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Review

Mycotoxin Monitoring, Regulation and Analysis in India: A Success Story

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Abstract: Mycotoxins are deleterious fungal secondary metabolites that contaminate food and feed, thereby creating concerns regarding food safety. Common fungal genera can easily proliferate in Indian tropical and sub-tropical conditions, and scientific attention is warranted to curb its growth. To address this, two nodal governmental agencies, namely the Agricultural and Processed Food Products Export Development Authority (APEDA) and Food Safety and Standards Authority of India (FSSAI), have developed and implemented analytical methods and quality control procedures to monitor mycotoxin levels in a range of food matrices and assess risks to human health over the past two decades. However, comprehensive information on such advancements in mycotoxin testing and issues in implementing these regulations is inadequately covered in recent literature. The aim of this review is thus to uphold a systematic picture of the role played by the FSSAI and APEDA for mycotoxin control at the domestic level and for the promotion of international trade along with certain challenges in dealing with mycotoxin monitoring. Additionally, it unfolds various regulatory concerns regarding mycotoxin mitigation in India. Overall, it provides valuable insights to the Indian farming community, food supply chain stakeholders, and researchers about India's success story in arresting mycotoxins throughout the food supply chain.

Keywords: aflatoxins; APEDA; EU-ML; FSSAI-ML; mycotoxins; ochratoxin A; patulin; regulation, method validation.

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1. Introduction

Mycotoxins, which are naturally-occurring secondary metabolites of fungi belonging to the Phylum Ascomycota [1], are released in a variety of foodstuffs including cereals, dried fruits, spices and milk, among many others. Exposure to mycotoxins might cause serious hazards to human and animal health. *Aspergillus*, *Penicillium*, *Fusarium*, *Myrothecium*, *Stachybotrys*, *Trychoderma*, *Trichotecium* and *Claviceps* are some of the frequently noticed fungal genera. So far, more than 500 mycotoxins have been reported as potentially toxigenic, which include aflatoxins (AFs), ochratoxins (OTs), trichothecenes (TCTs), fumonisins (FUMs), zearalenone (ZEN), patulin (PAT), citrinin (CT) and ergot alkaloids (EAs) [2, 3]. According to the Food and Agriculture Organisation (FAO) [4], one fourth of the world's food grains are estimated to be infected alone with AFs, affecting 4.5 billion lives in developing nations [5]. Out of the four variants of AFs, AFB1 has been identified as a group I carcinogen by the International Agency for Research on Cancer (IARC) [6] and has globally posed a serious menace [7]. As AFs continue to be a significant socioeconomic along with a health concern for both developing and industrialised nations, their screening and control in food items become increasingly important in terms of ensuring public health.

Every year, Indian agriculture faces a variety of stresses related to pest infestations, frequent fungicide applications, adverse weather, untimely harvest, hailstorm, water logging, all of which make it difficult to implement or maintain good agricultural practices.

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The Indian agro-climatic landscape is very conducive for development of fungal infections compared to temperate regions of North America, Canada and Europe that report relatively lesser cases of mycotoxin-induced diseases, primarily affecting humans [8, 9]. Pre- and post-harvest management practices (e.g. cropping pattern, weed management and handling of grains) have potential implications on health of stored crops [10]. It is expected that global climate change will continue increasing the occurrence of mycotoxins in foods, with the vulnerable population (e.g. infants and immunocompromised adults) being especially affected. After conducting numerous tests in India, it has been established that AFB1 is the most commonly encountered mycotoxin in foods, followed by AFB2 and finally AFG1 and AFG2, whose occurrence is almost negligible. Thus, the monitoring of mycotoxins, particularly AFs in foods (and feed) are extremely warranted.

Mycotoxin producing fungi associated with grains can be categorised into field fungi and storage fungi [11]. Storage fungi, chiefly *Penicillium, Aspergillus* and *Fusarium* are responsible for the loss of 25 to 40% of cereal grains [12], one key factor behind this infestation being the lack of advanced post-harvest storage facilities such as metal silos and aerated bins. Small holder farmers in developing nations are often seen resorting to jute bags, bamboo structures and baked earthen containers for storage of harvested grains, which are unregulated and ill-equipped for restricting moisture and oxygen migration, thus provide ideal conditions for microbial attack. In North Indian farms, for instance, grain sacks weighing 50 to 60 kg that are stacked and cushioned by rice straw have been reported to be affected by these toxins [10]. Additionally, there are problems related to inadequate drying (moisture content exceeding 12-15%) and faulty sealing. The production of various traditional Indian delicacies by small business operators with inadequate hygienic control further accelerates fungal growth. Identifying these shortcomings and addressing them are the first step towards implementing remedial strategies to safeguard public health.

According to the Rapid Alert System for Food and Feed's (RASFF) annual report, AFs were among the top 10 hazards detected and notified in nuts, nut products, fruits and vegetables, dried figs and seeds, from Turkey, the USA, Argentina and Spain in 2020 [13]. Way back in 2005, out of 993 mycotoxin-related notifications, 947 were contributed by AFs alone. Evidently, AF poisoning is one of the primary reasons for hepatocellular carcinoma, kidney disorder, neonatal jaundice and endocrine disruption in resource limiting countries [14]. One of the earliest instances of AF poisoning in India dates back to the 1970s, where people from approximate 200 villages in the states of Gujarat and Rajasthan reportedly suffered from hepatitis after consuming moldy maize [15]. Fast forward again to recent times, Mehta et al. [16] reported AFM1 in 41% of human milk samples and 93% of animal milk samples, in a risk assessment study conducted in Haryana, raising questions about infant health as this age group is heavily dependent on dairy products for nourishment. They also concluded that women who were predominantly on a rice and flour diet exceeded Provisional Maximum Tolerable Daily Intake (PMTDI) limit of AFB1 [16]. Obviously, both infants and mothers are in a high-risk situation, leading to more chances of child mortality, loss of valuable lives or other health complications.

