover, newer systems of detection and quantification have lower limits of detection. This review discusses these three new developments, namely, deviation from LC-MS-based systems, simultaneous determination of multiple toxins, and progress in reducing the limit of toxin detection. Only recent literature is reviewed, and perspectives for future work are laid out.
Keywords: *Fusarium spp., mycotoxin contamination, detection, grain, toxic secondary metabolites*

Introduction

Plant pathogens encounter plants and penetrate the plant surface mainly through natural openings. Upon entry into the plant, the pathogen forms structures for colonization, multiplies and produces various metabolites to weaken the plant. The plant may either resist the pathogen and survive or may progressively succumb to the persistent action of the invading pathogen. Various fungi and bacteria produce toxins when they infect their plant hosts (Wang et al., 2022a; Kumar et al., 2023). The pathogens produce the toxins to macerate and degrade plant tissue to trigger necrosis and death of tissue. The pathogen intrudes the cell, cell contents leak and it survives on the oozing sap and further advances to distant tissues to progress infection. Examples of bacteria which produce toxins include *Xanthomonas, Pseudomonas, Clavibacter* and many others (Strobel, 2012). Toxins produced by fungi are called mycotoxins and examples of mycotoxin-producing fungi include *Fusarium, Alternaria, Sclerotinia, Botrytis* and several others (Tian et al., 2022; Kumari et al., 2023; Şahin et al., 2023). Symptoms associated with pathogen attack of the host plant include necrosis, chlorosis, wilting, water soaking and tissue collapse, and this damage may be as a result of the production of toxins (Gurikar et al., 2022). The appearance of toxigenic pathogens and subsequent production of mycotoxins are more frequently observed in food and feed produced in developing countries due to climate, poor production practices and technologies and poor storage conditions for crops, but mycotoxin-contaminated food and feed can occur anywhere in the world through international trade. Scientists often find it difficult to delineate the role of toxins in pathogenicity and virulence because other factors such as cell wall-degrading enzymes, and effectors also contribute to pathogenicity and virulence. Sometimes the role of toxins may be subtle, it is often the manipulation of cellular processes of the host plant.

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Known actions of toxins are enhancing virulence by killing cells around the point of pathogen infection, as well as increasing the invasiveness of the pathogen in diverse organs or immune cells. The focus of this review is on the mycotoxin-producing *Fusarium*.

Various pathogens employ different modes of attack and *Fusarium* pathogens follow a necrotrophic mode since it degrades tissue and produces mycotoxins. Mycotoxins are naturally occurring, low molecular-weight secondary compounds which are produced by *Fusarium* and other fungi. They have diverse chemical structures which work to advance infection. Their production occurs during the habitation and parasitism executed by the fungus on the plant. Toxins are therefore regarded as virulence factors since they advance the infection of the plant host, making it prone to pathogen colonization. The detrimental effects of mycotoxins in plants range from light symptoms to the severity of plant tissue death, and crop products severely contaminated with mycotoxins are usually unfit for consumption by both animals and humans. The mycotoxin-producing members of the genus *Fusarium* comprise numerous toxigenic species that are pathogenic to plants (Wang et al., 2022b). *Fusarium* causes drastic yield losses of grain crops and the toxins produced contaminate harvested grain of its primary hosts which include wheat and maize (reviewed in Ntushelo et al., 2019). Ingestion of high amount of grain with mycotoxins poses various health risks in humans and animals. Among the known ailments and sicknesses caused by the ingestion of toxin-contaminated grain are vomiting, headache, and dizziness in humans. Animals may lose weight and suffer anorexia. Severe conditions of mycotoxin ingestion include leukoencephalomalacia in horses, pulmonary edema in swine, and kidney and liver cancers in mice (reviewed in Rauwane et al., 2020). In humans, serious conditions of toxicosis include cancer (Gurikar et al., 2022).

Among the major mycotoxins produced by *Fusarium* are trichothecenes, zearalenone (ZEN or ZEA), fumonisins and others (*Table 1*) (reviewed in Yazar and Omurtag, 2008; Alisaac and Mahlein, 2023). Trichothecenes are the most dominant group split into four subgroups A, B, C and D (Chen et al., 2019). They are derived from farnesyl diphosphate and their synthesis is regulated by the TRI cluster of genes. Trichothecenes A and B are produced by *Fusarium* spp. and the presence or absence of ketone (=O) at C_8 of thichothecenes backbone is the main difference between the two groups (Foroud et al., 2019). *F. graminearum* and *F. culmorum* are the main cause of trichothecene type B contamination in cereals (Pasquali et al., 2016). The mode of toxicity of trichothecenes is blocking protein synthesis in plants by interfering with peptidyl transferase from binding the 60S ribosomal subunit. This action inhibits polypeptide chain elongation and chain termination (Cundliffe et al., 1974, 1977). ZEN (also mainly produced by *F. graminearum* and *F. culmorum*), previously known as F-2 toxin, is a resorcyclic acid lactone [6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcyclic acid lactone (C18H22O5, MW: 318.36, CAS 17924–92-4)] which causes electrolyte leakage in maize, blocks H+ extrusion causing acidification and as a result causes reduction in root length. This mycotoxin is of low acute toxicity either *in Planta* or *in Animalia* compared with trichothecenses (McLean, 1995; Ferrigo et al., 2016). Fumonisins are derived from polyketides and their biosynthetic pathway is regulated by the *FUM* gene cluster (Proctor et al., 2003, 2006, 2008; Stepień et al., 2011). Species which produce fumonisins include *F. verticillioides*, *F. proliferatum*, *F. sacchari*, *F. subglutinans* and *F. fujikuroi* and others (Perincherry et al., 2019). Specific regulations for the amount of trichothecenes, zearalenone and fumonisins (among other) mycotoxins in food and feed have been set by

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various regulatory authorities, mainly because of the tremendous risks that they pose to food safety.

Table 1. *Major mycotoxins produced by Fusarium species*

Mycotoxin	*Fusarium* pathogen	References
Trichothecenes	*F. acuminatum, F. crookwellense, F. culmorum, F. equiseti, F. graminearum, F. poae, F. sambucinum, and F. sporotrichioides*	Goswami et al., 2011; McCormick et al., 2011; Chen et al., 2019; Foroud et al., 2019; Spanic et al., 2019
Zearalenone	*F. crookwellense, F. culmorum, F. equiseti, F. graminearum, F. semitectum, F. sporotrichioides*	McLean, 1995; Kuzdralinski et al., 2013; Tralamazza et al., 2016; Bertero et al., 2018; Li et al., 2019; Stanciu et al., 2019; Vogelgsang et al., 2019
Fusaric Acid	*F. oxysporum, F. proliferatum*	Shi et al., 2017; Lopez-Diaz et al., 2018
Fumonisins	*F. proliferatum, F. verticillioides*	Scott, 2012; Dalla'Asta and Battilani et al., 2016; Alshannaq and Yu, 2017; Braun and Wink, 2018
Enniatins and Beauvericin	*F. acuminatum, F. avenaceum, F. equiseti, F. lateritium, F. poae, F. proliferatum, F. sambucinum, F. sporotrichioides, F. subglutinans, and F. tricinctum*	Jestoi, 2008; Fraeyman et al., 2017; Prosperini et al., 2017
Fusaproliferin	*F. proliferatum, F. subglutinans*	Jestoi, 2008
Moniliformin	*F. acuminatum, F. avenaceum, F. oxysporum, F. proliferatum, F. sambucinum, F. subglutinans, F. tricinctum, and F. verticillioides*	Jestoi, 2008; Fraeyman et al., 2017

