Review Article

RECENT ADVANCES IN MYCOTOXIN DETECTION

Abstract

Mycotoxins, toxic compounds produced by fungi like *Aspergillus* and *Penicillium*, pose significant health risks even at low concentrations. Detecting these contaminants in food requires intricate methods due to their low levels and complex sample matrices. Historically, toxic fungi research began with mushrooms but expanded in the mid-1800s to include other fungi, such as *Claviceps purpurea*, which caused ergotism through contaminated rye. Mycotoxins severely impact both human and animal health, causing issues like cancer, immune suppression, and nervous system damage, while also disrupting global food trade, affecting around 25% of crops worldwide. Traditional detection methods include chromatoy (TLC, LC, GC, HPLC) and immunological assays like ELISA and LFI, though these methods have limitations. Recent advances in mycotoxin detection feature innovations like biosensors, fluorescence-based techniques, phage display technology, and smartphone-enabled systems, which offer enhanced sensitivity, specificity, and rapid detection capabilities. These methods represent a promising shift towards more efficient and precise mycotoxin analysis in food safety.

Keywords: mycotoxin, biosensors, chromatoy, fungal growth

INTRODUCTION

Mycotoxigenic fungal strains are the primary producers of mycotoxins, which are low molecular weight molecules and poisonous secondary metabolites*viz.*, *Aspergillus, Penicillium, Fusarium, Claviceps* and *Alternaria*[1].Animals and humans are seriously harmed by mycotoxin, even at low concentrations. It is difficult, but not impossible, to identify tiny mycotoxin compounds at such low quantities. It isn't feasibleto overestimate the importance of finding mycotoxins in food and animal feed because even at very low concentrations, they can have detrimental effects on both people and animals.Therefore, developing a sensitive, reliable, and accurate method for determining mycotoxin levels in a variety of food products is essential[2].Mycotoxin analysis is challenging due to three main factors: differences in their physical and chemical attributes, the complex specimen matrix, and the abnormally low

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mycotoxin concentrations present in food [3]. Thus, the process of mycotoxin detection in agricultural products involves several steps such as preparing the sample, matrix extraction, purification, and sampling[2] and evaluation itself. Mycotoxin levels as low as ppm can be detected using contemporary analytical techniques like mass spectroscopy and chromatoy. Advanced analytical techniques are typically costly and only available in specialised labs; using them necessitates highly qualified academic and technical staff, which drives up the cost and duration of the analysis. Portable, user-friendly biosensors and immunochromatoic instruments that can express and detect mycotoxins in the field would be a far better option.Due to their high thermal stability, many mycotoxin compounds are challenging to break down using standard methods [4]. Therefore, sophisticated management techniques are needed to prevent fungal growth and mycotoxin breakdown. Many mycotoxin measurement techniques have been developed to accomplish this goal, such as HPLC, lateral flow assay, biosensors, spectroscopic approaches, fluorescent polarisation immunoassay, immunological microspheres based on smart phone recognition, etc.

MYCOTOXINS PRESENT IN FOOD COMMODITIES

Aflatoxin (AF), ochratoxin A (OTA), patulin (PT), sterigmatocystin (STC), trichothecenes (TCTs), fumonisins (FBs), deoxynivalenol (DON), zearalenone (ZEA), alternariol (AOH), tenuazonic acid and alternariol monomethyl ether are the main categories of mycotoxins that are produced by fungus. The table lists some significant mycotoxins along with the food products they contaminate[5].

Table 1: Details regarding several significant mycotoxins and food items[5]

Mycotoxins	**Producer Organism**	**Commodities affected**
Aflatoxins	*Aspergillus flavus, A. parasiticus, Penicillium puberulum*	Nuts, spices, grains, milk, milk products
Zearalenone	*Fusarium roseum, F.verticillioides*	Cereals, maize, rice, beer
Deoxynivalenol	*Fusarium graminearum*	Cereals, Cereal products
Fumonisins	*Fusarium verticillioides*	Maize, Sorghum, Asparagus
Ochratoxins	*Aspergillus ochraceus, Penicillium viridicatum*	Cereals, wine, coffee, dried fruits, nuts, cheese

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Patulin	*Penicillium expansum*	Fruits & vegetables
T2 toxin	*Fusarium poae, Fusarium acuminatum*	Wheat, rye, maize, soybeans

OVERVIEW OF HISTORY

For a long time, it has been known that some fungi, such as mushrooms are hazardous. The possible health hazards that other toxic fungus could pose to both humans and animals, however, were not recognised until the 1850s. Around this period, scientists discovered a particular disease known as ergotism, which was connected to eating rye and other grains tainted with the fungus *Claviceps purpurea*[6].Following this, news from Russia brought to light further cases of human mycotoxicosis. For example, eating bread contaminated with *Fusarium graminearum* has been linked to human stachybotryotoxicosis, and eating cereals contaminated with *Fusarium poae* and *Fusarium sporotrichioides* has been linked to alimentary toxic aleukia (ATA). Although there had been rare reports of mycotoxicosis in domestic animals prior to 1960, the discovery of turkey X illness in England signalled a paradigm shift in mycotoxin research and aroused a great deal of interest among scientists[7].

MYCOTOXIN'S IMPACT ON THE HEALTH OF ANIMALS AND HUMAN

Mycotoxins are well known for their capacity to seriously impair human and animal health once they enter the body. They use a variety of methods to carry out their detrimental effects[8].These negative consequences, which result from consuming food or animal feed contaminated with mycotoxin, might appear as immunosuppressive, teratogenic, mutagenic and carcinogenic[9,10].Through immediate or long-term toxic effects, mycotoxins can harm organisms, causing issues with the liver, lungs, central nervous system, digestive system, and cardiovascular system.