Like other Indian food products, spices are also prone to fungal infection, as evidenced in a 2015 study by Jeswal and Kumar, who found *Aspergillus flavus* in the highest amount in black pepper among four other spices: green cardamom, turmeric, mace fennel [17]. As Indian spices are most commonly contaminated with AFs and OTAs [18], failure to arrest their spread in the food chain has far-reaching ramifications not only with respect to human health hazards and wellbeing but also non-compliances to food safety standards.

On the brighter side of things, over the past two decades, the food safety landscape in India has witnessed a wholesome change. The Indian government has taken measures and updated regulations to monitor safe mycotoxin levels in widely consumed food products. The maximum limits (ML) are now largely harmonised with the standards prescribed by Codex and the regulatory agencies of importing markets. Export control and surveillance agencies, including the Spices Board (SB) and the Export Inspection Council

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(EIC), both established by the Ministry of Commerce and Industry, are monitoring economically significant commodities such as ethnic spices and nuts (and nut-processed products) that are highly popular overseas. For export purpose, an Indian food business operator has to comply with the regulations of these governmental agencies, subject to which, a health certificate is issued, which is required for the customs clearance of the export food consignments.

To keep up with the ever-evolving requirements of trade activities, Indian regulators in cooperation with analytical scientists have introduced advanced testing methodologies involving high performance liquid chromatography (HPLC) with fluorescence (FLR) and mass spectrometry (MS)-based detection mechanism. Even though a great number of reviews have focused on AFs in the past five decades, a comprehensive information on such advancements in mycotoxin testing and issues in implementing these regulations in the Indian context is inadequately covered in recent literature. The present review thus upholds a systematic picture of the role played by the nodal Indian governmental agencies, namely the FSSAI and APEDA through the coordinated efforts of capacity building, disseminating knowledge, training laboratory professionals and creating traceability platforms in mycotoxin control both at domestic and international level. Additionally, it describes how these agencies have established a food safety network comprising primary, referral and reference laboratories which are actively involved in sampling and testing of mycotoxins using validated methodologies. The functions of web-based traceability platforms (e.g. Peanut.net of APEDA) and important governmental (EIC) agencies are also highlighted with an emphasis on how mycotoxins are being regulated in foodstuffs. Furthermore, it elucidates recent regulatory changes and alternative approaches to managing mycotoxin in India. This review is not intended to be an exhaustive list of mycotoxin analytical methods publications. Nonetheless, critical remarks on the selected approaches, their validation parameters, and applications are provided to help readers in appraising the significance of these breakthroughs. This review, the first of its kind, provides valuable insights into India's mycotoxin monitoring and testing systems, as well as specific challenges faced while exporting foods, and will be of interest to a wide range of readers.

2. Methodology

We referred to MDPI Publication's "Instructions for Authors" guidelines to prepare our review article. We decided on the review's aim and scope based on recent research and noteworthy inadequacies in India's mycotoxin surveillance. Initially, pertinent papers were selected by screening the 'Title' and 'Abstract' of published materials. All citations were exported to Microsoft Word and annotated as footnotes, with duplicates removed.

We searched for relevant data to:

a. Understand how monitoring and controlling mycotoxins in food influences public health.

b. Determine why Indian agricultural practices are prone to fungal infection and generation of mycotoxins in grains, nuts, spices and milk

c. Illustrate India's food safety framework for mycotoxin surveillance along with maximum level of major regulating countries

d. Enumerate the strategies acquired by export monitoring agencies of India (APEDA, EIC, and SB) to reduce consignment rejections and enhance trade of spices and nuts

e. Elucidate the fundamental role played by governmental and private laboratories in mycotoxin testing

f. Describe analytical methods developed by Indian reference laboratories

g. Identify the challenges associated with regulation and implementation of mitigation programs

h. Provide an overview of the impacts of these regulations on trade and commerce of India

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i. Emphasize and identify gaps in harmonising global safety standards and legislation for mycotoxins.

2.1. Study Design

Google scholar, Scopus, Wiley Online Library, Research Gate, and PubMed were used to conduct electronic searches. Furthermore, Google and Yahoo search engines were used to locate key international and federal entities linked with mycotoxins and related food safety standards. Additionally, we contacted regulatory specialists to gather authentic information on aflatoxin regulations and latest maximum limits.

Specific web-based resources which we referred to are included (not exhaustive):

- The Food Safety and Standards Authority of India (FSSAI).
- The European Union (EU)
- Agricultural and Processed Food Products Export Development Authority (APEDA)
- The Spices Board (SB)
- Export Inspection Council (EIC)
- National Dairy Development Board (NDDB)
- National Medicinal Plants Board
- The World Health Organization/Food and Agriculture Organization of the United Nations/The Joint FAO/WHO Expert Committee on Food Additives (JECFA)/ The Codex Alimentarius Commission.
- The United States Department of Agriculture (USDA)
- The Rapid Alert System for Food and Feed (RASFF)
- Trilogy Innovations Private Limited.

2.2. Inclusion Criteria

Only publications written in English language were considered and effort has been made to include recent literature. Analytical methods were included in this review, keeping in mind the Indian context.

2.3 Exclusion Criteria

Any publications with specific mycotoxins other than Aflatoxins, Ochratoxins, Patulin, Fumonisins and Zearalenone were excluded.

2.4. Search Strategy

The following keywords and search strings were applied.