Food safety regulations

Food safety is increasingly becoming a worldwide concern with various regulatory authorities prescribing food safety standards (Henson and Caswell, 1999). A plethora of studies attempt to address the concerns of the consumer in various aspects of food safety including contamination with pesticides and toxins. The World Health Organization (WHO) and the Food and Agricultural Organization (FAO) have recognized many toxins present in agricultural commodities. Various national regulatory bodies have instituted their safety standards to comply with trade expectations and for the safety of their local consumers. Certain countries have drafted guidelines on allowable limits of *Fusarium* mycotoxins to ensure safety of harvested grain products. Annually, 25-30% of the agricultural produce worldwide is contaminated with mycotoxins as mentioned in Ricciardi et al. (2013). Of greatest concern regarding mycotoxins is that they are not destroyed by the milling, baking and cooking processes. Crops with a significant amount of mycotoxins often have to be destroyed. However, there have been advances in technologies to eliminate or reduce toxins in food to guarantee food safety. *Fusarium*-produced mycotoxins of greatest concern are fumonisins, trichothecenes and zearalenone. Fumonisins are associated with human cancer and neural tube defects (Scott, 2012; Ahangarkani et al., 2014). They can also affect the process of liver cancer development in rats, can cause rabbit brain damage and nephrotoxicity to other animals. Other animals

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adversely affected are swine, poultry, and other livestock animals (Ahangarkani et al., 2014). The fumonisin FB_1 interferes with the synthesis of myelin and causes leukoencephalomalacia and liver necrosis in horses. Severity of these disorders may lead to death. FB_1 also causes a lung disfunction called pulmonary edema in swine (Scott, 2012). In its regulations, the Codex Alimentarius Commission set the maximum levels of fumonisin (FUM) at 4000 µg/kg for unprocessed maize and 2000 µg/kg for maize meal and these standards have been instituted in countries like South Africa (Shephard et al., 2019). There are similar restrictions in the EU with maize-based baby foods and other maize products (see review by Ji et al., 2019). Based on inclusive findings of nephrotoxicity in male rats by the joint FAO and WHO, the maximum allowable daily limit for fumonisins has been, temporarily, prescribed to 2 µg/kg bw/day. Regarding trichothecenes, deoxynivalenol (DON) is regarded as a teratogen, neurotoxin, and immunosuppressant agent by the WHO and therefore it was not categorized as a carcinogenic agent. Similarly, the International Agency for Research (IARC) on Cancer classified DON as a non-carcinogenic agent. However, the effects of DON on humans which include various chronic and fatal intoxications like cell and organ disfunction required grouping of DON under toxic substances, IARC group 3. Similarly, for another trichothecene nivalenol (NIV), a number of countries tolerate only its low levels in cereals. Studies have shown ZEN to cause liver lesions and other liver disorders in rabbits, rats, and gilts (Pistol et al., 2014) and it has also been suggested to advance breast cancer (Ahamed et al., 2001). In several countries there are prescribed allowable limits for ZEN in harvested grain (see review by Ji et al., 2019). All three types of mycotoxins, namely, FUM, trichothecenes and ZEN can be detected with established and standardized laboratory techniques.

Techniques for mycotoxin detection

The often very low maximum allowable limits of mycotoxins in foodstuffs requires accurate detection and quantification of the mycotoxins in grain and grain products. Analytical techniques to be used to ensure compliance with safety standards must be convenient, rapid, accurate, sensitive, selective, reliable and their throughput must be high for ease of handling many samples. To meet these requirements, mycotoxin analysis shifted from wet chemistry to the present advanced forms of analysis which offer these critical features. This development ensured various beneficial programs for wide-scale monitoring and prediction which unfortunately may be limited only in developed grain production systems. The various analytical tools for detection and quantitative determination of mycotoxins in grains can be classified as either established or emerging. The emergence of new techniques adds new and convenient features to the older established systems. These developments occur to respond to new needs such as expanding mycotoxin testing beyond the laboratory, simultaneous detection of multiple mycotoxins and sensitivity to mention a few. Chromatographic techniques such as high-performance liquid chromatography (HPLC) coupled with a number of detectors like fluorescence detectors, diode array, ultra-violet (UV), liquid chromatography-mass spectrometry (LC-MS), and LC-MS/MS have proven powerful in analysis of major mycotoxins in grain (Kinani et al., 2008; Soleimany et al., 2012; Ma et al., 2019). LC-MS-based techniques have become the standard against which newly developed applications are tested to prove the reliability of the results they produce. Recent developments in mycotoxin detection and quantification have been instrumental in

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ensuring accurate detection with repeatability of results. These have proven to be particularly effective in not only the accurate detection of mycotoxins, but also in detecting mycotoxin-producing fungi. Most notable recent developments are the studies by various toxins workers (Dzuman et al., 2015; Zhao et al., 2015; Dagnac et al., 2016; Miró-Abella et al., 2017; Qian et al., 2018; Jensen et al., 2019; Luo et al., 2020; Zhu et al., 2023) to mention a few. Vardali et al. (2023) has comprehensively reviewed recent advances in mycotoxin determination in grains. Traditionally, the analytical methods commonly used to determine *Fusarium* mycotoxins are chromatographic techniques and chromatography-mass spectrometry purified with solid phase extraction and usually with non-simultaneous protocols for detection. However, recent advances in mycotoxin research and development of applications include methods based on immunoaffinity, proteomic and genomic methods, molecular techniques, electronic nose, aggregation-induced emission dye, qualitative nuclear magnetic resonance (NMR) and hyperspectral imaging which all have brought an additive advantage to the field of mycotoxin determination in foodstuffs and grains. This is a deviation from the established traditional LC-MS-based systems. However, this deviation from LC-MS recognizes the analytical power of LC-MS and some new methods are validated with LC-MS and others are sometimes validated with other chromatographic and mass spectrometry applications. For an example, for screening and semi-quantitative analysis, enzyme-linked immunosorbent assay (ELISA) and thin-layer chromatography (TLC) are frequently used however, these are augmented by the use of the instruments with a higher resolving power like the gas chromatography (GC) and the HPLC-MS. Examples of LC-MS validation of results obtained with new techniques will be provided in a latter section of this manuscript. Recently, the immunoaffinity column based on Sepharose 4B gel polysaccharide matrix has been adopted and commercialized for detection of *Fusarium* toxins in feed and cereal grains (Chang et al., 2017; Kim et al., 2017; Park et al., 2018). Another recent trend has developed, and this is design of methods for detecting multiple toxins in a single analysis. Methods to detect the full range of classified and restricted mycotoxin provides the convenience which replaces the need to perform head-to-head analyses for different mycotoxins. This development added to the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) techniques for extraction of toxins. Following a QuEChERS extraction method, Facorro et al. (2020) developed a method for simultaneous analysis of multi-class mycotoxins in mixed rations. Method robustness was tested by the repeated detection and validation of 26 mycotoxins produced by different genera and method evaluation proved linearity, repeatability, accuracy, matrix effect, measurement uncertainties, limits of detection and recovery. Liu et al. (2020) also prepared multitarget immunoaffinity columns using poly(glycidyl methacrylate–divinylbenzene) as a matrix for the detection of *Fusarium* toxins ZEN, the trichothecenes DON, T-2 and HT-2. Several biosensing methods based on molecular recognition and signal amplification have also been developed for mycotoxin detection (Zhu et al., 2023). The recently developed methods aim to either maintain or reduce the limit of detection of *Fusarium*-produced mycotoxins. Deviation from LC-MS-based detection techniques, the quest and the development of multiple toxins in a single run and the maintaining and lowering of detection limits are discussed at length in a following section. This is an important feature in the recent advances in the determination of *Fusarium*-produced mycotoxins.

It has become apparent that the future moves towards a deviation from LC-MS techniques, simultaneous detection of multiple mycotoxins in a single analytical run as well as increasing sensitivity to detect low quantities of mycotoxins in grain. Numerous

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present challenges and other various recent research trends and the future of research in the detection of *Fusarium*-produced mycotoxins are important consideration to ensure that this research remains relevant.

