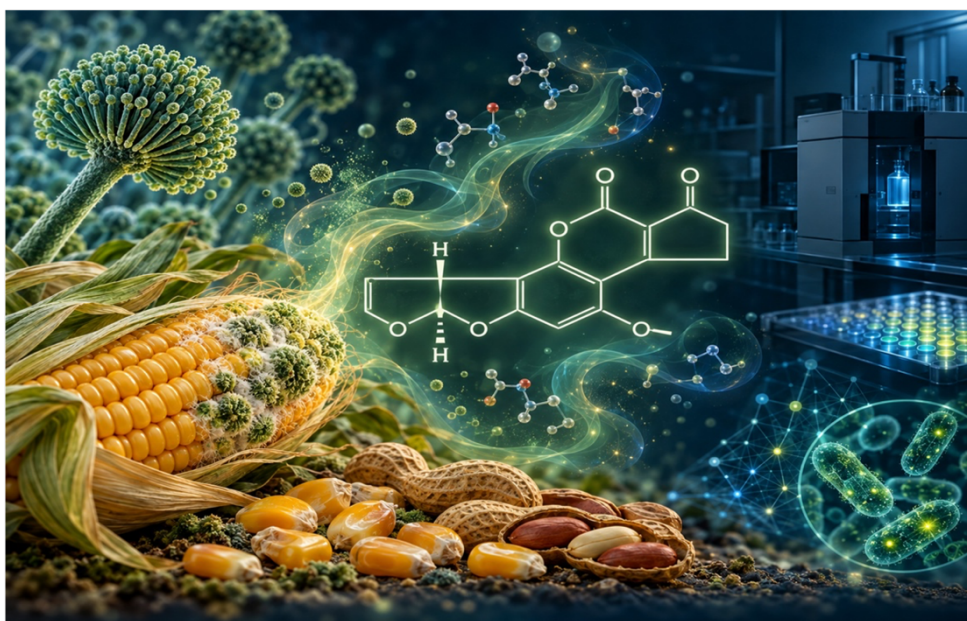


Early Warning and Risk Prediction of *Aspergillus flavus* Contamination and Aflatoxin B₁ Production in Maize and Peanut Kernels Using Volatile and Whole- Cell Biosensors



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Abstract

Mycotoxin contamination remains a major challenge for food and feed safety, particularly in postharvest systems where fungal growth may occur before visible spoilage or measurable toxin accumulation becomes evident. Among toxigenic fungi, *Aspergillus flavus* is of particular concern because of its ability to infect important agricultural commodities and produce aflatoxin B₁ (AFB₁), one of the most toxic and strictly regulated mycotoxins. This thesis aimed to develop early-warning and risk-monitoring strategies for *A. flavus* contamination by focusing on volatile organic compounds (VOCs), whole-cell biosensing, and regression-based prediction.

First, the thesis reviewed the occurrence and control of major mycotoxins in Asian food and feed systems, highlighting the need for preventive monitoring rather than late-stage intervention. It then examined VOC-based detection as a promising non-destructive approach for identifying postharvest deterioration. Building on this framework, experimental work was conducted to characterize early volatile changes in maize kernels infected with *A. flavus*. By integrating electronic nose analysis, gas chromatography–ion mobility spectrometry, gas chromatography–mass spectrometry, and exploratory GC×GC–MS screening, distinct VOC profile changes were observed during the asymptomatic stage of infection. These changes allowed infected maize samples to be differentiated from controls before visible mold development, indicating that early fungal colonization is accompanied by detectable metabolic disturbance in the headspace environment.

The second experimental part extended this early-warning concept toward toxin-related risk estimation. A transcriptome-guided whole-cell biosensor array was constructed by selecting promoter elements from *Escherichia coli* genes responsive to VOCs emitted by *A. flavus*-infected maize and peanut kernels. When combined with machine-learning regression models, especially XGBoost and CatBoost, the biosensor responses enabled accurate prediction of infection stages and AFB₁ accumulation in both commodities. The use of transcriptome-informed promoter selection improved not only predictive performance but also biological interpretability, linking biosensor outputs to cellular responses induced by mold-associated volatile environments.