- Mycotoxin AND Public health AND food safety OR regulation OR guideline OR risk AND management OR risk assessment
- Aflatoxins AND Peanut AND RASFF OR Spices OR food safety OR Maximum Limits OR cancer OR risk AND health OR risk AND assessment OR
- FSSAI AND mycotoxin manual AND Patulin
- FSSAI AND mycotoxin manual AND Aflatoxin and Ochratoxin
- Aflatoxins AND spices AND milk AND validation
- Export AND spices AND food AND Maximum Limit
- Peanut.net AND mycotoxins AND nuts AND ethnic spices
- Trade AND export AND regulatory body AND MRLs
- Export AND Codex AND Maximum Limit and consumers AND mycotoxin management AND food AND safety

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2.5. Critical Appraisal

We made certain that only relevant high-quality papers were included. Before publications were included in our study, the following citation, title, and abstract screening questions were answered.

- Are the title and abstract in English?
- Were the aim and objective/s of the study clearly communicated?
- What were the main findings of the paper and did they address the objectives of our review?
- Did the study address its aim?
- What were the strengths of the paper and limitations of the paper?
- Did the author acknowledge study limitations?
- Were the findings consistent with other published literature?

3. India's food safety framework for domestic surveillance of mycotoxin

In the following paragraphs, we have briefed how the government is bolstering consumer trust by establishing a hierarchy with respect to food testing laboratories to enhance accountability and transparency in the food supply chain.

As mentioned previously, FSSAI is the chief regulatory body concerned with building an effective food safety network to provide scientifically validated information to the consumers regarding safe and healthy food. On 20th October, 2020, the FSSAI published a revised manual on methods of mycotoxin analysis [19], compiling the ML of the concerned toxins in different food matrices and made it available in the public domain [20]. These include AFs in peanuts [21, 22], deoxynivalenol (DON) in wheat [23], zearalenone [24] and fumonisin [25] in maize and patulin in apple juice [26]. Similarly, APEDA, an export development organisation established by the Ministry of Commerce and Industry (under the APEDA Act of Government of India in 1985), has launched several traceability platforms such as HortiNet for export monitoring of fresh fruits (grapes, pomegranates, mangoes and oranges) and vegetables (okra, green chilli, etc.) [27], TraceNet to supervise organic foods export [28] and Peanut.Net [29], who's functioning has been briefed in the subsequent sections.

Despite making such commendable advances in mycotoxin testing and capacity building, these government regulatory bodies constantly face issues with implementation of regulations and management of mycotoxin mitigation programmes. This is because of diversity in food consumption pattern, heterogeneous nature of distribution of mycotoxins, transparency issues and executive hurdles (discussed later). Another point of concern is when domestic and international standards vary. The MLs of mycotoxins in agricultural commodities are established/utilised by the apex authorities worldwide such as United States Food and Drug Administration (USFDA), World Health Organisation (WHO), Food and Agriculture Organisation (FAO), European Food Safety Authority (EFSA) and the FSSAI. The MLs of traded commodities are tabulated below (Table 1).

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Table 1. MLs of AFs (B1 and Total) in various food commodities traded from India and major trading countries

Country/ region	Foodstuff	AFB1 (µg/kg)	Total AF (µg/kg)	Reference
India	Cereals and Nuts	10	15	[30]
	Spices	15	30	
European Union	Peanut and their products	2	4	[31]
	Rice	5	10	
	Spices	5	10	
Japan	All foods	-	10 (B1)	[32]
USA	All foods except milk	-	20	[33]
China	Peanut and their products	20	-	[34]
	Rice	10	-	

Under Section 43 of the Food Safety and Standards Act, 2006, FSSAI has approved several ISO/IEC17025:2017 accredited food testing laboratories. For ensuring domestic food safety from mycotoxins, three national food laboratories [35] (NFL) were established, which are located in Ghaziabad (Uttar Pradesh State) [36], Kolkata (West Bengal State) and Mumbai (Maharashtra State). These are the apex food laboratories of India dealing with quality testing of food under FSS Act 2006. The NFLs in Ghaziabad and Mumbai are operated through public-private partnerships. There are 227 NABL accredited primary food laboratories {accredited by National Accreditation Board for Testing and Calibration Laboratories (NABL)} [37], which are located in Northern, Southern, Eastern and Western zones of the country. Furthermore, 19 Referral Laboratories (RL) [38] have been recognised for analysing the appeal samples, which were previously rejected on account of above-ML residues. In addition, 12 National Reference Laboratories [39] are identified by FSSAI to set country-wide standards for routine analysis, validation of such testing methods, development of new and reliable methods and organising proficiency testing (PT) in the country. These are located at Mysore, Mohali, Kochi, Pune, Anand, Lucknow, Hyderabad, Kolkata and Gurugram. Additionally, two Ancillary laboratories in Chennai and Kolkata have also been identified to assist the National Reference Laboratories for organising PT. Among the FSSAI recognised National Reference Laboratories, ICAR-National Research Centre for Grapes (NRCG), Pune and Trilogy Analytical Laboratory Pvt. Ltd., Hyderabad are particularly assigned for mycotoxin analysis. These laboratories also support the FSSAI in analysing the import samples, in case those are rejected by any primary food laboratory on account of above-ML levels of contamination.

Concurrently, the Scientific Panel of the FSSAI on Methods of Sampling and Analysis makes insightful recommendations to the authority on existing testing methodologies and incorporating new test methods for the analysis of new parameters (commodity-ML com-

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binations). During the fiscal year 2019-20, 21 subject specific scientific panels were operational, which held a total of 67 meetings [40]. To build the capacity of laboratory personnel, the FSSAI conducts specialised training courses on microbiological and chemical aspects of mycotoxin testing [40], which are attended by analysts from FSSAI notified laboratories and state/central food laboratories. In 2019, two such "Training of Trainers" programme on mycotoxin analysis took place at ICAR-NRCG, Pune and Export Inspection Agency, Kochi [40]. The FSSAI has also provided portable lateral flow assay readers to the regulatory laboratories of various states and union territories for easy monitoring of mycotoxins. Under Section 16 (2) (f) of the FSS Act, 2006, FSSAI has published specific manuals on analytical methods on different food commodities with "one parameter-one method" approach. Its first mycotoxin analysis manual was released in 2016, with the primary goal of providing thorough and up-to-date methodologies for regulatory compliance. After several revisions, it came up with the latest version in 2021 in which several validated methods (mostly contributed by NRL, ICAR-NRCG) of AFs, OTA, patulin, etc. were incorporated. This analytical manual summarises the regulatory limits (MLs), safety requirements and 20 well defined analytical methods comprising sample preparation as well as instrumental analyses. By making these information accessible to the public, FSSAI has significantly improved the current mycotoxin testing practices.