Present challenges, recent research trends and the future

The scope on the research advances in mycotoxin detection and research in general is very broad. Research regarded as valuable is that which improves sensitivity, accuracy, and reliability among others. Mycotoxin analysis is always two-fold, first the extraction of mycotoxins from samples and then identification using such techniques as MS. Optimization of mycotoxin determination can take place in either of these two stages. Before method optimization for the determination of *Fusarium*-produced mycotoxins, attention is brought to various broader issues, some outside of the laboratory. Over the past few years leading topics in the determination of mycotoxins have been to understand the spectra of mycotoxins produced by the various *Fusarium* species. For example, Iwase et al. (2020) studied the less known *F. sambucinum* to understand the range of mycotoxins it produces. Many other studies are regional reports of toxins often targeted at analysis of samples representing either a country or an important grain producing region. Studies on grain products for animal nutrition have also been conducted. Masked and emerging toxins have also come to the spotlight as demonstrated by the study by Ekwomadu et al. (2020). Biological control of *Fusarium* and the fate of toxins from biological control is also another topic which was recently tackled. Of significant note is a promising result on the use of *Trichoderma* to bio-transform T-2 toxin, HT-2 toxin, diacetoxyscirpenol and neosolaniol into their glycosylated forms (Tian et al., 2020). Over and above these, there must still be an understanding on how changes in the climate and agronomic practices will change the spectrum of mycotoxins which can be expected. The changing climate dynamics may cause various combinations of mycotoxins produced in a single grain crop and elevation of mycotoxins in certain grains. This, coupled with the complexity of the samples, may present various challenges which may require new and fresh approaches. Researchers must constantly review guidelines and identify emerging toxins and set new grain quality guidelines. Another challenge is the availability of test kits in under-resourced communities to monitor occurrence and enforce compliance at various stages of the handling of grain and grain products. This is an issue which also requires attention. The future seems to focus on the foodomics, nanomaterials and other new techniques, prediction based on weather patterns and regional studies. This is just a snippet of the vast array of recent research topics on *Fusarium* mycotoxins research which commendably has not forsaken improving and advancing detection methods for optimal determination of *Fusarium* mycotoxins in grains and grain products both simple and complex. On the optimization front is a concern, among other things, about matrix effects on the extraction efficiencies in LC-based detection and quantification methods. Methods of detection and analysis keep evolving with focus placed on optimization to obtain higher recoveries and more sensitive detection, however still maintaining a low cost, simplicity, and quicker turnaround times. To achieve these goals, new techniques have been developed and they deviate from the traditional LC-MS-based techniques of detection. Among these is the use of a multiplex immunochromatographic assay based 25 nm colloidal gold for simultaneous qualitative detection of FB_1, DON and ZEN in grain (Hou et al., 2020) to achieve higher sensitivity, higher specificity, with less ambiguity of results and consistency with LC-MS/MS results. Besides sensitivity, low cost, simplicity and

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reliability of results, multiplicity is valued in quantitative analysis of mycotoxins. A reliable and sensitive method for analysis of 38 mycotoxins was developed based on QuEChERS-based extraction method (Rausch et al., 2020). The method which was able to detect multiple mycotoxins still maintained the integrity of high recovery rates and repeatability of results. Additionally, the quest for more sensitive methods still continues with Zhang et al. (2020a) achieving a low detection limit of 0.55 $ng.g^{-1}$ of ZEN in wheat using magnetic hyperbranched molecularly imprinted polymers for selective enrichment of ZEN. Only these last three aspects, deviation from LC-MS-based systems; determination of multiple toxins; as well as progress in maintaining or reducing the limit of mycotoxins detection will be discussed in greater detail. Some features of the newer methods of detection which include convenience, cost etc. suit the researcher and this may explain the deviation from the traditional, tried-and-tested LC-MS-based methods. Other features of the new methods are those which appease commercial applicators and regulatory bodies, and these are simultaneous detection of multiple toxins and sensitivity.

Deviation from LC-MS

Recent efforts in determining *Fusarium*-produced mycotoxins in grains include the use of other technologies besides the widely acceptable LC-MS approaches. Immunochromatographic assays, with major examples being ELISA, dipsticks, flow-through membranes and lateral-flow devices (LFDs), are becoming established and prove to be an alternative to LC-MS. Biosensing methods based such as aptamers based on molecular recognition and signal amplification are also gaining popularity. Among the recent studies using immunochromatography is the study of Hou et al. (2020) who developed a multiplex immunochromatographic assay based on 25 nm colloidal gold for the simultaneous detection of FB_1, DON and ZEN in wheat and corn. This is a quick method which had relatively low detection limits of 60, 12.5 and 6 ng/mL. Han et al. (2020) also developed an ELISA method for the detection of DON in food and feed grains and achieved low detection limits over and above reliability proven by validation with HPLC. Other studies which made use of ELISA/immunochromatography include those of notable workers (Wang et al., 2013; Han et al., 2019; Batrinou et al., 2020; Li et al., 2020; Pan et al., 2020). For aptamers, Zhu et al. (2023) developed a portable aptasensor based on magnetic microspheres (MMPs) and hybridization chain reaction (HCR) to detect T-2 and ZEN simultaneously. The superparamagnetism and good biocompatibility of the MMPs were utilized for enriching the targets, while the high affinity of the aptamers with the targets was employed to solve the specificity problem. HCR is used for signal amplification and constant temperature fluorescence detector is used for fluorescence detection. Under the optimal conditions, T-2 and ZEN exhibited good fluorescence response in the dynamic range of 0.001–10 ng mL^{-1} and 0.01–100 ng mL^{-1} with detection limits of 0.1 pg mL^{-1} and 1.2 pg mL^{-1}, respectively. Some of these studies proved to be either sensitive or reliable if validated using LC-MS. Methods based on the use of nanomaterials have also been developed and used. Xu et al. (2020) developed a gold nanoparticle @aptamer-functionalized hybrid affinity monolithic column for the determination of ZEN and achieved results similar to those generated based on LC-MS with a low detection limit of 0.05 ng/mL. Jiang et al. (2020) also developed a method for the detection of FB_1 using multiple metal nanoparticles. Another study based on the use of nanomaterials for determining mycotoxins is that of Ramadan et al. (2020) and it promises to provide good and reliable measures for mycotoxin determination. Progress and promises on the use nanotechnology-based detection methods is reflected on a review

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manuscript (Shan et al., 2020). Other methods not based on LC-MS are reviewed in Jia et al. (2020) and these are methods which use the vibrational spectroscopic techniques of infrared spectroscopy, Raman spectroscopy, and hyperspectral imaging to determine fungal infections and mycotoxin contamination in cereals. Moreover, fluorescence-based detection of FB_1 (Ekwomadu et al., 2020), the use of a DNA hydrogel (Sun et al., 2020) are among some of the recent developments in mycotoxin research. Despite the progress in establishing non-LC-MS based methods, the simultaneous detection of multiple toxins remains paramount, convenient and cuts both the cost and the time required to do back-to-back analyses.