Along with the detrimental impacts on human and animal health, mycotoxin contamination has an adverse influence on the trade of food and animal feed[11,12].According to the United Nations Food and Agriculture Organisation (FAO), mycotoxins are found in around 25% of agricultural products worldwide, putting almost 4.5–5 billion people at risk of chronic exposure. In less developed countries, this burden is more evident[13].

CONVENTIONAL MYCOTOXIN DETECTION METHODS

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The traditional methods for detecting mycotoxin depend on either immunological or chromatoic approaches. Commonly used techniques included ELISA, PCR, lateral flow assay, liquid chromatoy–mass spectrometry (LC-MS), gas chromatoy–mass spectrometry (GC-MS), and high-performance liquid chromatoy (HPLC). These techniques maintain a high throughput capacity while providing a number of benefits, including as high sensitivity, low limit of detection (LOD), and superior selectivity. Consequently, these chromatoic approaches have emerged as the benchmark by which other mycotoxin detection techniques are evaluated[14].

1.Chromatoy

Mycotoxins can be measured using a variety of chromatoic techniques. These methods use either gas chromatoy (GC) or liquid chromatoy (LC) in conjunction with detectors such as mass spectrometry (MS), fluorescence (FLD), or ultraviolet (UV) in order to precisely measure mycotoxin levels. These techniques can detect a wide range of mycotoxins with great sensitivity by utilising sophisticated instrument configurations and meticulous sample preparation.

1. Thin layer chromatoy (TLC)

One of the first methods for detecting mycotoxin is conventional Thin Layer Chromatoy (TLC). It is prized for its low cost, ease of use, and capacity to identify mycotoxins via fluorescent signals when exposed to ultraviolet light. It is difficult to accurately quantify mycotoxins, nevertheless, because of its shortcomings in sensitivity and precision. Although TLC is a recognised technique for identifying aflatoxins (AFs), High-Performance Liquid Chromatoy (HPLC) has essentially substituted TLC in the quantification of mycotoxins. Nonetheless, TLC has shown progress in detecting ochratoxin A (OTA). With just 2 µl of the material on the TLC plate, 0.2 µg of OTA detected. Similar to LC procedures, this technique has a limits of detection as low as parts per billion (µg/kg), and is more sensitive than using a UV lamp[15].

2. Liquid chromatoy (LC)

The thin-layer chromatoy (TLC) method's drawbacks have been addressed by the development of liquid chromatoy (LC). Because TLC is an open system, it has limitations including a restricted plate length and is vulnerable to temperature and humidity changes. Pre-column or post-column derivatisation is frequently used in conjunction with LC ina range of detecting methods, such as UV absorption, amperometric detection, and fluorescence

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detection (FLD). The fluorescent characteristics of aflatoxins (AFs) are used for measurement in LC combined with FLD. It is commonly acknowledged that the best standard methods for mycotoxin detection are LC-MS and LC-FLD[16].However, the particular mycotoxins, matrices, ionisation processes, and sensitivity of the used procedures can all affect the sensitivity, precision, and accuracy of LC-MS approaches. Because of problems including matrix effects and ion suppression, LC-MS frequently produces less-than-ideal results for the quantitative detection of mycotoxins.

3. Gas chromatoy (GC)

The separation of compounds between a stationary liquid phase and a mobile gas phase is the basis of gas chromatoy (GC) analysis. GC is frequently used to evaluate food samples both qualitatively and quantitatively. It is widely used in the identification and measurement of mycotoxins in food items[4].It is especially favoured for trichothecenes analysis because of their variable polarity, lack of fluorescence, and restricted absorption in the UV-visible region[17].Chemicals such as insecticides, oils and steroids that are thermally stable, non-polar, semi-polar, volatile and semi-volatile can all be examined by GC[18].

A derivatisation procedure is usually used to increase the volatility and detectability of mycotoxins in GC systems because the majority of them are not volatile [17, 4]. Trimethylsilyl (TMS) for TMS esters, pentfluoropropyl (PFP), heptafluorobutyl (HFB), or trifluoroacyl (TFA) for fluorination, and acetic anhydride for acetylation are some of the agents that react with the hydroxyl groups of mycotoxins during this derivatisation process to form the corresponding esters[17].The particular mycotoxins being studied and the kind of detection instrument being used, determine which derivatisation agent is best[19].When evaluating type A and type B trichothecenes, fluorinating agents are recommended so as to achieve improved sensitivity and selectivity[20].

4. High performance liquid chromatoy (HPLC)

One widely used chromatoic method for mycotoxin analysis is High-Performance Liquid Chromatoy (HPLC), which provides a variety of detection choices.Utilising a variety of adsorbents appropriate for the specific mycotoxin's chemical and physical characteristics, HPLC is a contemporary analytical method for analysing mycotoxins [4]. HPLC is a quantitative technology that can be used to clean up online sample extracts. It can be

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combined with various detectors, each of which has a different degree of selectivity and sensitivity.

In HPLC, ultraviolet (UV) and fluorescence (FLD) detectors are the most often utilised types. Analytes are recognised by UV detectors, which measure the sample's absorption of light at different wavelengths. However, in order to identify the analyte, fluorescence detectors depend on the particles' chromophore. Because of their inherent fluorescence characteristics, certain toxins, such as aflatoxins (AFs), can be directly detected using HPLC-FLD.Two highly accurate detection techniques employed for quantitative analyses in HPLC are electrochemical and fluorescence detection[21].