4. **Mycotoxin surveillance for export facilitation**

After focusing on the FSSAI's pivotal role in domestic mycotoxin monitoring, this section of our review describes how monitoring authorities are responding to rapid alerts along with the role of APEDA and the NRLs in export facilitation, testing and validation.

It is well documented that the European Commission (EC) and its Member States maintain a high level of safety and ensure quick responses to any threats pertaining to food and feed. One key tool used to rapidly react to such safety crises is – RASFF, as stated previously. When imported goods are found to be non-compliant with the European food legislation, individual such cases are reported through the RASFF and this information is freely accessible for the general public. The EU has defined rules (Commission Implementing Regulation (EC) No.2019/1793) on the frequency of identity checks and physical checks for the consignments of food entering from India. The consignments of rice, husked rice, semi-milled or wholly milled rice are subjected to 10% checks at the port of entry by the EU for AF and OTA as hazards. Peanut and peanut products are subjected to a more rigorous 50% check for AFs. In 2020, two AF related RASFF notifications were reported in rice, 10 notifications for herbs and spices and 19 notifications for peanuts and peanut products. In the following year (2021), one notification was reported in rice with reference to OTA along with 7 notifications for AFs in herbs and spices and 14 notifications of AFs in peanuts and peanut products. In response to an increasing number of RASFF notifications of AF contamination in peanuts imported from India, the EC/Directorate-General for Health and Food Safety has recently conducted certain inspections, which indicate a steady improvement in implementing the mycotoxin management practices at field (pre-harvest) and pack-houses (post-harvest).

It is an established fact that trading agricultural commodities is an established way of revenue generation and economic progress. During 2020-21, India had exported 680000 tonnes of peanuts, and some of its top importers were Indonesia, Vietnam, China, Malaysia, Nepal, Russia, Philippines and Thailand [41]. Like peanuts, other nuts, spices, medicinal herbs and milk are rich substrates for fungal attack, and hence it is imperative that rigorous surveillance be in place to minimise product recalls and food safety concerns upon reaching the importing countries. To accomplish this, the Indian government has introduced the following schemes, platforms and monitoring programmes to oversee export of these food matrices.

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4.1 APEDA-Peanut.Net

Peanut.Net is APEDA's most recent initiative to facilitate transparency in the peanut supply chain as well as to facilitate compliance with MLs of AFs and quality parameters of importing countries. This programme authorises 40 ISO/IEC17025:2017 accredited laboratories for sampling and analysis of export consignments. As of April 2022, 26 laboratories are designated for the EU, Singapore, Malaysia and the Russian Federation, while 14 of them are solely meant for Indonesia [42]. For this purpose, an elaborate manual on export procedures for peanuts has been laid down [43]. Under Peanut.Net, stakeholders can register their peanut processing units or warehouses, shelling units, grading units and apply for a Certificate of Export (COE) from APEDA. AF analysis by approved laboratories, monitoring by NRL, consignment creation, reimbursement of laboratory testing charges for export and the verification of COE are among the other services which can be availed under this platform. Only an ISO/IEC17025:2017-approved laboratory with AFs in its scope that can successfully perform in the PT round of NRL is granted access to Peanut.net.

Importantly, the exporter and export units need to be registered with APEDA. Then, the exporters contact an approved laboratory for AF testing once materials arrive at the pack houses. An NRL-trained, authorised sampling official then visits the unit and collects samples in accordance with the Peanut.net export manual, which conforms to Commission regulation (EC) No. 401/2006. In the sampling training, a trainee receives a detail exposure to the provisions of the EU regulation (Commission regulation (EC) No. 401/2006). The Regulation specifies the weight and number of samples to be collected depending upon the size of the lot and the place of sampling. A lot or sub-lot is only accepted for export if the laboratory sample meets the MLs after adjusting for recovery (%) and measurement uncertainty. On the contrary, a product is rejected if the laboratory sample exceeds the ML beyond a reasonable point, usually, within +50% measurement uncertainty. Once collected, the samples are taken to the laboratory and tested for AFB1, AFB2, AFG1 and AFG2 using an HPLC-Fluorescence or LC-MS/MS instrument. Once a sample complies with ML, a COE and Health certificate are issued to the exporter.

In this way, APEDA encourages accurate labelling practices and ensures that the contamination levels of AFs in the Indian-origin products are within the MLs of the trade-partner countries. Most importantly, it provides e-monitoring via this platform for tracing and tracking consignments while reducing the time-consuming paperwork associated with traceability.

4.2 Role of National Reference/Referral Laboratories (NRL) in testing and method validation

The APEDA has nominated and funded two National Referral Laboratories, namely ICAR-NRCG and the Central Food Technology Research Institute (CFTRI), Mysore, for testing plant and poultry products respectively [44]. These laboratories are responsible for supervising activities that require them to do AF testing within the scope of ISO/IEC 17025:2017 accreditation.

The NRL of ICAR-NRCG suggests approval of the commercial food testing laboratories based on the physical assessment of their method validation records and performance in PT rounds. The candidate laboratories are expected to validate the analytical methods in accordance with Commission regulation (EC) No. 625/2017, with special reference to the performance criteria outlined in the Commission regulation (EC) No. 401/2006 [45] and SANTE/12089/2016 [46]. By ensuring accuracy and precision, these validated methods help to ensure the authenticity of mycotoxin analysis data.