Simultaneous detection analysis of multiple toxins

Fungi produce a multitude of mycotoxins when they invade the plant. Up to 400 compounds which include aflatoxins, ochratoxin A, FUM, NIV, DON, ZEN, T-2 toxin, and HT-2 toxin are produced by fungi (Binder et al., 2007). Given this high number of mycotoxins and the possibility of multiple contamination of grain products, it is beneficial to detect multiple mycotoxins in a single run to minimize cost, increase convenience and to collect as much information as possible from a single sample. Methods of mycotoxin detection and quantification which analyse multiple mycotoxins simultaneously will be favoured by analysts, commercial applicators and regulators as long as they do not compromise other valuable qualities in instruments such as sensitivity, accuracy and reliability. A multitude of methods have been developed/used recently. A flow injection mass spectrometry was developed to detect seven mycotoxins in samples of food and grain still using the QuEChERS extraction approach. This method achieved a detection limit below established regulatory levels, a remarkable achievement considering the absence of chromatography. This method still maintained the high speed, sensitivity, high throughput and simplicity (Sapozhnikova et al., 2020). Still on analysis of multiple toxins, a method using an aqueous solution of acetic acid solution, acetonitrile and QuEChERS with vibrational shaking was developed (Colli et al., 2020). This method which used UHPLC-MS/MS was able to detect 42 mycotoxins in oats. Gbashi et al. (2020) also estimated 15 different mycotoxins in three stable cereals from Nigeria using a 'novel' green extraction of pressurized hot water, breaking the established tradition of the use of solvents for extraction. Data analysis using principal component analysis and orthogonal projection was able to, as expected, distinguish between samples as predicted thus establishing the pressurized hot water extraction (PHWE) method as an alternative to mycotoxin/metabolite extraction using solvents. A similar study still using PHWE successfully extracted for analysis 15 different mycotoxins but this time on maize (Gbashi et al., 2020) and this affirmed PHWE for the extraction of multiple mycotoxins in grains. Another method based on MALDI-TOF MS was able to detect six mycotoxins including DON and ZEN (Hleba et al., 2021). Simultaneous detection of mycotoxins was advanced by another study by Zhang et al. (2020b) who were able to detect six mycotoxins in maize in a single test. The mycotoxins included ZEN, FUM B and DON and made use of a Raman scattering (SERS)-based lateral flow immunosensor. Low limits of detection, 6.2 pg/mL for ZEN, 0.26 ng/mL for FUM B1, 0.11 ng/mL for DON, were achieved over and above the short turnaround time of less than 20 minutes and there was consistency with LC-MS. Simultaneous determination of multiple toxins remains valuable only if it does not compromise such good principles of analysis as sensitivity. Newly developed analytical platforms must either maintain or lower detection limits to meet the ever-increasing demands placed by regulatory bodies.

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Maintaining low or lowering detection limits

The desire to detect very low amounts of mycotoxins in grains and grain products necessitates the development of highly sensitive detection and quantitative methods for mycotoxin analysis in grains and grain products. Expectations for sensitive techniques keep rising and this has propelled research and development of applications with lower detection limits. Sensitive applications include those based on GC-MS, HPLC-fluorescence detection, HPLC-MS, ELISA, radioimmunoassay, and fluorescent immunoassay to mention some. Studies which have recently been concluded are herein reviewed, all with a relatively low detection limit. No benchmark is provided when the phrase "low detection limit" is provided because various scientists and regulatory bodies may have different preferences. These detection limits are regarded as relatively low because they may be useful for some application. First to be presented is a study by Zhang et al. (2020a) who after optimizing the amount of adsorption, extraction time and elution solvent achieved a detection limit of ZEN of 0.55 $ng.g^{-1}$ in wheat using magnetic hyperbranched molecularly imprinted polymers for selective enrichment and determination of ZEN. Similarly, using a multiplex immunochromatographic assay based on 25 nm colloidal gold for multiple detection of FB_1, DON and ZEN in wheat and corn (Hou et al., 2020) detection limits for the three mycotoxins were 60, 12.5 and 6 ng/mL and the results were consistent with those of the widely accepted application of LC-MS/MS. Rausch et al. (2020) used a QuEChERS-based extraction method coupled with high performance liquid chromatography tandem mass spectrometry to determine mycotoxins in cereals and achieved detection limits from 0.05 to 150 µg/kg for 38 mycotoxins which included DON, NIV and ZEN. A low detection limit of 0.05 ng/mL was achieved for ZEN using a new gold nanoparticle @aptamer-functionalized hybrid affinity monolithic HPLC column (Xu et al., 2020). The results of this analysis were similar to those obtained using LC-MS and showed an improvement to those obtained using the immunoaffinity column or molecularly imprinted polymer. With a test on grain a low detection limit for DON was achieved using a self-assembled direct competitive enzyme-linked immunosorbent assay. This low limit of detection of 0.62 ng/ml was comparable to those achieved using HPLC proving dcELISA to have a good performance meeting the relevant technical requirements with good accuracy, reliability, convenience, and high throughput (Han et al., 2020). Determining FB_1 by membrane solid phase extraction coupled with solid-phase fluorescence analysis (Li et al., 2020) were able to achieve a detection limit of 0.119 µg/L and confirmed method accuracy with LC-MS. This method still maintained the characteristics of simplicity, sensitivity, reliability for determination of FB_1 in corn. Using a novel method based on DNA hydrogels and etched gold nanorods to detect T-2 toxin, Sun et al. (2020) achieved a low limit of detection (0.87 pg mL^{-1}) in corn with a relatively short assay time. Liu et al. (2020) designed a smartphone-based quantitative dual detection mode device and integrated it with gold nanoparticles (GNPs) and time-resolved fluorescence microspheres (TRFMs) lateral flow immunoassays (LFIA) to determine multiple toxins in cereals. This application achieved visible limits of detection of 10/2.5/1.0/10/0.5 and 2.5/0.5/0.5/2.5/0.5 µg/kg for AFB_1, ZEN, DON, T-2 and FB_1 respectively for the respective two methods of the smartphone-based quantitative dual detection mode device, integrated with GNPs and TRFMs LFIA. Validation of results was done using LC-MS/MS and the results showed consistency and reliability and this method is a promising on-point detection system for mycotoxins in cereals. With a magneto-controlled aptasensor for quantitative analysis of ochratoxin and FB_1 using ICP-MS with multiple metal nanoparticles as element labels Jiang et al. (2020)

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limits of detection of 0.10 and 0.30 ng mL^{-1} were achieved for OTA and FB1 respectively and the method demonstrated specificity and succeeded in wheat flour. Luo et al. (2020) constructed a photoelectrochemical (PEC) aptasensor based on simply *in-situ* conjugated composites of zinc oxide-nitrogen doped graphene quantum dots (ZnO-NGQDs) and applied it to determine ZEN on mildewing cereal crops and attained a low detection limit of 3.3 × 10−14 g mL^{-1}. This method has promise for early diagnosis and forecasting of ZEN in wheat. Another method that achieved low limits of detection for ZEN in cereals was a lateral flow immunochromatographic assay using colloidal Au sphere and nanorods as signal markers. The detection limits achieved were 5.0 μg L^{-1} and 60 μg kg^{-1} for colloidal AuSP immunochromatographic analysis (AuSP-ICA) in solution and spiked cereal sample and for AuNRs immunochromatographic analysis (AuNRs-ICA) the two lower limit of detection (LODs) achieved 3.0 μg L^{-1} and 40 μg kg^{-1} in solution and spiked cereal samples, respectively (Pan et al., 2020). These are the achievements the future of mycotoxin research has to be built on.

Future perspectives

Over the decades, mycotoxin analysis shifted from the slow process of wet chemistry to simultaneous detection of multiple toxins in grains and grain products even detecting masked toxins. From a human health perspective, regulation and compliance rapid detection on-point is crucial in the monitoring of transported grain for imports and exports. However, such testing kits must still maintain sensitivity and reliability of results and focus on only regulated mycotoxins. There is progress in this area with commercial kits available for detection of mycotoxins in minutes. Ease-of-use is critical for such kits because experts cannot be deployed to ports of entry of goods to test grain consignments, therefore port officials with basic training should be able to use these kits and understand the result. The challenge is not necessarily the development of kits but to ensure access of these kits in various ports of entry, grain storage facilities and in the milling setup in the under-resourced parts of the world. As the new developments have emphasized the use of non-LC-MS methods this may facilitate on-point testing and mobile services. Handheld devises will prove valuable in the future, and this may also expand testing and the scope may move from field testing of unharvested grain to samples of grain products already on the shelf ready for sale. Simultaneous detection of *Fusarium*-produced mycotoxins is also of great worth when considering the future, especially a future in which testing will be expanded. It would be inconvenient to do back-to-back testing with probably different devices. Finally, all future developments must maintain or improve on the low limits of detection. This feature of testing services must not be compromised because it encapsulates the whole essence of determination of *Fusarium*-produced mycotoxins.