2.ELISA – Enzyme Linked Immunosorbent Assay

Immunological tests like ELISA have become quite popular for screening for mycotoxinsbecause they are inexpensive and simple to use. The ELISA assay does not require cleanup steps and may analyse mycotoxin extract immediately. Although the quantity of matrices assessed is restricted and these immunoassays frequentlylack accuracy when taking little amounts, they generally offer quick and affordable measurements. The competitive assay format used by commercially available ELISA kits for mycotoxin detection uses a primary antibody specific to the target molecule or an enzyme-target conjugate. A chromogenic substrate will interact with the complex to generate a measurable result. These are easy to use and highly accurate. Because of their size, most mycotoxins require the development of a carrier molecule in order to induce immunogenicity and the formation of antibodies. Quantifiable mycotoxin measurements may be subject to inaccuracies due to conjugate and antibody binding being inhibited by the presence of structurally related mycotoxins or matrice interference. Using the same antibody to prepare an immunoaffinity column sample, an indirect ELISA detection method was found to be highly accurate at 0.02 µg/L[4].The majority of researches focused on altering the standard ELISA methodology in order to restore high sensitivity. ELISA formats (direct, indirect, competitive, and sandwich) are known to be quite precise and successful for screening for mycotoxin, but they require specialised plate readers, are not appropriate for field testing and take a lot of time.

3. Lateral Flow Strip

lateral flow immunoassay (LFI) is aspecifically ideally suited method for on-site detection of contamination with mycotoxin in food. A liquid sample is combined with a specific antibody and labelled, and the mixture then passes across a membrane. Its initial site of

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contact is with the test line, also known as the T-line, which is an antigen coated onto the membrane. The labelled antibodies bind to the coated antigen and concentrate at the T-line, which becomes visible and identifiable when the sample lacks the target material (as in a negative sample). On the other hand, When the target material is present in the sample in amounts below the lower detection limit (in a positive sample), the labelled antibody binding sites become saturated and lose their ability to bind to the coated antigen.Consequently, the T-line stays undetected and invisible. The process typically includes a second control line, or C-line. Secondary antibodies unique to the species develop this line, which is used to detect excess particular antibodies. The C-line ensures the integrity of the materials and reagents by validating that the experiment was carried out correctly. Additionally, it can be used as an internal standard for measuring the T-line's intensity and deciding whether the result is positive or negative, or tt is useful for calculating the T/C signal ratio, which aids in identifying differences between various test strips [22]. Despite their ease of use, the results are qualitative rather than quantitative.

4. Polymerase Chain Reaction

PCRis a highly accurate physico-chemical technique for analysing mycotoxins in agricultural products. Before or even after processing, this can be utilised for testing agricultural products for the presence of mycotoxin producers. A sample should be nearly free of mycotoxins if the assay yields negative results. Standard physico-chemical techniques are used to analyse only positive samples for the presence of mycotoxins. To detect fungi that produce mycotoxin, certain DNA sequences of the corresponding organism must be selected as primer binding sites. Mycotoxin biosynthesis pathway genes provide the ideal framework for a precise and targeted detection approach for mycotoxigenic strains in foods, animal feed, and agricultural products. It is believed that those genes are only found in organisms that have the capacity to produce mycotoxins[23].Aflatoxigenic Aspergilli can be detected using PCR techniques based on the regulatory gene aflR, the norsolerinic acid reductase-encoding gene nor 1, the Versicolorin A dehydrogenase-encoding gene ver 1, and the sterigmatocystin o-methyltransferase-encoding gene omt A[24].The target sequence for identifying penicillium strains that produce patulin is found in the gene IDH, which codes for isoepoxydon dehydrogenase[25].

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Despite their accuracy and sensitivity, many conventional approaches have limitations, such as being expensive, time-consuming, and dependent on specialised personnel and sophisticated equipment. These techniques might not be appropriate for performing on-site screenings or monitoring a high volume of samples.Rapid detection technology, on the other hand, differs from conventional laboratory techniques. It frequently incorporates ideas from a variety of disciplines, including electrochemistry, spectroscopy, molecular biology and immunology. Rapid detection just requires a portable equipment with a brief detection duration and offers simplicity, cost and convenience of use. In the field of food safety, this method satisfies the need for real-time on-site mycotoxin screening [26]. Current developments in the detection of mycotoxin include

I. Biosensors
II. Spectroscopic techniques
III. Fluorescent polarization immunoassay
IV. Phage display
V. Multicolorquantum dot nanobeads for simultaneous multiplex immunochromatoic detection
VI. Aggregation induced emission
VII. Smartphone recognition-based immune microspheres

I. Biosensors

Biosensors are analytical devices used to measure analyte concentration. Because biosensors offer several advantages, including quick, simple and affordable sample analysis, accuracy, stability and repeatability, as well as on-site sample testing, their usage in food industries can help limit the level of mycotoxins present[27].The three primary transducers utilised for mycotoxin detection are electrochemical (impedimetric, potentiometric, and amperometric), optical (surface plasmon resonance, or SPR and fluorescence), and piezoelectric (quartz crystal microbalance, or QCM). Peptides, enzymes, antibodies, cells, and nucleic acids are examples of common recognition elements; however, aptamers, molecularly imprinted polymers, and recombinant antibodies are all possible materials. To increase the biosensor's sensitivity, metal nanoparticles, carbon nanotubes, and nanofibers have all been explored. These materials havespecial physicochemical characteristics, such as a high ratio of surface to volume, and are biocompatible. These materials interact to cause physical and