Today, ICAR-NRCG is also a PT provider (PTP) as per the accreditation of ISO/IEC 17043:2010. As an advanced procedure of inter-laboratory comparison (ILC), a PT round allows external quality control and assurance wherein individual laboratories can prove their competency in analysis by participating in PT rounds conducted by a PTP. This enables them to identify systematic gaps in their measurement systems and improve their analytical capacity. Every year, NRL organises at least two PT rounds on AFs in peanuts, the plan of which is notified in the website of ICAR-NRCG well in advance. After the

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completion of a PT, the list of successful laboratories (with z scores within ±2) is communicated to APEDA, who in turn provides them the login access details for participation in Peanut.net. The NRL also checks 5% of the counter samples that are randomly collected from the laboratories as a routine quality assurance measure.

4.3 Mycotoxins' impact on spice commerce, quality improvement and surveillance

As previously stated, the unregulated spread of fungal toxins in agricultural produces is a direct loss of trade opportunity, resulting in poor reputation for the exporting country. As per the World Trade Organisation (WTO), India produces a substantial quantity of its ethnic spices, close to 3 million tonnes per year [47]. In comparison to the quantity generated and despite the immense popularity of pepper, cardamom, dried ginger, turmeric, cumin, fennel and chillies in the USA, the UK, Germany, Japan, Canada and other EU nations, the spice trade is hardly realising its true potential. The EU has established the MLs for AFs in imported spices as already stated. Many times, Indian authorities have been perplexed as to why, despite meeting all official export inspection requirements, consignments are rejected in the EU. In addition to MLs, several parameters, including the number of dead insects in a sample, the presence of foreign objects, mammalian waste and percentage of weight of spoilt items, are also critically inspected. These exported consignments must also periodically be evaluated to check whether they qualify for the Defect Action Level of the USFDA and comply with the cleanliness mandates set by the American Spice Trade Association (ASTA).

Since, a moist food sample attracts fungal infection and induces toxin release, therefore, it is imperative that moisture content of spices and other food commodities be maintained as low as possible so that they may remain unharmed by mycotoxins during long durations of transport. As per the current FSSAI specifications, moisture in black dried pepper, a popular Indian spice, must not exceed 13% by weight. Similarly, the safe moisture level for storing dried ginger is 8 to 10% [48]. For this, drying operation should be carried out in controlled environment on clean surfaces preferably in solar or artificial dryers to avoid contamination with extraneous matter. As far as storage is concerned, grains should be well dried, free from any physical contaminants, packed in fresh gunny bags and should ideally be placed 50 to 60 cm away from the wall to restrict moisture ingress. Adherence to such stringent quality parameters not only ensures the best quality of spices being exported, but also amounts to a good reputation in the international market.

To promote and propagate the sale of high-quality Indian spices in the international markets, the SB was founded by the Union of Cardamom Board and the Spices Export Promotion Council in 1986. Currently, the Board is managed by a network of Quality Evaluation Laboratories (QELs), located in Kochi, Mumbai, Kandla, Kolkata, Guntur, Chennai, New Delhi and Tuticorin. The Board lays down mandatory sampling and testing protocols of export consignments under its *Quality Evaluation System* in which MLs of AFs are listed corresponding to the importing countries. Through its annual trainings at the Kochi Centre, the Board also disseminates information on physical and chemical analysis of spices and spice products. All laboratories at the QELs are well equipped with the instrumentation facilities that are required for AF testing [49]. After this discussion, we portray how Indian testing methods have evolved over the past two decades and what current high-throughput technologies are in use in mycotoxin analysis.

5. Evolution of mycotoxin analysis techniques

It is worth noting that Indian scientists and food analysts have adapted to fast changing testing methodologies. Till 2006, mycotoxin testing in India was primarily carried out through immunochemical approaches such as the enzyme-linked immunoassay (ELISA). However, ELISA based approaches seemed to be time consuming [50] and suffering from a lack of selectivity due to matrix interferences, resulting in the possibilities of false negative and false positive results in tested samples. With technological progress, the EU came up with a regulation (EC 401/2006) to check their domestic and imported food products

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for mycotoxins. In response to that, a number of Indian laboratories also adopted the quality control criteria of EC 401/2006 to comply with the export-related quality-compliance requirements and manage the exports of peanuts and processed peanut commodities. The regulation prescribed chromatographic analysis with fluorescence and/or mass spectrometric detection for selective and accurate testing. Consequently, the laboratories in India, under the purview of APEDA and EIC, had initially implemented the standard AOAC Official methods, wherever those were applicable**.** The NRL of ICAR-NRCG played a decisive role in coming with innovative methods of analysis in Indian food products, which have been adopted by several laboratories, who modified the AOAC methods at their in-house level to suit the matrix-specific requirements, and implemented those for routine analysis after appropriate validation. These methods also gained popularity due to their simple workflows, selectivity, sensitivity, high throughput and cost-effectiveness.

When FSSAI became operational in 2011, the MLs of 7 classes of mycotoxins (commonly found in the Indian foods) were gazette notified. Out of these, except AFs and OTA, the other mycotoxins were not amenable to fluorescence detection. Thus, the use of LC-MS came into practice and has been a reliable tool for multi-class, multi-mycotoxin analysis. To further consolidate the food testing mechanisms, FSSAI signed a Memorandum of Undertaking (MOU) with AOAC INTERNATIONAL in 2018 [51], which made the AOAC Official methods applicable to all regulatory testing and dispute resolution purposes in the country.