Conclusions

LC tandem spectroscopy is traditionally most utilized for the quantitative analysis of small molecules including mycotoxins. It has proven to be highly selective, sensitive, robust with a multi-analyte capability which make possible the detection and quantification of hundreds of mycotoxins in a single run. However, we have demonstrated in this review article that a shift away from LC-MS systems is taking place with the emergence of immunoaffinity-based detection kits, proteomic and genomic approaches, use of nanomaterials etc. Moreover, recent achievements include the detection of multiple

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toxins simultaneously relieving analysts of the burden to perform back-to-back tests trying to capture multiple toxins in a single sample. The newer tools either maintain or lower detection limits which assists in further ensuring lowered risks of contamination in grain and grain products. The notable achievements of analysis of grain and grain products for toxin determination must maintain all the characteristics of accuracy and reliability, low cost, sensitivity, short testing during, on-point testing etc for usefulness to commercial applicators and bodies to regulate toxin levels in grain and grain products. Furthermore, it is emphasized that the future must both build on the new developments and capitalize on the good features of non-LC-MS based detection methods, simultaneous detection of multiple Fusarium-produced mycotoxins and finally the capability to either maintain or improve on low detection limits by the newer systems of determination of *Fusarium*-produced mycotoxins.

REFERENCES

[1] Ahamed, S., Foster, J. S., Bukovsky, A., Wimalasena, J. (2001): Signal transduction through the ras/Erk pathway is essential for the mycoestrogen zearalenone-induced cell-cycle progression in MCF-7 cells. – Molecular Carcinogenesis: Published in cooperation with the University of Texas MD Anderson Cancer Center 30(2): 88-98. https://doi.org/10.1002/1098-2744(200102)30:2<88::AID-MC1017>3.0.CO;2-E.

[2] Ahangarkani, F., Rouhi, S., Gholamour Azizi, I. (2014): A review on incidence and toxicity of fumonisins. – Toxin Reviews 33(3): 95-100. https://doi.org/10.3109/15569543.2013.871563.

[3] Alshannaq, A., Yu, J. H. (2017): Occurrence, toxicity, and analysis of major mycotoxins in food. – International journal of environmental research and public health 14(6): 632. https://doi.org/10.3390/ijerph14060632.

[4] Batrinou, A., Houhoula, D., Papageorgiou, E. (2020): Rapid detection of mycotoxins on foods and beverages with enzyme-linked immunosorbent assay. – Quality Assurance and Safety of Crops & Foods 12(1): 40-49. https://doi.org/10.15586/QAS2019.654.

[5] Bertero, A., Moretti, A., Spicer, L. J., Caloni, F. (2018): *Fusarium* molds and mycotoxins: Potential species-specific effects. – Toxins 10(6): 244. https://doi.org/10.3390/toxins10060244.

[6] Binder, E. M., Tan, L. M., Chin, L. J., Handl, J., Richard, J. (2007): Worldwide occurrence of mycotoxins in commodities, feeds, and feed ingredients. – Animal feed science and technology 137(3-4): 265-282. https://doi.org/10.1016/j.anifeedsci.2007.06.005.

[7] Braun, M. S., Wink, M. (2018): Exposure, occurrence, and chemistry of fumonisins and their cryptic derivatives. – Comprehensive Reviews in Food Science and Food Safety 17(3): 769-791. https://doi.org/10.1111/1541-4337.12334.

[8] Chang, H., Kim, W., Park, J. H., Kim, D., Kim, C. R., Chung, S., Lee, C. (2017): The occurrence of zearalenone in South Korean feedstuffs between 2009 and 2016. – Toxins 9(7): 223. https://doi.org/10.3390/toxins9070223.

[9] Chen, Y., Kistler, H. C., Ma, Z. (2019): *Fusarium graminearum* trichothecene mycotoxins: biosynthesis, regulation, and management. – Annual Review of Phytopathology 57: 15-39. https://doi.org/10.1146/annurev-phyto-082718-100318.

[10] Cundliffe, E., Cannon, M., Davies, J. (1974): Mechanism of inhibition of eukaryotic protein synthesis by trichothecene fungal toxins. – Proceedings of the National Academy of Sciences 71(1): 30-34. https://doi.org/10.1073/pnas.71.1.30.

[11] Cundliffe, E., Davies, J. E. (1977): Inhibition of initiation, elongation, and termination of eukaryotic protein synthesis by trichothecene fungal toxins. – Antimicrobial Agents and Chemotherapy 11(3): 491-499. https://doi.org/10.1128/AAC.11.3.491.

© 2023, ALÖKI Kft., Budapest, Hungary

[12] Dagnac, T., Latorre, A., Fernández Lorenzo, B., Llompart, M. (2016): Validation and application of a liquid chromatography-tandem mass spectrometry-based method for the assessment of the co-occurrence of mycotoxins in maize silages from dairy farms in NW Spain. – Food Additives & Contaminants: Part A 33(12): 1850-1863. https://doi.org/10.1080/19440049.2016.1243806.

[13] Dall'Asta, C., Battilani, P. (2016): Fumonisins and their modified forms, a matter of concern in future scenario? – World Mycotoxin Journal 9(5): 727-739. https://doi.org/10.3920/WMJ2016.2058.

[14] De Colli, L., Elliott, C., Finnan, J., Grant, J., Arendt, E. K., McCormick, S. P., Danaher, M. (2020): Determination of 42 mycotoxins in oats using a mechanically assisted QuEChERS sample preparation and UHPLC-MS/MS detection. – Journal of Chromatography B 1150: 122187. https://doi.org/10.1016/j.jchromb.2020.122187.

[15] Dzuman, Z., Zachariasova, M., Veprikova, Z., Godula, M., Hajslova, J. (2015): Multi-analyte high performance liquid chromatography coupled to high resolution tandem mass spectrometry method for control of pesticide residues, mycotoxins, and pyrrolizidine alkaloids. – Analytica Chimica Acta 863: 29-40. https://doi.org/10.1016/j.aca.2015.01.021.

[16] Ekwomadu, T. I., Dada, T. A., Nleya, N., Gopane, R., Sulyok, M., Mwanza, M. (2020): Variation of *Fusarium* free, masked, and emerging mycotoxin metabolites in maize from agriculture regions of South Africa. – Toxins 12(3): 149. https://doi.org/10.3390/toxins12030149.

[17] Facorro, R., Llompart, M., Dagnac, T. (2020): Combined (d) SPE-QuEChERS extraction of mycotoxins in mixed feed rations and analysis by high performance liquid chromatography-high-resolution mass spectrometry. – Toxins 12(3): 206. https://doi.org/10.3390/toxins12030206.

[18] Foroud, N. A., Baines, D., Gagkaeva, T. Y., Thakor, N., Badea, A., Steiner, B., Bürstmayr, M., Bürstmayr, H. (2019): Trichothecenes in cereal grains-An update. – Toxins 11(11): 634. https://doi.org/10.3390/toxins11110634.

[19] Fraeyman, S., Croubels, S., Devreese, M., Antonissen, G. (2017): Emerging *Fusarium* and *Alternaria* mycotoxins: Occurrence, toxicity and toxicokinetics. – Toxins 9(7): 228. https://doi.org/10.3390/toxins9070228.