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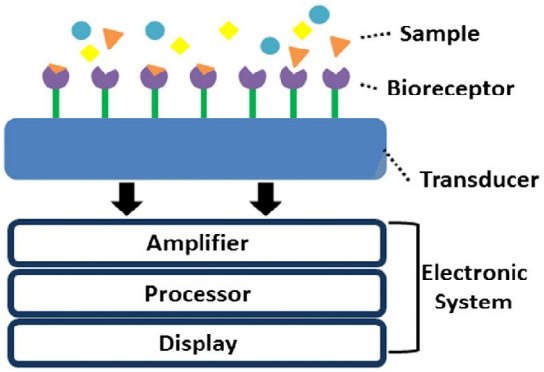
chemical changes, which a transducer detects and transforms into an electrical signal. The analyte concentration in the sample is determined by interpreting and converting this signal.



Components of biosensor[27]

TYPES OF BIOSENSORS

A. Based on receptors used

1. Immunosensors
2. Aptasensors
3. Molecularly imprinted polymers

B. Based on transducers used

1. Electrochemical biosensors
   - Impedimetric biosensors
   - Potentiometric biosensors
2. Optical biosensors
   - Surface plasma resonance
   - Planar waveguide biosensors
3. Pieozoelectrics biosensors
   - Quartz crystal microbalance

A. Based on receptors used

1. Immunosensors

A. Labelled Immunosensors

The competitive immunoassay format is typically used for the detection of tiny analytes, ormolecules with low molecular weights. In this experiment, the label coated analyte or analyte-protein conjugate competes with the sample analyte for a limited number of antibody

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binding sites. As the analyte level in the sample increases, more labelled analytes are displaced. Competitive assays use the "indirect" approach, where a saturation level of concentration of labelled analyte-protein conjugate is applied to the electrode surface. Free analyte and immobilised labelled analyte-protein conjugate compete for a fixed quantity of antibody. The concentration of the sample analyte is inversely correlated with the amount of labelled antigen that is detected. The format of labelled non-competitive immunoassay is sandwich. Secondary antibodies that are tagged with enzymes, fluorescent materials, or nanomaterials produce a signal when they capture antigen. The BSA-mycotoxin conjugate is the most frequently observed analyte-protein. To lessen the steric impact, a linker molecule such as polyethylene glycol (PEG) is used as a spacer between the OTA and ovalbumin (OVA) conjugation (OTA-PEG-OVA)[28].

B. Label Free Immunosensors

There are two types of label-free immunosensor innovation: competitive and non-competitive. The main advantages of these label-free immunosensors are the ease of use and one-step, reagent-free operation. Such immunosensors often fall short of the sensitive detection criterion. Because immunosensors are quick and easy to use, the non-competitive approach is favoured over the competitive one. The major drawback of non-competitive label-free immunosensors is that nonspecific binding causes false positive results. The interaction between the analyte and immobilised antibodies is revealed by the transducer in a label-free non-competitive immunosensor. The enhanced sensitivity of the label-free immunosensor depends on the transducer and detection technique. By adding a metal (PtCo NP), metal oxide nanoparticles (CeO2, Fe3O4, TiO2, ZnO, etc.), conducting polymer, etc., to a specific matrix, the electrochemical immunosensor's sensing capacity can be improved[29].

2. Aptasensors

The aptamer, a synthetic oligonucleotide ligand that can be either single stranded DNA (ssDNA) or RNA with 10–50 variable bases, is the crucial element of the aptasensor. It is known to have a high binding affinity and high specificity. Aptamers can attach to their ligands byfold into unique three-dimensional formations and interact with similar shapes. An aptamer has a number of benefits over an antibody, including stability, cost-effectiveness, and simplicity of production and modification with a range of chemical groups. The aptamers exhibit strong characteristics and preferences of binding towards a wide range of targets (from macro to micro molecules, such as toxins, medicines, peptides, proteins, and whole cells, etc.)

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and may retain their structures during repeated rounds of denaturation. Aptamers are thought to be a superior substitute for antibodies in a variety of biological applications[30].

A. Labelled Aptasensors

As the target (mycotoxin) molecules are added to the reaction chamber, aptamer conformational changes cause the signalling in aptasensors. Depending on the assay type, the transducer can detect using either the "signal on" or "signal off" form. Fluorescence materials (such as fluorescein, luminols, and QDs), enzymes HRP, GO and ALP, as well as electroactive substances (such as ferrocyanide, methylene blue (MB), Pt and Cds QDs and other metal nanoparticles (NPs), are frequently used labels. Target identification in the "signal on" style is predicated on the signal that is amplified following contact with the target. On the other hand, the term "signal off" describes a decrease in signal caused by the target-aptamer complex[30].