Overall, this thesis demonstrates that VOC changes can provide early-warning information during asymptomatic *A. flavus* infection, while transcriptome-guided biosensors coupled with regression models can further translate these biological signals into quantitative estimates of infection progression and AFB₁-related risk. The proposed platform is better positioned as a front-line screening and risk-triage tool for repeated, low-cost, and minimally invasive monitoring in postharvest systems. Further validation under complex storage conditions, mixed fungal backgrounds, and broader commodity systems will be necessary before practical deployment. Nevertheless, this work provides a biologically informed approach for moving food safety monitoring from endpoint detection toward earlier, risk-oriented intervention.

Keywords: *Aspergillus flavus*; aflatoxin B₁; volatile organic compounds; maize; peanut; electronic nose; GC-IMS; GC-MS; whole-cell biosensor; machine learning.

Résumé

La contamination par les mycotoxines demeure un défi majeur pour la sécurité des denrées alimentaires et des aliments pour animaux, en particulier dans les systèmes post-récolte, où la croissance fongique peut survenir avant que l'altération visible ou l'accumulation mesurable de toxines ne devienne évidente. Parmi les champignons toxigènes, *Aspergillus flavus* suscite une préoccupation particulière en raison de sa capacité à infecter d'importantes denrées agricoles et à produire l'aflatoxine B₁ (AFB₁), l'une des mycotoxines les plus toxiques et les plus strictement réglementées. Cette thèse visait à développer des stratégies d'alerte précoce et de surveillance du risque lié à la contamination par *A. flavus*, en s'appuyant sur les composés organiques volatils (COV), les biocapteurs à cellules entières et la prédiction fondée sur des modèles de régression.

Dans un premier temps, cette thèse a examiné l'occurrence et le contrôle des principales mycotoxines dans les systèmes alimentaires et les aliments pour animaux en Asie, en soulignant la nécessité d'une surveillance préventive plutôt que d'une intervention à un stade avancé. Elle a ensuite étudié la détection fondée sur les COV comme une approche non destructive prometteuse pour identifier la détérioration post-récolte. Sur cette base, des travaux expérimentaux ont été menés afin de caractériser les changements volatils précoces dans des grains de maïs infectés par *A. flavus*. En intégrant l'analyse par nez électronique, la chromatographie en phase gazeuse couplée à la spectrométrie de mobilité ionique, la chromatographie en phase gazeuse couplée à la spectrométrie de masse et un criblage exploratoire par GC×GC-MS, des modifications distinctes des profils de COV ont été observées au cours du stade asymptomatique de l'infection. Ces changements ont permis de différencier les échantillons de maïs infectés des témoins avant le développement visible de moisissures, indiquant que la colonisation fongique précoce s'accompagne d'une perturbation métabolique détectable dans l'environnement de l'espace de tête.

La deuxième partie expérimentale a étendu ce concept d'alerte précoce à l'estimation du risque lié aux toxines. Un réseau de biocapteurs à cellules entières guidé par le transcriptome a été construit en sélectionnant des éléments promoteurs issus de gènes d'*Escherichia coli* sensibles aux COV émis par des grains de maïs et d'arachide infectés par *A. flavus*. Lorsqu'elles ont été combinées à des modèles de régression fondés sur l'apprentissage automatique, en particulier XGBoost et CatBoost, les réponses des biocapteurs ont permis une prédiction précise des stades d'infection et de l'accumulation d'AFB₁ dans les deux matrices. L'utilisation d'une sélection de promoteurs informée par le transcriptome a amélioré non seulement la performance prédictive, mais aussi l'interprétabilité biologique, en reliant les sorties des

biocapteurs aux réponses cellulaires induites par des environnements volatils associés aux moisissures.

Dans l'ensemble, cette thèse démontre que les changements de COV peuvent fournir des informations d'alerte précoce durant l'infection asymptomatique par *A. flavus*, tandis que les biocapteurs guidés par le transcriptome, couplés à des modèles de régression, peuvent traduire davantage ces signaux biologiques en estimations quantitatives de la progression de l'infection et du risque lié à l'AFB₁. La plateforme proposée se positionne davantage comme un outil de dépistage de première ligne et de triage du risque, adapté à une surveillance répétée, peu coûteuse et minimalement invasive dans les systèmes post-récolte. Une validation supplémentaire dans des conditions de stockage complexes, en présence de communautés fongiques mixtes et dans un éventail plus large de matrices sera nécessaire avant toute application pratique. Néanmoins, ce travail fournit une approche biologiquement informée permettant de faire évoluer la surveillance de la sécurité alimentaire d'une détection en point final vers une intervention plus précoce et orientée vers le risque.