[20] Gbashi, S., Njobeh, P. B., Madala, N. E., De Boevre, M., Kagot, V., De Saeger, S. (2020): Parallel validation of a green-solvent extraction method and quantitative estimation of multi-mycotoxins in staple cereals using LC-MS/MS. – Scientific Reports 10(1): 10334. https://doi.org/10.1038/s41598-020-66787-z.

[21] Gbashi, S., Njobeh, P. B., De Saeger, S., De Boevre, M., Madala, N. E. (2020): Development, chemometric-assisted optimization and in-house validation of a modified pressurized hot water extraction methodology for multi-mycotoxins in maize. – Food Chemistry 307: 125526. https://doi.org/10.1016/j.foodchem.2019.125526.

[22] Goswami, R. S., Kistler, H. C. (2005): Pathogenicity and in planta mycotoxin accumulation among members of the *Fusarium graminearum* species complex on wheat and rice. – Phytopathology 95(12): 1397-1404. https://doi.org/10.1094/PHYTO-95-1397.

[23] Gurikar, C., Shivaprasad, D. P., Sabillón, L., Gowda, N. N., Siliveru, K. (2022): Impact of mycotoxins and their metabolites associated with food grains. – Grain & Oil Science and Technology. https://doi.org/10.1016/j.gaost.2022.10.001.

[24] Han, L., Li, Y. T., Jiang, J. Q., Li, R. F., Fan, G. Y., Lv, J. M., Zhou, Y., Zhang, W. J., Wang, Z. L. (2019): Development of a direct competitive ELISA kit for detecting deoxynivalenol contamination in wheat. – Molecules 25(1): 50. https://doi.org/10.3390/molecules25010050.

[25] Han, L., Li, Y., Jiang, J., Li, R., Fan, G., Lei, Z., Wang, H., Wang, Z., Zhang, W. (2020): Preparation and characterisation of monoclonal antibodies against deoxynivalenol. – Italian Journal of Animal Science 19(1): 560-568. https://doi.org/10.1080/1828051X.2020.1763861.

© 2023, ALÖKI Kft., Budapest, Hungary

[26] Henson, S., Caswell, J. (1999): Food safety regulation: an overview of contemporary issues. – Food policy 24(6): 589-603. https://doi.org/10.1016/S0306-9192(99)00072-X.

[27] Hleba, L., Císarová, M., Shariati, M. A., Tančinová, D. (2021): Detection of mycotoxins using MALDI-TOF mass spectrometry. – Journal of Microbiology, Biotechnology and Food Sciences, pp. 181-185. 10.15414/jmbfs.2017.7.2.181-185.

[28] Hou, S., Ma, J., Cheng, Y., Wang, H., Sun, J., Yan, Y. (2020): One-step rapid detection of fumonisin B1, dexyonivalenol and zearalenone in grains. – Food Control 117: 107107. https://doi.org/10.1016/j.foodcont.2020.107107.

[29] Iwase, C. H., Piacentini, K. C., Giomo, P. P., Čumová, M., Wawroszová, S., Běláková, S., Minella, E., Rocha, L. O. (2020): Characterization of the *Fusarium sambucinum* species complex and detection of multiple mycotoxins in Brazilian barley samples. – Food Research International 136: 109336. https://doi.org/10.1016/j.foodres.2020.109336.

[30] Jensen, T., De Boevre, M., Preußke, N., De Saeger, S., Birr, T., Verreet, J. A., Sönnichsen, F. D. (2019): Evaluation of high-resolution mass spectrometry for the quantitative analysis of mycotoxins in complex feed matrices. – Toxins 11(9): 531. https://doi.org/10.3390/toxins11090531.

[31] Jestoi, M. (2008): Emerging *Fusarium*-mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin-A review. – Critical reviews in food science and nutrition 48(1): 21-49. https://doi.org/10.1080/10408390601062021.

[32] Ji, F., He, D., Olaniran, A. O., Mokoena, M. P., Xu, J., Shi, J. (2019): Occurrence, toxicity, production, and detection of *Fusarium* mycotoxin: A review. – Food Production, Processing and Nutrition 1(1): 1-14. https://doi.org/10.1186/s43014-019-0007-2.

[33] Jia, B., Wang, W., Ni, X. Z., Chu, X., Yoon, S. C., Lawrence, K. C. (2020): Detection of mycotoxins and toxigenic fungi in cereal grains using vibrational spectroscopic techniques: A review. – World Mycotoxin Journal 13(2): 163-178. https://doi.org/10.3920/WMJ2019.2510.

[34] Jiang, D., Huang, C., Shao, L., Wang, X., Jiao, Y., Li, W., Chen, J., Xu, X. (2020): Magneto-controlled aptasensor for simultaneous detection of ochratoxin A and fumonisin B1 using inductively coupled plasma mass spectrometry with multiple metal nanoparticles as element labels. – Analytica Chimica Acta 1127: 182-189. https://doi.org/10.1016/j.aca.2020.06.057.

[35] Kim, D. H., Hong, S. Y., Kang, J. W., Cho, S. M., Lee, K. R., An, T. K., Lee, C., Chung, S. H. (2017): Simultaneous determination of multi-mycotoxins in cereal grains collected from South Korea by LC/MS/MS. – Toxins 9(3): 106. https://doi.org/10.3390/toxins9030106.

[36] Kinani, S., Bouchonnet, S., Bourcier, S., Porcher, J. M., Aït-Aïssa, S. (2008): Study of the chemical derivatization of zearalenone and its metabolites for gas chromatography–mass spectrometry analysis of environmental samples. – Journal of chromatography A 1190(1-2): 307-315. https://doi.org/10.1016/j.chroma.2008.02.115.

[37] Kumar, P., Kamle, M., Mahato, D. K. (eds.) (2023): Mycotoxins in Food and Feed: Detection and Management Strategies. – CRC Press.

[38] Kumari, N., Srivastava, A., Bansal, S. (2023): Green Technologies for Reduction of Toxins in Food Production and Processing. – Green Chemistry in Agriculture and Food Production 225.

[39] Kuzdraliński, A., Solarska, E., Muszyńska, M. (2013): Deoxynivalenol and zearalenone occurence in beers analysed by an enzyme-linked immunosorbent assay method. – Food Control 29(1): 22-24. https://doi.org/10.1016/j.foodcont.2012.06.003.

[40] Li, M., Yang, C., Mao, Y., Hong, X., Du, D. (2019): Zearalenone contamination in corn, corn products, and swine feed in China in 2016–2018 as assessed by magnetic bead immunoassay. – Toxins 11(8): 451. https://doi.org/10.3390/toxins11080451.

[41] Li, L., Chen, W., Li, H., Iqbal, J., Zhu, Y., Wu, T., Du, Y. (2020): Rapid determination of fumonisin (FB1) by syringe SPE coupled with solid-phase fluorescence spectrometry. –

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 226: 117549. https://doi.org/10.1016/j.saa.2019.117549.

[42] Liu, H., Zhang, J., Ding, K., Chen, X., Han, T. (2020): Preparation of multitarget *Fusarium* toxin (zearalenone, deoxynivalenol, T-2, and HT-2) immunoaffinity columns using poly (glycidyl methacrylate–divinylbenzene) as a matrix. – Polymer Bulletin 77: 4507-4522. https://doi.org/10.1007/s00289-019-02991-w.

[43] Liu, Z., Hua, Q., Wang, J., Liang, Z., Li, J., Wu, J., Shen, X., Lei, H., Li, X. (2020): A smartphone-based dual detection mode device integrated with two lateral flow immunoassays for multiplex mycotoxins in cereals. – Biosensors and Bioelectronics 158: 112178. https://doi.org/10.1016/j.bios.2020.112178.

[44] López-Díaz, C., Rahjoo, V., Sulyok, M., Ghionna, V., Martín-Vicente, A., Capilla, J., Di Pietro, A., López-Berges, M. S. (2018): Fusaric acid contributes to virulence of *Fusarium oxysporum* on plant and mammalian hosts. – Molecular Plant Pathology 19(2): 440-453. https://doi.org/10.1111/mpp.12536.