In general, FSSAI does not adopt any method in its manuals unless its single laboratory and inter-laboratory validation data are reviewed and accepted by the Scientific Panel. Hence, for any new method, its performance in terms of recovery, intra-lab precision and reproducibility in the test matrices are required to be submitted, which should ideally comply with the EC 401/2006 and SANTE/12089/2016 criteria for analytical quality control. The inter-laboratory validation is expected to include successful performance of at least 7 laboratories. The methods prescribed in the updated manual offers highly sensitive analysis of all regulated mycotoxins with LOQs much lower than the MLs. These developments suggest the remarkable progress that India has made with respect to food testing and analysis on a national scale.

6. Make in India analytical methods

6.1 Fit-for-purpose analytical methods

Now, we discuss some simple, reproducible and cost-effective analytical methods for mycotoxin analysis which have been indigenously standardised to comply with the FSSAI and other regulations. As stated previously, over more than a decade, the NRL of ICAR-NRCG has contributed multiple effective methods for mycotoxin testing in export commodities, e.g. peanuts, cereals, spices and various processed products. The first published method from ICAR-NRCG targeted a direct analysis of AFs (B1, B2, G1 and G2) without involving any post-column derivatisation [52]. This method highlighted the need of developing a slurry of the sample to provide a satisfactory extraction-efficiency and improved precision in comparison to dry-homogenisation. In brief, peanuts were homogenised after adding equal amount of water. The method workflow involved subjecting the slurry to methanol-water (80:20) extraction, immunoaffinity column (IAC) cleanup through AF-specific cartridges and final analysis by ultra-HPLC with fluorescence detection (FLD). The use of a large volume flow cell (13 µL) could avoid the requirement of any post-column derivatisation, which is otherwise practised in conventional methods. Once this method was standardised and validated in peanuts, its performance was successfully evaluated in peanut-processed products and selected cereals (millets, rice and corn). Subsequently, the scope of the HPLC analysis was extended to include OTA also in the same method [53]. Here, a wavelength switching step was included in which, the excitation wavelength was changed from 365 to 333 nm after the elution of all AFs. This allowed high-sensitivity estimation of all AFs and OTA in a single HPLC-FLD run with selectivity established through compound-specific excitation-emission wavelength combinations. It may be noted here that while using a regular FLD flow cell, the estimation of AFB1 and

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AFG1 required an additional step of derivatisation to compensate their limited fluorescent properties and provide the desired method LOQs. Derivatisation could be carried out by either electrochemical reactor (Kobra Cell) or a "Photochemical Reactor for Enhanced Detection" (PHRED).

Contamination of spices with AFs could be a potential hazard for health of consumers in India [54]. AFs were found in five spices {turmeric, black pepper, garam masala (a blend of ground spices), chilli and tandoori masala} in a research on marketed spice samples in Doha, and except for garam masala, the other four varieties surpassed the MLs for AFB1 and/or total AF. The presence of fungal propagules was the highest in chilli powder [55]. For this, NRL-NRCG has been doing its best to develop analytical methods to detect mycotoxins in spices. ICAR-NRCG has published a multi-mycotoxin analytical technique that enables the simultaneous assessment of all 4 AFs and OTA in chilli powder with excellent sensitivity, accuracy and precision [56]. The sample preparation was optimised by extraction of powdered sample (25 g) with methanol-water (100 mL, 80:20). An aliquot (3 mL) was cleaned on an IAC and analysed in a single chromatographic run utilising UHPLC-FLD and tandem mass spectrometric (LC-MS/MS) detection. The method's performance was assessed using intra- and inter-laboratory validation (ILV) investigations, as well as an examination of a certified reference material. These indigenously developed, authentic, labour-saving advancements are helping food analysts and laboratory personnel, across the country to rapidly deliver accurate results.

A validated method has also been reported for the LC-MS/MS analysis of patulin in apple and apple juice [26]. The scientists at NRL-NRCG have also validated an LC-MS/MS method for the simultaneous analysis of AFs, OTA, ZEA, T2 and HT2 in medicinal herbs [57] and also their separate analysis in specified commodities, e.g. cereals. Nevertheless, information on marketed grounded spice mixes, for example, garam masala (comprising cardamom, cinnamon, cloves, and black pepper and others), pav bhaji masala (mainly made of roasted red chillies, coriander, cloves, cumin, fennel, cinnamon and cardamom) and sambar masala (consisting of coriander, cumin, mustard, black pepper, red chilies, fenugreek seeds, among many others), just to name some, are yet unavailable, which need further attention.

Herbs, like spices, have been used in food preparation for ages to create a distinct flavour in many countries. Dried herbs can be a major source of microbiological risks, especially because they are frequently added to recipes with little processing before consumption [58]. As a result, Indian dry herbs such as kasoori methi (dried fenugreek leaves) and curry leaf powder, among others, require attention because we do not yet have a method for testing mycotoxins in them.

6.2 Automated AF testing in rice and peanuts

The food testing laboratories in India sometimes receive a large number (50+) of samples a day. This poses a big challenge to the labs who find it difficult to manage the analysis of all samples within the expected time of 1-2 days. In mycotoxins analysis, the major bottleneck is immunoaffinity (IAC) column cleanup, which takes a substantial period of time and also involves a dedicated human resource for operation and supervision. Dhanshetty, Thorat & Banerjee optimised, developed and validated a semi-automatic method for aflatoxins analysis in rice, peanuts, sorghum and their processed products, in which, the extraction step was only manually performed [59]. The IAC cleanup and HPLC analysis were performed automatically. The method utilised a special kind of IAC cartridge, which could be reused for 15 times without a loss in recovery. Automation in the cleanup and HPLC analysis steps provided a higher precision (RSD=10%) than the traditionally practiced manual cleanup (RSD=12-15%) and hence find wide acceptance in future for high-throughput mycotoxins testing in food commodities.