Mots-clés : *Aspergillus flavus* ; aflatoxine B₁ ; composés organiques volatils ; maïs ; arachide ; nez électronique ; GC-IMS ; GC-MS ; biocapteur à cellules entières ; apprentissage automatique.

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List of Acronyms

1. ***A. flavus*** — *Aspergillus flavus*
2. **AFs** — Aflatoxins
3. **AFB₁** — Aflatoxin B1
4. **AFB₂** — Aflatoxin B2
5. **AFG₁** — Aflatoxin G1
6. **AFG₂** — Aflatoxin G2
7. **AFM₁** — Aflatoxin M1
8. **AI** — Artificial intelligence
9. **ANOVA** — Analysis of variance
10. **aw** — Water activity
11. **CatBoost** — Categorical boosting
12. **CFDA** — China Food and Drug Administration
13. **CK** — Control group
14. **CMOS** — Complementary metal–oxide–semiconductor
15. **DAHP** — 3-Deoxy-D-arabino-heptulosonate 7-phosphate
16. **DEGs** — Differentially expressed genes
17. **DMAPP** — Dimethylallyl diphosphate
18. **DNA** — Deoxyribonucleic acid
19. **DON** — Deoxynivalenol
20. **dpi** — Days post-inoculation
21. **DVB/CAR/PDMS** — Divinylbenzene/carboxen/polydimethylsiloxane
22. **EC** — European Commission
23. **EDTA** — Ethylenediaminetetraacetic acid
24. **EI** — Electron ionization

25. **ELISA** — Enzyme-linked immunosorbent assay
26. **E-nose** — Electronic nose
27. **EOs** — Essential oils
28. **EU** — European Union
29. **E4P** — Erythrose-4-phosphate
30. **FAO** — Food and Agriculture Organization
31. **FDA** — Food and Drug Administration
32. **FB₁** — Fumonisin B1
33. **FB₂** — Fumonisin B2
34. **FB₃** — Fumonisin B3
35. **FT-ICR** — Fourier transform ion cyclotron resonance
36. **FTIR** — Fourier transform infrared spectroscopy
37. **GC** — Gas chromatography
38. **GC-AED** — Gas chromatography–atomic emission detection
39. **GC-ECD** — Gas chromatography–electron capture detector
40. **GC-IMS** — Gas chromatography–ion mobility spectrometry
41. **GC-MS** — Gas chromatography–mass spectrometry
42. **GGPP** — Geranylgeranyl diphosphate
43. **GFPP** — Geranylgeranyl diphosphate
44. **GPP** — Geranyl diphosphate
45. **HCA** — Hierarchical cluster analysis
46. **HS** — Headspace
47. **HS-GC-IMS** — Headspace gas chromatography–ion mobility spectrometry
48. **HS-SPME** — Headspace solid-phase microextraction
49. **IMS** — Ion mobility spectrometry

- 50. **IoT** — Internet of Things
- 51. **IPP** — Isopentenyl diphosphate
- 52. **IR** — Infrared spectroscopy
- 53. **KFDA** — Korea Food and Drug Administration
- 54. **KNN** — k-nearest neighbors
- 55. **LB** — Luria–Bertani medium
- 56. **LD** — Ludan 513
- 57. **LDA** — Linear discriminant analysis
- 58. **LIF** — Laser-induced fluorescence
- 59. **LOD** — Limit of detection
- 60. **LOQ** — Limit of quantification
- 61. **MAE** — Mean absolute error
- 62. **MEP** — Methylerythritol phosphate pathway
- 63. **MIMS** — Membrane introduction mass spectrometry
- 64. **ML** — Machine learning
- 65. **MOS** — Metal oxide semiconductor
- 66. **mRNA** — Messenger RNA
- 67. **MVA** — Mevalonate pathway
- 68. **m/z** — Mass-to-charge ratio
- 69. **NIR** — Near-infrared spectroscopy
- 70. **NIST** — National Institute of Standards and Technology
- 71. **NN** — Neural network
- 72. **OPLS-DA** — Orthogonal partial least squares-discriminant analysis
- 73. **OTA** — Ochratoxin A
- 74. **PCA** — Principal component analysis