[45] Luo, L., Liu, X., Ma, S., Li, L., You, T. (2020): Quantification of zearalenone in mildewing cereal crops using an innovative photoelectrochemical aptamer sensing strategy based on ZnO-NGQDs composites. – Food Chemistry 322: 126778. https://doi.org/10.1016/j.foodchem.2020.126778.

[46] Ma, S., Wang, M., You, T., Wang, K. (2019): Using magnetic multiwalled carbon nanotubes as modified QuEChERS adsorbent for simultaneous determination of multiple mycotoxins in grains by UPLC-MS/MS. – Journal of Agricultural and Food Chemistry 67(28): 8035-8044. https://doi.org/10.1021/acs.jafc.9b00090.

[47] McCormick, S. P., Stanley, A. M., Stover, N. A., Alexander, N. J. (2011): Trichothecenes: from simple to complex mycotoxins. – Toxins 3(7): 802-814. https://doi.org/10.3390/toxins3070802.

[48] McLean, M. (1995): The phytotoxicity of selected mycotoxins on mature, germinating *Zea mays* embryos. – Mycopathologia 132: 173-183. https://doi.org/10.1007/BF01103984.

[49] Miró-Abella, E., Herrero, P., Canela, N., Arola, L., Borrull, F., Ras, R., Fontanals, N. (2017): Determination of mycotoxins in plant-based beverages using QuEChERS and liquid chromatography–tandem mass spectrometry. – Food Chemistry 229: 366-372. https://doi.org/10.1016/j.foodchem.2017.02.078.

[50] Ntushelo, K., Ledwaba, L. K., Rauwane, M. E., Adebo, O. A., Njobeh, P. B. (2019): The mode of action of *Bacillus* species against *Fusarium graminearum*, tools for investigation, and future prospects. – Toxins 11(10): 606. https://doi.org/10.3390/toxins11100606.

[51] Pan, M., Ma, T., Yang, J., Li, S., Liu, S., Wang, S. (2020): Development of lateral flow immunochromatographic assays using colloidal Au sphere and nanorods as signal marker for the determination of zearalenone in cereals. – Foods 9(3): 281. https://doi.org/10.3390/foods9030281.

[52] Park, J., Chang, H., Kim, D., Chung, S., Lee, C. (2018): Long-term occurrence of deoxynivalenol in feed and feed raw materials with a special focus on South Korea. – Toxins 10(3): 127. https://doi.org/10.3390/toxins10030127.

[53] Pasquali, M., Beyer, M., Logrieco, A., Audenaert, K., Balmas, V., Basler, R., ... & Vogelgsang, S. (2016): A European database of *Fusarium graminearum* and *F. culmorum* trichothecene genotypes. – Frontiers in Microbiology 7: 406. https://doi.org/10.3389/fmicb.2016.00406.

[54] Perincherry, L., Lalak-Kańczugowska, J., Stępień, Ł. (2019): *Fusarium*-produced mycotoxins in plant-pathogen interactions. – Toxins 11(11): 664. https://doi.org/10.3390/toxins11110664.

[55] Pistol, G. C., Gras, M. A., Marin, D. E., Israel-Roming, F., Stancu, M., Taranu, I. (2014): Natural feed contaminant zearalenone decreases the expressions of important pro-and anti-inflammatory mediators and mitogen-activated protein kinase/NF-κB signalling molecules in pigs. – British Journal of Nutrition 111(3): 452-464. https://doi.org/10.1017/S0007114513002675.

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DOI: http://dx.doi.org/10.15666/aeer/2104_32573273
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[56] Proctor, R. H., Brown, D. W., Plattner, R. D., Desjardins, A. E. (2003): Co-expression of 15 contiguous genes delineates a fumonisin biosynthetic gene cluster in *Gibberella moniliformis*. – Fungal Genetics and Biology 38(2): 237-249. https://doi.org/10.1016/S1087-1845(02)00525-X.

[57] Proctor, R. H., Plattner, R. D., Desjardins, A. E., Busman, M., Butchko, R. A. (2006): Fumonisin production in the maize pathogen *Fusarium verticillioides*: genetic basis of naturally occurring chemical variation. – Journal of Agricultural and Food Chemistry 54(6): 2424-2430. https://doi.org/10.1021/jf0527706.

[58] Proctor, R. H., Busman, M., Seo, J. A., Lee, Y. W., Plattner, R. D. (2008): A fumonisin biosynthetic gene cluster in *Fusarium oxysporum* strain O-1890 and the genetic basis for B versus C fumonisin production. – Fungal Genetics and Biology 45(6): 1016-1026. https://doi.org/10.1016/j.fgb.2008.02.004.

[59] Prosperini, A., Berrada, H., Ruiz, M. J., Caloni, F., Coccini, T., Spicer, L. J., Perego, M. C., Lafranconi, A. (2017): A review of the mycotoxin enniatin B. – Frontiers in Public Health 5: 304. https://doi.org/10.3389/fpubh.2017.00304.

[60] Qian, M., Yang, H., Li, Z., Liu, Y., Wang, J., Wu, H., Ji, X., Xu, J. (2018): Detection of 13 mycotoxins in feed using modified QuEChERS with dispersive magnetic materials and UHPLC–MS/MS. – Journal of Separation Science 41(3): 756-764. https://doi.org/10.1002/jssc.201700882.

[61] Ramadan, M. M., Mohamed, M. A., Almoammar, H., Abd-Elsalam, K. A. (2020): Magnetic nanomaterials for purification, detection, and control of mycotoxins. – In Nanomycotoxicology, Academic Press, pp. 87-114. https://doi.org/10.1016/B978-0-12-817998-7.00005-7.

[62] Rausch, A. K., Brockmeyer, R., Schwerdtle, T. (2020): Development and validation of a QuEChERS-based liquid chromatography tandem mass spectrometry multi-method for the determination of 38 native and modified mycotoxins in cereals. – Journal of Agricultural and Food Chemistry 68(16): 4657-4669. https://doi.org/10.1021/acs.jafc.9b07491.

[63] Rauwane, M. E., Ogugua, U. V., Kalu, C. M., Ledwaba, L. K., Woldesemayat, A. A., Ntushelo, K. (2020): Pathogenicity and virulence factors of *Fusarium graminearum* including factors discovered using next generation sequencing technologies and proteomics. – Microorganisms 8(2): 305. https://doi.org/10.3390/microorganisms8020305.

[64] Ricciardi, C., Castagna, R., Ferrante, I., Frascella, F., Marasso, S. L., Ricci, A., Canavese, G., Loré, A., Prelle, A., Gullino, M. L., Spadaro, D. (2013): Development of a microcantilever-based immunosensing method for mycotoxin detection. – Biosensors and Bioelectronics 40(1): 233-239. https://doi.org/10.1016/j.bios.2012.07.029.

[65] Şahin, S., Eyupoğlu, O. E., Yaman, M., Doğan, T. Ç., Korkmaz, B. İ. O., Omurtag, G. Z. (2023): Investigation of the deoxynivalenol and ochratoxin A levels by high-performance liquid chromatography of cereals sold in the markets in Türkiye. – Food Science and Technology 43. https://doi.org/10.1590/fst.89822.

[66] Sapozhnikova, Y., Zomer, P., Gerssen, A., Nuñez, A., Mol, H. G. (2020): Evaluation of flow injection mass spectrometry approach for rapid screening of selected pesticides and mycotoxins in grain and animal feed samples. – Food Control 116: 107323. https://doi.org/10.1016/j.foodcont.2020.107323.

[67] Scott, P. M. (2012): Recent research on fumonisins: a review. – Food Additives & Contaminants: Part A 29(2): 242-248. https://doi.org/10.1080/19440049.2010.546000.