B. Label Free aptasensors

The transducer in a label-free aptasensor utilises the change in the transducer signal to measure the direct interaction between the aptamer and the analyte[31].The aptamer may experience conformational or configurational changes as a result of the development of the aptamer-target complex. Label-free aptasensing techniques can be divided into the following categories based on this change:(1) structure switchable aptamer assays; (2) aptamer construct assembly/disassembly based assays (3) target-induced variation in charge transfer transistance.

a. Structure switchable aptamer assays

Aptamers are initially folded into a stable three-dimensional structure via hydrogen bonds, van der Waals contacts, weak and non-covalent connections, and hydrophobic effects in apta-switching detection techniques. Aptamer destabilisation or attachment to a complementary strand then triggers the switchable event. Ultimately, a detectable signal is produced directly from the conformational transition[31].

b.Aptamer construct assembly/disassemblybased assays

The basis of this kind of label-free aptamer-assays is the alteration of the sensor's configuration. During the aptamer-target complex's development, the biosensing construct either associates or dissociates. A sandwich structure containing a secondary aptamer is created as a result of the association or assembly. However, a DNA strand is released as a result of the

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dissociation or disintegration. By producing a signal that may be translated by various identification techniques, both methods alter the surface electrode[31].

c. Target-Induced Variation in Charge Transfer Resistance

This aptasensing format is the simplestas it relies on the clear view of the interaction between the aptamer and its target. It is independent of both conformational and configurational changes to the aptamer structure or build. The target's binding creates a strong barrier on the sensing surface in this type of detection system, preventing electrons from moving to the electrode. The concentation of the target in the sample determines how much the charge resistance changes[31].

3. Molecularly Imprinted Polymers

MIPs are a proven analytical technique for mycotoxin identification that is based on bioreceptors. These are more adaptable to changes in temperature, pH, organic solvents, and other variables. comparatively less expensive to develop, easier to use, and multipurpose. made to resemble biological receptors and natural recognition entities like antibodies, which have specificities identical to those of antibody-antigen interactions. Cross-linked polymers are produced during molecular imprinting when functional monomers and a cross-linker co-polymerize free radicals while an analyte (such as mycotoxins) acts as a template[32].Following the removal of the original template, a three-dimensional network with distinct recognition cavities that complement the target in size and form is produced. It can identify a specific target molecule that mimics the biological function of naturally occurring receptors. Regarding mycotoxins, the template is costly and unsafe[33].

B. Based on transducers used

A. Electrochemical Biosensor

Electrochemical biosensors work by identifying changes in electrical signals produced by chemical reactions between the target analytes and recognition components that are immobilised on electrodes. Potentiometric and impedimetric techniques are two of the categories into which these sensors can be divided according to the kind of electrical signals that can be detected.

(1) Impedimetric Sensors

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The electrochemical impedance spectroscopy (EIS) method was created to detect mycotoxins. This method analyses the changes in the electrode-redox probe interface that are evident[34].An impedimetric sensor is made up of three electrodes: the working, reference, and counter electrodes. Samples were dropped onto the electrode's detecting region. A portable detector is used to measure impedance. The detector's LED panel displayed the results. Tests of impedimetric sensors for AFB1, AFM1, OTA, and PAT have been performed accurately[35].

(2) Potentiometric Sensors

Ion-selective electrodes are used in potentiometric sensors. Two electrode systems (working and reference) or three electrode systems (working, reference, and counter) may be used for this technique. The variations in circuit voltage between the working and reference electrodes provide information about the recognition event. The mycotoxin level of food has been determined using differential-pulse voltammetry (DPV), cyclic voltammetry (CV), and square-wave voltammetry (SWV). The basic idea is that the target causes the aptamer to separate from the linker on the electrode surface. Potentiometric sensors for AFB1 in maize powder, OTA in grape juice and red wine, PAT in juice and ZEN in maize have all undergone successful testing[36].

Flexible Dispense-Printed Electrochemical Immunosensor Development for Milk Aflatoxin M1 Detection[36]

In this study, single walled carbon nanotubes (SWCNTs) were used to functionalise flexible biosensors made using inkjet printed electrodes. To increase their sensitivity, the biosensors were then coated with certain antibodies. The immunosensor then employed a chronoamperometric approach to test for AFM1 in buffer solution and a spiked milk sample. For analysis, the milk was centrifuged for 15 minutes at 4˚C at 6000 rpm to defatten it. For the experiment, skim milk was gathered while fat and cream were thrown away. Spiking with known concentrations of AFM1 (0, 0.01, 0.02, 0.05, 0.1, 0.5 and 1 µg/L) allowed for the analysis of AFM1 in skimmed milk.

The integration of the antibody antigen (analyte) for varying concentrations of AFM1 affected the sensor's response in terms of produced current. Because of its superior detection capabilities, TMB (3,3',5,5'-tetramethylbenzidine dihydrochloride) was selected to evaluate HRP (horseradish peroxidase) activity as a mediator for H2O2. Chronoamperometry was used for the study, with the AFM1–horseradish peroxidase conjugate (AFM1–HRP) used in the

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immunoassay, at a potential of −100 mV. TMB changed colour to blue as a result of the HRP-catalyzed oxidation, and the degree of this change was inversely correlated with the AFM1 concentration. The current produced was negatively correlated with the AFM1 concentration; stronger TMB oxidation and higher current were the results of increased conjugate attachment with the antibody at lower AFM1 concentrations. Higher AFM1 concentrations, on the other hand, decreased conjugate attachment, which in turn decreased TMB oxidation and decreased current. With a detection range of 0.01 to 1 µg/L for both buffer and milk samples, the sensor's limit of detection (LOD) was 0.02 µg/L for buffer samples and 0.0259 µg/L for milk samples. Because of its affordability, speed and convenience of use, this sensor has the potential to protect consumer health and can be used in milk collection locations or on milk processing lines[36].

B. Optical Biosensor

When molecular recognition processes take place on the sensing element, Variations in the optical signals produced by transducers are what optical sensors rely on. Additionally, there are several prospects for the concurrent identification of different mycotoxins with optical biosensor systems[37].