- 75. **PCA-DA** — Principal component analysis-discriminant analysis
- 76. **PCR** — Polymerase chain reaction
- 77. **PDA** — Potato dextrose agar
- 78. **PEP** — Phosphoenolpyruvate
- 79. **PHL** — Postharvest losses
- 80. **PID** — Photoionization detection
- 81. **PLS** — Partial least squares
- 82. **PLS-DA** — Partial least squares-discriminant analysis
- 83. **PLSR** — Partial least squares regression
- 84. **PTR-MS** — Proton transfer reaction–mass spectrometry
- 85. **RASFF** — Rapid Alert System for Food and Feed
- 86. **RF** — Random forest
- 87. **RI** — Retention index
- 88. **RMSE** — Root mean square error
- 89. **RNA** — Ribonucleic acid
- 90. **RNA-seq** — RNA sequencing
- 91. **ROC** — Receiver operating characteristic
- 92. **RSD** — Relative standard deviation
- 93. **RT** — Retention time
- 94. **SGB** — Stochastic gradient boosting
- 95. **SIFT-MS** — Selected-ion flow-tube mass spectrometry
- 96. **SPLS-DA** — Sparse partial least squares-discriminant analysis
- 97. **SPME** — Solid-phase microextraction
- 98. **SVM** — Support vector machine
- 99. **THz** — Terahertz

- 100. **TOF** — Time of flight
- 101. **UHT** — Ultra-high temperature
- 102. **UV-Vis** — Ultraviolet-visible spectroscopy
- 103. **VIP** — Variable importance in projection
- 104. **VOC** — Volatile organic compound
- 105. **VOCs** — Volatile organic compounds
- 106. **WCBA** — Whole-cell biosensor array
- 107. **WHO** — World Health Organization
- 108. **WY** — Wuyue 78
- 109. **XGBoost** — Extreme gradient boosting
- 110. **ZD** — Zhengdan 958
- 111. **ZEN** — Zearalenone

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Chapter 1

General Introduction, Objectives, Rationale and Research Hypotheses

1.1. Overview of Postharvest Fungal Contamination and Mycotoxin Risks

Cereals, oilseeds, and their derived products are susceptible to contamination by mycotoxigenic fungi during harvesting, transportation, and storage. Fungal colonization, growth, and mycotoxin production are influenced by multiple factors, including temperature, water activity, storage duration, kernel damage, and storage management practices (Mongkon et al., 2017). Such contamination can impair the sensory and nutritional quality of agricultural commodities, reduce their market value, and pose serious risks to food safety, animal health, and international trade.

The major genera of mycotoxigenic fungi include *Aspergillus*, *Penicillium*, and *Fusarium*, which are responsible for the production of several important mycotoxins, including aflatoxins, ochratoxin A, zearalenone, deoxynivalenol, and fumonisins (Pouris et al., 2024; Glenn et al., 2007;). Aflatoxins are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* and commonly occur in maize, peanuts, tree nuts, spices, and dried fruits. Ochratoxin A is produced by several *Aspergillus* and *Penicillium* species and frequently contaminates cereals, coffee, dried fruits, grapes, wine, and spices. Zearalenone and deoxynivalenol are produced primarily by *Fusarium* species and are commonly detected in maize, wheat, barley, and oats. Fumonisins, produced predominantly by *Fusarium verticillioides* and *Fusarium proliferatum*, are particularly associated with maize and maize-based products.

Among these fungi, *A. flavus* is particularly relevant to the present thesis because it is an important contaminant of maize, peanuts, and other susceptible commodities (Eskola et al., 2020). Certain strains of *A. flavus* can produce aflatoxins, among which aflatoxin B₁ (AFB₁) is of greatest concern because of its potent hepatotoxic, mutagenic, and carcinogenic effects. Owing to its high toxicity and chemical stability, strict maximum levels have been established for AFB₁ and total aflatoxins in food and feed.

However, *A. flavus* infection and AFB₁ production do not necessarily occur synchronously. The capacity and extent of toxin production depend on multiple factors, including fungal strain, commodity matrix, infection stage, and storage environment. Consequently, the detection of *A. flavus* does not necessarily indicate a high level of AFB₁ contamination, while a low toxin concentration at a given time does not exclude the possibility of subsequent accumulation. Early detection of fungal infection and quantitative determination of AFB₁ should therefore be regarded as two related but distinct monitoring objectives. This distinction provides the rationale for combining early-warning approaches for fungal activity with methods for estimating toxin-related risk.