[68] Shan, H., Li, X., Liu, L., Song, D., Wang, Z. (2020): Recent advances in nanocomposite-based electrochemical aptasensors for the detection of toxins. – Journal of Materials Chemistry B 8(27): 5808-5825. https://doi.org/10.1039/D0TB00705F.

[69] Shephard, G. S., Burger, H. M., Rheeder, J. P., Alberts, J. F., Gelderblom, W. C. (2019): The effectiveness of regulatory maximum levels for fumonisin mycotoxins in commercial and subsistence maize crops in South Africa. – Food Control 97: 77-80. https://doi.org/10.1016/j.foodcont.2018.10.004.

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DOI: http://dx.doi.org/10.15666/aeer/2104_32573273
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[70] Shi, W., Tan, Y., Wang, S., Gardiner, D. M., De Saeger, S., Liao, Y., Wang, C., Fan, Y., Wang, Z., Wu, A. (2016): Mycotoxigenic potentials of *Fusarium* species in various culture matrices revealed by mycotoxin profiling. – Toxins 9(1): 6. https://doi.org/10.3390/toxins9010006.

[71] Soleimany, F., Jinap, S., Abas, F. (2012): Determination of mycotoxins in cereals by liquid chromatography tandem mass spectrometry. – Food Chemistry 130(4): 1055-1060. https://doi.org/10.1016/j.foodchem.2011.07.131.

[72] Spanic, V., Zdunic, Z., Drezner, G., Sarkanj, B. (2019): The pressure of *Fusarium* disease and its relation with mycotoxins in the wheat grain and malt. – Toxins 11(4): 198. https://doi.org/10.3390/toxins11040198.

[73] Stanciu, O., Juan, C., Berrada, H., Miere, D., Loghin, F., Mañes, J. (2019): Study on trichothecene and zearalenone presence in Romanian wheat relative to weather conditions. – Toxins 11(3): 163.

[74] Stępień, Ł., Koczyk, G., Waśkiewicz, A. (2011): FUM cluster divergence in fumonisins-producing *Fusarium* species. – Fungal Biology 115(2): 112-123. https://doi.org/10.1016/j.funbio.2010.10.011.

[75] Strobel, G. A. (2012, December): Toxins of plant pathogenic bacteria and fungi. – In Biochemical Aspects of Plant-Parasite Relationships: Proceedings of The Phytochemical Society Symposium University of Hull, England April, 1975, No. 13, p. 135, Elsevier.

[76] Sun, Y., Li, S., Chen, R., Wu, P., Liang, J. (2020): Ultrasensitive and rapid detection of T-2 toxin using a target-responsive DNA hydrogel. – Sensors and Actuators B: Chemical 311: 127912. https://doi.org/10.1016/j.snb.2020.127912.

[77] Tian, Y., Yu, D., Liu, N., Tang, Y., Yan, Z., Wu, A. (2020): Confrontation assays and mycotoxin treatment reveal antagonistic activities of *Trichoderma* and the fate of *Fusarium* mycotoxins in microbial interaction. – Environmental Pollution 267: 115559. https://doi.org/10.1016/j.envpol.2020.115559.

[78] Tian, Y., Zhang, D., Cai, P., Lin, H., Ying, H., Hu, Q. N., Wu, A. (2022): Elimination of *Fusarium* mycotoxin deoxynivalenol (DON) via microbial and enzymatic strategies: Current status and future perspectives. – Trends in Food Science & Technology. https://doi.org/10.1016/j.tifs.2022.04.002.

[79] Tralamazza, S. M., Bemvenuti, R. H., Zorzete, P., de Souza Garcia, F., Corrêa, B. (2016): Fungal diversity and natural occurrence of deoxynivalenol and zearalenone in freshly harvested wheat grains from Brazil. – Food Chemistry 196: 445-450. https://doi.org/10.1016/j.foodchem.2015.09.063.

[80] Vardali, S., Papadouli, C., Rigos, G., Nengas, I., Panagiotaki, P., Golomazou, E. (2023): Recent Advances in Mycotoxin Determination in Fish Feed Ingredients. – Molecules 28(6): 2519. https://doi.org/10.3390/molecules28062519.

[81] Vogelgsang, S., Beyer, M., Pasquali, M., Jenny, E., Musa, T., Bucheli, T. D., Wettstein, F. E., Forrer, H. R. (2019): An eight-year survey of wheat shows distinctive effects of cropping factors on different *Fusarium* species and associated mycotoxins. – European Journal of Agronomy 105: 62-77. https://doi.org/10.1016/j.eja.2019.01.002.

[82] Wang, Y. K., Shi, Y. B., Zou, Q., Sun, J. H., Chen, Z. F., Wang, H. A., Li, S. Q., Yan, Y. X. (2013): Development of a rapid and simultaneous immunochromatographic assay for the determination of zearalenone and fumonisin B1 in corn, wheat, and feedstuff samples. – Food Control 31(1): 180-188. https://doi.org/10.1016/j.foodcont.2012.09.048.

[83] Wang, Y., Pruitt, R. N., Nürnberger, T., Wang, Y. (2022a): Evasion of plant immunity by microbial pathogens. – Nature Reviews Microbiology 20(8): 449-464. https://doi.org/10.1038/s41579-022-00710-3.

[84] Wang, J., Gu, Y., Zhang, Y., Chen, S., Li, L., Liao, Z., Shan, X., He, L., Chen, J. (2022b): Toxigenic potential analysis and fumigation treatment of three *Fusarium* spp. strains isolated from *Fusarium* head blight of wheat. – Food Science and Technology 42. https://doi.org/10.1590/fst.53822.

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DOI: http://dx.doi.org/10.15666/aeer/2104_32573273
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[85] Xu, J., Chi, J., Lin, C., Lin, X., Xie, Z. (2020): Towards high-efficient online specific discrimination of zearalenone by using gold nanoparticles@aptamer-based affinity monolithic column. – Journal of Chromatography A 1620: 461026. https://doi.org/10.1016/j.chroma.2020.461026.
[86] Yazar, S., Omurtag, G. Z. (2008): Fumonisins, trichothecenes and zearalenone in cereals. – International Journal of Molecular Sciences 9(11): 2062-2090. https://doi.org/10.3390/ijms9112062.
[87] Zhang, W., Tang, S., Jin, Y., Yang, C., He, L., Wang, J., Chen, Y. (2020a): Multiplex SERS-based lateral flow immunosensor for the detection of major mycotoxins in maize utilizing dual Raman labels and triple test lines. – Journal of Hazardous Materials 393: 122348. https://doi.org/10.1016/j.jhazmat.2020.122348.
[88] Zhang, Y., Liu, D., Peng, J., Cui, Y., Shi, Y., He, H. (2020b): Magnetic hyperbranched molecularly imprinted polymers for selective enrichment and determination of zearalenone in wheat proceeded by HPLC-DAD analysis. – Talanta 209: 120555. https://doi.org/10.1016/j.talanta.2019.120555.
[89] Zhao, Z., Liu, N., Yang, L., Deng, Y., Wang, J., Song, S., Lin, S., Wu, A., Zhou, Z., Hou, J. (2015): Multi-mycotoxin analysis of animal feed and animal-derived food using LC–MS/MS system with timed and highly selective reaction monitoring. – Analytical and Bioanalytical Chemistry 407: 7359-7368. https://doi.org/10.1007/s00216-015-8898-5.
[90] Zhu, W., Ji, G., Chen, R., Xiang, Y., Ji, S., Zhang, S., Gao, Z., Liu, H., Wang, Y., T. (2023): A fluorescence aptasensor based on hybridization chain reaction for simultaneous detection of T-2 toxins and zearalenone1. – Talanta 124249. https://doi.org/10.1016/j.talanta.2022.124249.

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