6.3 Analytical methods developed on medicinal plants and botanicals

India's long association with traditional medicine, in particular Ayurveda, is widely acknowledged [60]. The overall market demand for raw herbal pharmaceuticals for the

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fiscal year 2014-15 was projected to be 5,12,000 MT [61]. Unfortunately, plant products are frequently contaminated with naturally occurring toxins, which puts the aged population at risk because they consume a lot of health boosting supplements manufactured from such medicinal herbs [62]. Previously, Roy et al. (1988) reported 14 out of 15 Indian drug plants to be positive for AFB1 at levels 0.1 to 1.2 μg/kg [63]. There have been recent incidences of Indian medicinal herbs being contaminated with emerging mycotoxins-beauvericin (BEA) and enniatins (ENs) [64] and these toxins currently have no risk assessment based MLs, which is extremely concerning. Although such plant derived health supplements are much less affected by mycotoxins than the grains and nuts, they are an integral part of the Asian culture as a rich source of phenolic compounds which possess antioxidant, anticancer and anti-inflammatory properties [64]. For this, their testing for mycotoxin contamination assumes high significance.

The consumption of medicinal plant species, namely *tulsi (Ocimum sanctum), ashwagandha (Withania somnifera), safed musli (Chlorophytum borivilianum), satavari (Asparagus racemosus) and giloy (Tinospora cordifolia),* for healing purposes has been a customary practice in the country. But there was no method available to test mycotoxins in these matrices. To address the gap, an important breakthrough in multi-mycotoxin analysis in herbs was achieved by developing a method for detecting 9 regulated mycotoxins in these five prominent Indian medicinal plants [56]. This simple, robust and precise method of analysis employed FLD and MS based determination techniques and proved to be apt for regulatory testing purposes because of a wise-spectrum application.

Regardless, there are hundreds of botanical products and dried fruits which still have not received enough attention from food chemists. These include ginseng, ginger, liquorice, kava-kava, herbal tea, dried figs, raisins, sultanas, currants, prunes, dates and apricots to name a few. This calls for a meticulous understanding and systematic study to detect and quantify mycotoxins in these lesser studied matrices.

6.4 Methods developed for AFM1 detection in milk

Apart from nuts, spices and natural products, milk is especially susceptible to AF M1 contamination, which is a carcinogenic hydroxylated metabolite, relatively stable to detoxification attempts such as sterilisation, pasteurisation and boiling [65].

Within a few hours of ingesting the contaminated feed, close to 0.3 to 6.2% of AFB1 in the feed is metabolized to AFM1 in milk [66]. Furthermore, the EU guidelines direct that barring AFM1, none of the other AFs are found in milk. The IARC of WHO classifies AFM1 as a Group I human carcinogen [66]. EU mandates that AFM1's concentration in milk must not surpass 0.05 μg/kg (0.05 ppb) and 0.025 μg/kg (0.025 ppb) for infant milk-based products [31]. The United States regulated this limit to 0.5 μg/kg (0.5 ppb) for milk [67]

In India, AFM1 is mainly analysed by the FSSAI methods FSSAI 07.013:2020 and FSSAI 07.014:2020, which are based on the AOAC Official Methods 986.16 and 2000.08, respectively [19]. In both cases, AFM1 is estimated by HPLC-FLD. More recently, a quick method [68] of detecting AFMI in milk based on atmospheric pressure-matrix assisted laser desorption/ionisation (AP-MALDI) has been reported, in which AFM1 was determined by selected reaction monitoring (SRM). Since feed is the main source of AFB1, which subsequently gets converted to M1 in the animal body, it is also necessary to monitor AFB1 level in feed. A recent paper from NRL of ICAR-NRCG has demonstrated how AFs can be estimated in a wide range of animal feeds by UHPLC-FLD without a step of derivatisation [69]. The monitoring of AFs in feed is important because feed is the primary source of AFs to the animals. Such economically feasible and time-saving analysis techniques have revolutionised existing laboratory capacities and are producing accurate results within limited timespan.

6.4 Mycotoxin detoxification through food processing

Since, AFs are heat-resistant, they can withstand exposure to cooking temperatures and microwave treatment. In order to considerably lessen toxin loads in peanuts in a

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household setting, Dhanshetty et al. evaluated the effect of 3 robust Indian cooking methods on AFB1 content [21]. For this, certain positive samples of peanut were subjected to frying, roasting and pressure cooking. Following a pattern, the processing techniques lowered the B1 content to varying degrees: roasting with a solution of sodium chloride and citric acid; pressure cooking with a solution of sodium chloride and citric acid; and frying. In order to decontaminate or reduce the level of B1 for a safer consumption of peanuts at the household level without altering the organoleptic qualities, the cooking techniques did not require any difficult steps or specialised equipment, so these could be easily adopted.

In addition to detection and quantitation, mycotoxin reduction strategies are being looked into for quite some time now. Since, bread and bakery items are nutritionally rich with high amounts of carbs, proteins and vitamins B and E [70], they are a popular staple meal enjoyed all over the world. It has been discovered that conditions like time and temperature of baking and toasting significantly affect the levels of mycotoxins in bakery products, whereas, fermentation has not shown any such results [71].

7. Concerns in mycotoxin control

As already stated, AF production is heavily influenced by moisture and oxygen content in the environment. Typically, AF B1 and B2 are produced at 11-37 °C, whereas AF G1 and G2 production usually occurs at 28 °C [72]. It is well known that toxic fungal metabolites are ubiquitous in nature and heat stable [73]. As a result, most thermal processing techniques that work at temperatures ranging from 80 to 121°C are ineffective against these heat-resistant toxins. For example, AFs show thermal stability even at temperatures ranging 237 to 306°C [74]. Although traditional home-based cooking methods of India such as roasting, pressure cooking and frying have shown considerable promise in reducing AF load, a complete elimination or reduction to below ML are the matters of concern when the AF load is high [21].