1.2. Structural Overview of AFB₁

The major mycotoxins discussed in this thesis belong to several structurally distinct chemical classes. Aflatoxins are difuranocoumarin derivatives consisting of a

coumarin nucleus fused to a bifuran system. AFB₁, the principal mycotoxin investigated experimentally in this thesis, contains an 8,9-double bond in its terminal furan ring. Cytochrome P450-mediated epoxidation of this double bond generates a highly reactive metabolite that plays a central role in AFB₁ genotoxicity (Caceres et al, 2020).

1.3. Limitations of Conventional Monitoring and the Potential of VOC-Based Early Warning

Conventional monitoring of fungal contamination relies primarily on visual inspection, culture-based methods, molecular identification, and instrumental or immunological analysis of mycotoxins. Although these approaches are essential for contamination confirmation and regulatory control, they generally require sample collection, preparation, and laboratory analysis and therefore provide only a snapshot of contamination at the time of measurement. By the time visible fungal growth or substantial AFB₁ accumulation is detected, fungal colonization may already be well established, leaving limited opportunities for preventive intervention.

Volatile organic compounds (VOCs) offer a promising basis for earlier detection. During *A. flavus* infection, fungal metabolism, host stress responses, lipid oxidation, and host–pathogen interactions can alter the headspace VOC profile before visible mold development. Because headspace sampling causes minimal disturbance to the commodity, it is suitable for rapid and repeated monitoring. Infection-associated VOCs—including alcohols, aldehydes, ketones, esters, furans, sulfur-containing compounds, aromatic compounds, and terpenoids—may therefore serve as early indicators of altered biological activity and support timely decisions.

1.4. Research Questions

The overarching research question of this thesis is whether and how volatile signals associated with *A. flavus* infection can be integrated into a staged monitoring framework for the early detection of fungal contamination and the subsequent estimation of infection progression and AFB₁-related risk. This overarching question was addressed through the following specific research questions:

RQ1. Does *A. flavus* infection induce detectable, reproducible, and time-dependent changes in the headspace VOC profile of maize kernels before visible mold development?

RQ2. Can E-nose, GC-IMS, GC-MS, and GC×GC-MS provide complementary evidence for distinguishing infected kernels from non-infected controls and identifying candidate early-warning VOCs?

RQ3. Can the complex volatile environment generated by *A. flavus*-infected maize and peanut kernels induce systematic and informative transcriptional responses in immobilized *E.coli*, and can promoters selected from these responses be used to construct a bioluminescent whole-cell biosensor array?

RQ4. Can the multivariate responses of the whole-cell biosensor array be translated through machine-learning models into reliable estimates of infection stage and AFB₁ accumulation in maize and peanut kernels, and how robust are these predictions under internal and external validation?

1.5. Research Hypotheses

Hypothesis 1: Early infection alters VOC profiles before visible symptoms develop

It was hypothesized that the colonization of maize kernels by *A. flavus* during the first few days after inoculation would induce time-dependent changes in the headspace VOC profile before visible mold development, with some signals remaining informative across the tested maize cultivars.

Hypothesis 2: Different analytical platforms provide complementary evidence

E-nose, GC-IMS, GC-MS, and GC×GC-MS were expected to capture different but complementary aspects of the infection-associated volatile response. The integration of pattern-level and compound-level information was expected to provide broader chemical coverage and increase confidence in the identification of candidate early-warning signals compared with the use of a single analytical platform.

Hypothesis 3: The mold-associated volatile environment induces informative cellular responses

The volatile environment generated by *A. flavus*-infected maize and peanut kernels was expected to induce systematic transcriptional responses in immobilized *Escherichia coli*. Promoters selected on the basis of these responses were expected to generate measurable bioluminescent signals and form a biologically relevant whole-cell biosensor array.

Hypothesis 4: Biosensor response patterns can predict infection stage and AFB₁ accumulation

The combined signals generated by the transcriptome-guided reporter array were expected to contain sufficient information for selected regression models to estimate infection stage and AFB₁ concentration in the tested maize and peanut systems. Tree-based ensemble algorithms were expected to effectively model the nonlinear relationships between reporter responses and contamination progression, while external validation was expected to reveal the limitations of model generalization across fungal strains, commodities, and experimental conditions.