Surface Plasmon Resonance

The high specificity, sensitivity, speed, and affordability of optical biosensing make it a promising technological substitute for conventional analytical methods. Real-time direct detection is also possible with optical biosensors. Fluorescence resonance energy transfer and surface plasmon resonance (SPR) are key techniques in the area of optical biosensors. SPR is a simple, cutting-edge analytical technique that produces quick, highly sensitive findings. Additionally, label-free detection and real-time qualitative and quantitative analysis of multiplexed contaminants are accomplished using this technology[38].

C. Piezoelectric Biosensors

A class of analytical tools known as piezoelectric biosensors works on the basis of capturing affinity interactions. The sensing element of these sensors is a piezoelectric platform or crystal, which detects variations in oscillations brought on by a mass being attached to the piezoelectric crystal's surface.

Quartz Crystal Microbalance (QCM)

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QCM-based biosensorshave been studied forboth pathogen surveillance and mycotoxin analysis. The gold-plated quartz crystal in the QCM transducer alters the resonance frequency by transmitting an electrical signal. There is a sensory layer of interest on the surface of quartz that causes mass change and certain vibrations. QCM-based biosensors have been evaluated for OTA in red wine and for AFB1 in peanuts, pistachios, rice and wheat[39].

Zearalenone Detection in Food Samples Using a Portable, Label-Free, Reproducible Quartz Crystal Microbalance Immunochip[40]

A portable, robust, and reliable Quartz Crystal Microbalance (QCM) immunochip was created in this study to test the quantities of ZEN (zearalenone) in actual food samples quickly, affordably and sensitively. The ZEN in a variety of food items, such as corn, wheat flour, soy sauce and milk, was accurately and consistently detected by the QCM immunochip. There was a good connection (R2 = 0.9844) between the HPLC–MS/MS technique findings and the QCM immunochip results.

Using an immunosuppressive format, the QCM immunosensing chip is reusable and portable, and it is intended for quantitative ZEN analysis in actual food samples. To track the oscillation frequency response, Princeton Applied Research (Oak Ridge, TN, USA) provided a Type-922 QCM device. Testing the chip against five ZEN structural mimics (α□zearalenol, β□zearalenol, α□zearalanol, β□zearalanol, and zearalanone) and other mycotoxins (AFB1, OTA, FB1, and DON) at different doses verified its specificity for ZEN analysis. The ultimate decision was to use a 0.05 $molL^{-1}$ NaOH solution for QCM chip regeneration and an anti-ZEN antibody concentration of 100 µg mL^{-1} for ZEN detection. Within 30 minutes, the complete measurement procedure could be finished, including sample pretreatment (around 20 minutes), QCM measurement (5 minutes), and regeneration (5 minutes)[40].

When applied to specific food samples, our recently proposed QCM immunochip showed great sensitivity (Limit of Detection: 0.37 μgL^{-1}) and reasonable accuracy (76.6%–92.5%). Its benefits of quick, accurate, sensitive, and economical detection are highlighted by the fact that it may be used at least six times and that each analysis can be finished in just five minutes. Additionally, this QCM immunochip's design and functionality can be modified to analyse additional target compounds in a variety of sectors[40].

II. Spectroscopic Techniques

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spectrometry uses the target molecules in the sample to absorb ultraviolet or visible light, in order to produce fluorescence and determine a sample's chemical makeup. It has strong sensitivity and specificity for detection.

A. Surface-Enhanced Raman Spectroscopy

SERS has been investigated as a quick examiningtechnique that may allow for on-site measurement using a portable gadget. It is a sophisticated Raman spectroscopy method that improves the chemical fingerprint of the target mycotoxin when roughened metal particles and nanoscale surfaces are present. By using portable instruments and on-site measurements, SERS can be used for quick and easy OTA investigation in food systems like wines. The sole drawback is that in order to obtain satisfactory repeatability, an easy extraction technique is required[41].

B. Hyperspectral Imaging

A new technology called Hyper specral imaging uses the sample's combined spectral and spatial information. Each pixel in the captured image has spectral information at a particular wavelength range[42].Hence, it is based on three axes: a third spectral axis (λ) and two spatial axes (X, Y), producing tri-dimensional data known as a hypercube. The data cube is used to choose the samples' intended areas. Following that the spectra associated with those areas are taken out and utilised for additional chemometric adjustments. Whole kernel evaluation would allow the differentiation of severely infected kernels in order to build a mitigation strategy, thereby overcoming the contamination variability of cereal batches. The recently released HSI experiments that intended to identify mycotoxin and fungal contamination in individual cereal kernels. The most pertinent results demonstrated that the discrimination accuracy for mycotoxin levels and fungal infection was over 90% and 80%, respectively[43].

III. Fluorescent Polarization Immunoassay

One of the most used homogeneous competitive immunoassays for target analyte measurement is fluorescence polarisation immunoassay (FPIA). FPIA is frequently utilised in food analysis, clinical, and biological applications due to its quick measurement time and ease of implementation.A portable fluorescence polariser analyser equipped with a

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microdevice for target analyte measurement is used for FPIAs. The competitive binding response between the fluorescently labelled target analyte (tracer) and an antibody is the foundation of FPIA. The quantities of tracer and antibody molecules are fixed as constants in FPIA. The majority of tracer molecules attach to the antibody when the concentration of the analyte molecule is low, increasing the binding efficiency. However, a high analyte concentration causes the majority of the analyte molecules to attach to the antibody, resulting in a low binding efficiency and the presence of free tracer molecules. Analyte, tracer, and antibody solutions must be mixed in order to perform the measurements. As a result, unlike heterogeneous immunoassays, FPIA does not require antibody immobilisation and washing procedures, which increase handling complexity and measurement time[44].