8. Implementation challenges

Apart from limitations in controlling management, regulation of mycotoxin also faces other discerning challenges such as variation in MLs across the trade-partner (exporting and importing) countries, lack of disposal protocol for non-compliant lots, dearth of protocol for preventing non-compliant lots from proliferating in the retail market, want of methods for analysing processed commodities, inhomogeneity of toxins within commodities and finally sampling non-uniformity and errors, which are discussed in brief.

The MLs put forward by national and international agencies depend, to a large extent, upon the generic lifestyle of a population and its demography. A particular population might be more susceptible and less tolerant to fungal food poisoning than another, giving rise to diverse perceptions of tolerable health risks around the world [33], and MLs of individual countries vary accordingly. For instance, ML for patulin in apple juices in India is 50 μg/kg while in the EU, it is 10 μg/kg [30, 31]. For such discrepancies, when the EU enforced new mycotoxin standards in the early 1980s, India's exports of peanut meal to the EU declined by more than $30 million per year [33]. Such disparities in food laws between advanced and emergent markets lead to loss in economy and trade opportunities for the latter.

The disposal of border rejections poses a matter of concern because there is very little structured surveillance with respect to what should be done with them. In the absence of vigilance, these consignments are often released into the domestic markets at a competitive price, thereby creating public health alarm. In multiple occasions, it has been often observed that items that have passed quality control checks before export, fail to comply with MLs in the destination country. This leads to several rapid alerts, consignment failures, product recalls and huge economic losses. Such incidents of non-compliance bring into the picture inter-laboratory variations, heterogeneity of toxins, variation during sampling, in-transit storage conditions and analytical differences.

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Despite significant efforts to reduce fungal toxin contamination of food and feed products, Indian peanut consignments to the EU nations are frequently subjected to border rejections due to AF levels above the EU MLs. This alarming situation has led authorities to investigate RASFF notifications for the presence of mycotoxins in Indian-origin food and feed products shipped to these countries for the fiscal year 2020-2021.

That being said, India is divided into numerous agro-climatic regions, characterised by unique indigenous foods which play a pivotal role in the cultural ethnicity of its natives. Most of the time, such locally manufactured food items vary in composition. This makes testing and analysis all the more complex because of non-uniformity of matrices. Moreover, most testing protocols provided in the Indian guidelines have been developed on raw agricultural commodities (RAC). There is scarcely any validated methods for several locally processed street foods, for instance, *bhel* (savoury from puffed rice served with sauces, dried leaves and oils) and *daal badis* (pulse fritters). Aflatoxins of multiple kinds have been found in a variety of street foods, the most frequent of which being AF B, G, and M [77]. The concerning aspect about this is that a large segment of the middle-class Indian population consumes these food products due to convenience, taste and affordability. To address this, FSSAI's Eat Right India movement is spearheading the Clean Street Food Hub Initiative to educate vendors on hygienic practices [78]. Nevertheless, global regulatory bodies such as Codex and the EU also do not have provisions for sampling and analysis for such locally manufactured foods. Even if methods were to be developed, this would require huge data generation and survey. The dearth of pertinent toxicological data is also a significant hindrance to the appropriate testing of mycotoxins in food and feed. With a hot and humid subtropical monsoon climate, which is conducive to the propagation of AF or other mycotoxins, milk products such as indigenous cheese need to be tested. In addition, Chinese and Korean sauces (e.g. soy sauce) are becoming popular in India, which were earlier reported to have been infected with AFs [79]. So, all these commodities need to be tested since these are teeming with moisture and nutrients, ideal for mould attack.

Finally, scientists across the globe agree that sampling is the most crucial step for accurate mycotoxin analysis as it is characterised by large variation resulting in false negatives and false positives [80]. The high incidents of sampling errors may be attributed to very low concentrations present in food grains and heterogeneous distribution in the grain/nut lot. Since fungi does not develop on each and every kernel in a grain/nut lot, not all kernels show visual infection. In turn, some food articles are contaminated although appearing visually healthy. The heterogeneous and ubiquitous nature of mycotoxins makes monitoring and regulatory activities of utmost importance to safeguard public health and retain healthy trade relations in the foreign market.

8. Conclusion

This review has outlined how nodal Indian governmental agencies are assisting researchers and regulators to progress towards a mycotoxin-free food supply chain and highlights crucial hurdles they are facing to achieve that goal along with areas of improvement in the existing monitoring system. Despite the geographical conditions of India being supportive of fungal growth, the country has come a long way in setting up a robust export inspection regime with a strong complaint handling system and that has been appropriately covered in this review. Over the years, the government has introduced traceability networks (Peanut.Net) and organisations (FSSAI, SB, and EIC), who are constantly supervising mycotoxin intervention programs. Indian testing methods are ever-evolving as per the requirements of the international market; and much has been achieved in this regard, but more harmonisation of trade activities between regulators such as APEDA and EIC are demanded. The standardisation of testing and analytical methods is the first step towards freeing our food chain from the deleterious effects of these naturally occurring toxins. Indeed, a lot has been already been achieved in this front. However, food matrices such as mix grounded spices, dry herbs, and other street foods should be brought to mainstream study and more scientific attention should be devoted to them. The future

holds enormous opportunities to modernise and automate mycotoxin testing procedures similar to the ones already developed in case of spices. The next few years shall be crucial as scientists observe how weather patterns give rise to novel fungal genotypes with elevated resistance to decontamination. As far as the administration is concerned, the governmental agencies need to follow a systematic approach to the disposal mechanism of border rejected consignments. Trading countries need to work out amiable ways to strike a balance in prioritising between health benefits and economic costs; only then can we achieve economic progress and ameliorate the health status of Indian citizens and the world. We recommend adopting robust agricultural practices to arrest mycotoxin spread on farms rather than invest in detoxification attempts. It will be interesting to see how climate change affects global agricultural practices in the future, and how India will play a key role in mycotoxin control and crop loss reduction.

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