1.6. Research Rationale and Objectives

The central premise of this thesis is that postharvest contamination monitoring should not rely solely on endpoint indicators, such as visible mold growth or final toxin concentrations, but should also target early biological changes occurring before obvious quality deterioration and substantial AFB₁ accumulation. Early alterations in

VOC profiles may provide warning of emerging fungal activity, while biologically responsive whole-cell biosensor arrays may further integrate information from complex volatile environments. Combining these signals with machine-learning approaches could enable quantitative estimation of contamination progression and AFB₁-related risk.

Accordingly, the overall objective of this thesis was to develop and evaluate an early-warning and risk-monitoring framework for *A. flavus* contamination in postharvest commodities. Specifically, the thesis investigated whether infection-associated VOC changes could reveal fungal colonization during the asymptomatic stage and whether whole-cell biosensor responses could be translated through regression models into estimates of infection progression and AFB₁ accumulation.

The specific objectives were:

To review the occurrence and control of major mycotoxins in Asian food and feed systems and establish the broader food-safety context for postharvest mycotoxin monitoring;

To evaluate current advances, analytical technologies, practical applications, and limitations of VOC-based monitoring of postharvest deterioration;

To identify characteristic VOC fingerprints associated with asymptomatic *A. flavus* infection in maize using complementary analytical platforms;

To identify *E. coli* genes responsive to the volatile environment generated by *A. flavus*-infected maize and peanut kernels through transcriptomic analysis and to select the corresponding promoters for biosensor development;

To construct a bioluminescent whole-cell biosensor array and characterize its time-dependent response patterns during infection progression;

To develop, compare, and evaluate machine-learning regression models for the quantitative prediction of infection stage and AFB₁ accumulation in maize and peanut kernels; and

To integrate VOC analysis, whole-cell biosensing, and machine-learning prediction and assess their potential as a front-line screening and risk-triage strategy for postharvest contamination monitoring.

1.7. Research Outline

This thesis follows a logical progression from defining the food-safety problem and establishing the analytical basis to identifying early chemical signals, detecting biological responses, predicting contamination-related risks, and integrating the resulting evidence.

Chapter 2 reviews the occurrence of major mycotoxins in Asian food and feed systems and the corresponding prevention and control strategies, thereby establishing the broader food-safety context of this thesis.

Chapter 1

Chapter 3 reviews the origins of VOCs associated with the postharvest deterioration of agricultural commodities, the principal detection technologies, practical applications and current limitations of VOC-based monitoring, and recent developments in intelligent monitoring systems.

Chapter 4 investigates dynamic changes in VOC profiles during the asymptomatic stage of *Aspergillus flavus* infection in maize kernels using an electronic nose (E-nose), GC–IMS, GC–MS, and GC×GC–MS. It compares VOC responses across maize cultivars and analytical platforms and identifies candidate signals for early warning.

Chapter 5 uses transcriptomic analysis to identify *Escherichia coli* promoters responsive to the volatile environments generated by *A. flavus*-infected maize and peanut kernels. These promoters are used to construct a whole-cell biosensor array, whose response patterns are combined with machine-learning regression models to predict the infection stage and AFB₁ accumulation.

Chapter 6 integrates and discusses the findings of the preceding chapters, examines the relationship between VOC-based early warning and biosensor-based risk estimation, evaluates their practical applicability and limitations, and identifies the further validation required for their translation to realistic storage conditions.

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Chapter 2

Current status of major mycotoxin contamination in food and feed and its control strategies in Asia

This chapter is adapted from the published article:

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Introduction

Mycotoxins are toxic secondary metabolites mainly produced by filamentous fungi (Hussein & Brasel, 2001). As insidious poisons, mycotoxins are difficult to detect in food and feed, and their contamination is of great concern to public health and causes economic losses to crops and the food sector. The toxicity expressed by mycotoxins can be acute, chronic, or both depending on the type and dose ingested. Adverse health effects could be caused by consuming mycotoxin contaminated food/feed, such as liver and kidney damage and genotoxic, mutagenic, teratogenic, and carcinogenic effects (Bhat & Miller, 1991; Pocsí et al., 2018). Mycotoxins are classified in various ways based on their structural and functional variations. Liver cancer is more common in some regions of southeastern Asia than in other parts of the world, and it may appear with increased exposure to aflatoxins (WHO, 2020). Moreover, the economic losses caused by mycotoxin contamination could not be ignored. Mycotoxin contamination has adverse effects on the food chain, especially severely affecting corn and maize yields, which eventually disturbs economic outcomes (Mitchell et al., 2016).