IV. Phage Display

The synthesis of mycotoxin conjugates with protein molecules requires pure toxin, which is harmful by nature and could endanger the environment, consumers, and manufacturers. To improve laboratory and environmental safety, it is beneficial to substitute a harmless chemical for a harmful one when using it as an immunochemical reagent. Using the conjugate has additional drawbacks, such as the release of the analyte moiety, which could provide the transducer with an incorrect signal. Mimotopes are an alternative to mycotoxin conjugates. Mimotopes are amino acid peptide sequences that exhibit a strong affinity for toxins; they were chosen from random phages and showed peptide libraries. Scientists have created mimotopes for OTA, ZEN, and DON, among other mycotoxins, and used them to detect mycotoxins quickly and precisely[45].

V. Multicolorquantum dot nanobeads for simultaneous multiplex immunochromatoicdetection

Due to its simplicity, rapidity, cost-effectiveness, and ease of use, the immunochromatoic assay (ICA) is one of the most popular point-of-care testing (POCT) tools. It has multiple applications in clinical diagnostics, environmental investigation, and food safety screening. Compared to utilising many separate test strips, employing a single ICA test strip to detect numerous target chemicals in a single sample at the same time can save sample volume, minimise testing costs, and drastically reduce overall analysis time. Recently, a variety of multiplexed ICA strips for concurrent mycotoxin testing have emerged. Amorphous carbon nanoparticles and gold nanoparticles are two of the coloured nanoparticles used as markers in these multiplexed ICA strips. Additionally quantum dots (QDs)-based multiplexed ICA strips

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have been created to monitor several target analytes at once. Quantum dot nanobeads (QBs) are novel luminous nanomaterials made of polymer nanobeads with multiple QDs embedded in them. Compared to QDs, QBs have higher fluorescence intensity (FI) and are more resilient to environmental changes like pH and ion strength. Therefore, QBs help to further improve sensitivity and stability of ICA[46].

VI. Aggregation Induced Emission

The fluorescence of the AIE group of fluorescent dyes is noticeably increased in the aggregation state and glows faintly in the diluted solution condition. In the aggregate state, restricted intramolecular rotations may be the cause of intense dye's fluorescence. Tetraphenylethene (TPE), silacyclopentadiene (silole) and 9,10-distyrylanthracene (DSA) are the three most widely used AIE dyes. An aptasensor based on AIE dye was effectively created to detect OTA in coffee and wine and AFB1 in peanut oil and wide bean sauce[47].

Mycotoxin detection via label-free fluorescent aptasensing using aggregation-induced emission dye

The primary design of aptasensor makes use of AIE dyes (DSAI) in conjunction with the enzymatic digestion process. Aptamers' reactivity to target molecules would be exploited by the enzymatic digestion process. Exo. A nucleic acid enzyme called I was able to catalyse the nucleotide removal process from single-stranded DNA that started at the 3′ terminal. The aptamer would be coupled to the target OTA if it was present in the solution, which would allow it to withstand the enzyme digestion process. Therefore, there would be a greater reserve of aptamer sequences following the target OTA binding event. The negatively charged aptamer sequences would have a high binding ability due to the positively charged DSAI. The binding and aggregation of DSAI molecules might effectively light up the OTA-binding aptamer because of the AIE effect. However, Exo □ could digest aptamer sequences in the absence of the target OTA. It wouldn't cause DSAI to aggregate, which might lead to a weak fluorescence signal[48].

Detection of Fumonisin B1 by Ultrasensitive Lateral Flow Immunoassay Utilising Highly Luminescent Aggregation-Induced Emission Microbeads[49]

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Lateral Flow Immunoassay (LFIA), a fluorescent microbead-based technique, has drawn a lot of interest due to its quick and accurate use in food safety testing. However, the quenching effect brought on by the aggregation of loaded fluorescent molecules limits the use of conventional fluorescent microbeads, leading to poor sensitivity and weak signals in LFIA. In this study, an emulsification approach wasused to insert a green-emitting fluorophore with aggregation-induced emission (AIE) properties into polymer nanoparticles creating highly visible fluorescent microbeads known as AIEMBs. Fumonisin B1 (FB1) was subsequently quickly and highly sensitively detected in actual maize samples using these AIEMBs as signal indicators in a competitive LFIA (AIE-LFIA).

For quick FB1 detection, the AIEMBs were first altered with anti-FB1 monoclonal antibodies (mAbs) and used as AIEMB probes in a competitive LFIA (AIE-LFIA). The immobilised antigen (BSA-FB1) on the test (T) line and goat anti-mouse IgG on the control (C) line, respectively, caught the AIEMB probes when FB1 was absent from the sample, producing two bright green bands on the test strips. On the other hand, the presence of FB1 prevented AIEMB probes from binding to BSA-FB1 on the T line, which caused the T line to appear fainter or invisible. As the FB1 concentration increased from 0 to 100 ng/mL, the T line's brilliance progressively diminished until it was undetectable at 3.15 ng/mL.In competitive LFIA for FB1 detection, the produced green-emitting AIEMBs performed better as reporters than AuNPs, providing increased sensitivity, accuracy, and dependability. A highly sensitive competitive AIE-LFIA for quick FB1 screening was developed using these AIEMBs as signal indicators, and it was able to find FB1 in actual maize samples with a limit of detection (LOD) of 0.024 ng/mL. Compared to traditional AuNP-LFIA, this LOD is almost five times lower.