It is difficult to prevent and control mycotoxin contamination in the food chain. The infection of molds can occur at various stages of food and feed production. Mycotoxin-producing fungi have a chance to invade crops in different periods. During preharvest, harvest, postharvest, and even in storage conditions, toxigenic fungi may invade grains. The toxigenic fungi can be divided into field fungi, storage fungi, and advanced deterioration fungi according to the respective periods of fungi infection of grains (Maitree Suttajit, 2002). In addition, there are numerous factors affecting the growth of molds, such as food species/feed types, water activity (a_w), temperature, the availability of nutrients and oxygen, food processing, and chemical additives (Driehuis, 2015; Mongkon et al., 2017). In order to prevent the production of mycotoxins, it is essential to control these parameters during various stages of food production and storage. Given the hot and humid climate of South and Southeast Asia, it is suitable for fungal growth and mycotoxin production (Nugraha et al., 2018). In addition, most of Asia is developing countries with high sociocultural differences and high variability in food system dynamics (Wenndt et al., 2021). Each region has its own unique risk factors, and it is necessary to establish a suitable prevention and control modeling framework to reduce mycotoxin contamination based on its own reality.

The high incidence of mycotoxins leads to serious issues in Asia. Asia is the largest producer and consumer region of rice; about 87% of the world's total rice production is from the Asian region. In China (East Asia), more than 65% of the population consumes rice as a staple food (Sun et al., 2017). Rice can be contaminated with aflatoxins (AFs) and ochratoxin A (OTA), as well as fumonisins, which could cause serious damage to the liver and kidneys (Ali, 2018; Elaridi et al., 2019). Moreover, the co-occurrence of mycotoxins has been found in grains and may result in additive or synergistic toxicity (Föllmann et al., 2014). In South Asia,

biomonitoring data in Bangladeshi adults indicated a frequent exposure to the nephrotoxic mycotoxins such as OTA. The results showed that their exposure levels were significantly higher than those of Europeans. This may be related to the diet difference and food contamination such as rice and spices (Ali et al., 2016). In Southeast Asia, although efforts and measures have been made to reduce AF contamination, the high incidence of aflatoxins is still paralleled with the high occurrence of aflatoxicosis (Benkerroum, 2019). In Iran (West Asia), 76.6% of white wheat flour was found contaminated with OTA (Shahbazi & Shavisi, 2019). The exposure of deoxynivalenol (DON) in Asia is worth noting due to cumulative health risks related to DON (Wang et al., 2019). A previous study revealed that 70.2% of feed samples in Taiwan, China (East Asia) were detected to be contaminated with zearalenone (ZEN) (Yang et al., 2019).

According to FAO data in 2010, Asian cereal production accounts for about 49.62% of global cereal production, making it one of the world's largest cereal food and feed producing regions (FAO, 2010). However, mycotoxin contamination causes about 1 billion metric tons of food loss annually, which influences a serious decline in economic development and public health in Asia. In order to reduce the food and feed loss caused by mycotoxin contamination in Asia, it is essential to summarize findings from previous reports and research. This review is aimed at (1) researching the current status of the major mycotoxins causing food and feed contamination in Asia and (2) providing some possible prevention and control strategies for mycotoxins contamination in Asia.

2.1. Major Mycotoxins Causing Food and Feed Contamination in Asia.

The contamination caused by AFs, OTA, ZEN, DON, and fumonisins is a serious problem which should be taken into consideration as having an impact on public health and the food trade in Asia. Not only are they associated with health problems, but they are also among the few mycotoxins considered the most important from an economic point of view (Schatzmayr & Streit, 2013). These five kinds of mycotoxins are widely found in the main food and feed ingredients in Asia and are subject to legal regulations in various countries of Asia.