This work offers an adaptable technique for quickly and accurately screening small substances such as mycotoxins, pesticide residues and other chemical hazards. Additionally, it demonstrates that AIEMBs can be employed as signal probes to boost competitive LFIA's sensitivity[49].

VII. Smartphone Recognition-Based Immune Microspheres

Microsperes are tiny, solid or hollow, spherical particles composed of polymers, glass, or ceramic. Microspheres are doped with upconversion nanoparticles. The microsphere emits a distinct colour when excited at 980 nm.The competitive reaction and computerised analysis of the outcomes can be done by the system in a brief period of time. (1) The immunological competition approach is the basis for multiple detections. This section employs a hydrogel

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solid phase particle-based direct immune-competitive strategy. The solid-phase carrier hydrogel particles can be made in a variety of shapes. According to theory, the analyte encoding function might be accomplished by using a solid-phase carrier covered with a mycotoxin monoclonal antibody.Developing coding hydrogel on a large scale provides a framework for high-throughput simultaneous detection. For multi-target inspections to be completed quickly, this is necessary. (2) Competitive ELISA can be modified to detect analytes with the naked eye. According to theory colorimetric ELISA with an acid-base indicator is a technique for visually identifying a variety of hazardous compounds by using the conjugated catalysis of the label (such as urease or alkaline phosphatase) and the matching substrate to cause a modest pH change in the solution system. (3) A smartphone analysis app for instantaneous test result digitisation. The detecting method eliminates the need for sophisticated equipment entirely. Field detection is now simple to set up because to smartphones that combine image collecting, analysis, and processing capabilities[50].

Quick and instantaneous detection of mycotoxins using immunological microspheres based on smartphone recognition[51]

Hydrogel microspheres are used in a smartphone-based immunoassay device designed to quickly and simultaneously detect two mycotoxins. Three essential steps make up this effective detection system:

1. Rapid Separation: Using hydrogel solid-phase carrier particles, unbound compounds are quickly separated after a direct competitive response.
2. Effective Detection: To achieve effective detection, the system makes use of enzymes' catalytic properties.
3. Quick Image Capture and Analysis: Smartphone software allows for quick image capture and analysis.

OTA (ochratoxin A) and ZEN (zeralenone) were used as model mycotoxins to confirm the practicality of this intelligent quick detection method. The system provides an intelligent detection system by combining smartphone picture processing with direct competitive enzyme immunoassay. Equipped with a mycotoxin monoclonal antibody, a unique kind of particle coating functions as a solid-phase carrier that can encode the analyte. Colorimetric ELISA can detect numerous mycotoxins because mycotoxin-labeled urease serves as the competitive antigen. The test findings are rapidly digitised by a smartphone analysis app, which uses the

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brightness value derived from the photos to determine the mycotoxin content. The average brightness value rises steadily as the mycotoxin concentration rises because the particle's colour development lightens.

It only takes 30 minutes to complete the detection process, and it only takes 10 seconds to analyse the data. At 0.7711 ng L−1 and 1.0391 ng L−1, respectively, the detection limits for ZEN and OTA are remarkably low. For both mycotoxins, the recovery rates vary from 76.72% to 122.05%[51].This detection device provides a convenient solution for food safety monitoring while drastically reducing the amount of time needed for analysis. Crucially, this smartphone-based quick detection technology is adaptable and has uses in environmental monitoring, disease diagnostics, food pollutant detection, and many other areas.

CONCLUSION

Because of their varied characteristics and low amounts in samples, mycotoxins are extremely difficult to detect in food. As a result, numerous testing techniques for individual mycotoxins or particular mycotoxin groupings in diverse food kinds have been developed. We require quicker and more precise testing techniques to handle the rising complexity of food samples and the expanding number of mycotoxins [52]. Rapid screening approaches that employ ELISA, biosensors (such protein chips and electrodes covered with antibodies), spectroscopy, fluorescence polarisation immune tests, and aggregation-induced emission have been developed in response to this need.Mycotoxin identification is becoming more accurate and precise because to new methods that eliminate the need for laborious sample preparation and cleanup. Multiple groups of mycotoxins have been detected and measured simultaneously because some mycotoxins can have higher impacts when they occur together. Techniques that can identify multiple mycotoxin types at once are very desirable. In addition to expediting the testing procedure, this greatly improves food safety by screening for different kinds of mycotoxins.

FUTURE THRUST

The next task is to accurately detect changed mycotoxins in the food chain, evaluate the toxicity of many mycotoxins in a single sample and develop rules for new developing mycotoxin types. The future improvements in fast immunoassay technology for the detection of numerous mycotoxins will focus the following features:

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- Creating a platform for quantitative immunoassay to detect hazardous mycotoxins in the fields simultaneously.
- Sending assay findings to reasonably priced portable devices or generally available smartphones.
- Determining various mycotoxins simultaneously using a single dilution factor.
- Making sample preparation easier for quick analysis.
- Reporting the signals to widely used smartphones or low-cost portable devices.

- Amplifying signals using nanomaterials or eco-friendly, cost-effective novel molecules.
- To lower expenses and address ethical issues, bacteriophages, aptamers, and molecular imprinting polymers are animal-free substitutes for antibodies as recognition elements.
- Making use of microfluidic technologies and 3D printing for precise and simple analysis.

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