2.1.1. The Contamination of AFs in Food and Feed.

AFs are secondary metabolites primarily produced by several species of *Aspergillus* genus, mainly *Aspergillus flavus* and *Aspergillus parasiticus* (Daou et al., 2020). The Asian continent is large in area (44 million square kilometers), spanning a wide range of latitudes and crossing frigid zones, temperate zones, and tropical zones, which results in a complex and diverse climate. The tropical climate with characteristics of high temperature and humidity in west Asia, south Asia, and southeast Asia favors the growth of fungi from *A. flavus* (Norlia et al., 2018). *Aspergillus* species produce different types of AFs, including the aflatoxins B₁, B₂, G₁, and G₂ under natural conditions and the others as biotransformation metabolites

such as AFB₁, AFB_{2a}, AFG_{2a}, AFGM₁, and AGM₂ (Mamo et al., 2021). The characteristics of climate and poor hygienic storage conditions in some regions of Asia result in a high incidence of AF contamination in a wide range of food and feed.

In East Asia, a report in China indicated that fermented bean sauces have a high exposure risk of AFB₁, and the contamination level varies significantly according to different production channels. For example, the content of AFB₁ in homemade bean sauce samples (1.26–16.41 $\mu\text{g/kg}$) was higher than that in workshop and factory samples (1.35–8.67 $\mu\text{g/kg}$). The contamination difference between the samples was wide, and some samples exceeded the maximum allowable level of commercial fermented bean paste (5 $\mu\text{g/kg}$) (Zhang et al., 2020). Therefore, environmental hygiene in the production process may be the main reason for the difference of mycotoxin contamination in bean sauce samples. A study by Zhao et al. (2021) conducted in China from 2018 to 2020 found that the average levels of AFB₁ in feedstuff and complete feeds were 1.2–27.4 $\mu\text{g/kg}$, and the incidence was 81.9–100%, with 0.9% of raw feed ingredients contaminated by AFB₁ exceeding the Chinese safety standard concentration. Notably, the maximum value of AFB₁ contamination in corn was significantly different in different years. For example, it was 221.0 $\mu\text{g/kg}$ in 2018 and 2019 but 11.8 $\mu\text{g/kg}$ in 2020. These are also far from the average level of 4.0 $\mu\text{g/kg}$. These differences may be due to the fact that the feed samples were obtained randomly from different regions with different environmental factors during harvest, such as substantially higher temperatures and humidity in southern regions than in northern regions.

In Japan, from 2010 to 2015, the detected rate of AFB₁ contamination in corn, soybean meal, and formula feed was 46%, 30%, and 47%, respectively. Through the observation of the mean level of AFB₁ contamination in the formula feed and corn, it was possible that the AFB₁ contamination of corn ingredients passed to the feed as contamination amounts were relatable among both commodities (Nomura et al., 2018). Mycotoxin-producing fungi can survive during storage under natural conditions in Japan, and the lower rainfall, and higher temperatures may cause a higher concentration of AFB₁. It is important to apply suitable storage conditions to prevent mycotoxin contamination in food and feed.

In South Asia, there has been a significant prevalence of mycotoxin contamination in food and feed. For the poultry industry in Pakistan, mycotoxicosis has been the second most serious problem in breeding because of the contamination of feed. AFB₁ concentrations in all feeds before storage were generally significantly lower than after storage (Saleemi et al., 2020). Aside from the hot and humid climate in Pakistan, poor management and hygiene of poultry feed storage may also contribute to AF infection. In an assessment of aflatoxin contamination in dairy animal concentrate feed in India, 59% of samples were found positive for AFs of the 189 tested samples, while 44% of samples were detected with total AF levels higher than the tolerance limit of 20 $\mu\text{g/kg}$ established by the Food and Drug Administration (FDA). 58% of samples were detected with AFB₁ levels above the European

Commission (EC) legal limit of 5 $\mu\text{g/kg}$ (Saleemi et al., 2020). The high incidence of AFs in feed may be related to the contamination of feed raw materials, especially cereals. Aflatoxin levels in the feed component not only have a direct impact on animals but also contaminate their dairy products. Consequently, in addition to continuous monitoring of aflatoxin in feed, it is also necessary to monitor the source of contaminants and to regulate and control the degree of contamination of feed components.

In West Asia, Turkey is one of the world's top fig exporters. Figs are susceptible to AF contamination because of their characteristics. Thus, it is extremely crucial to investigate AFs in figs. Between 2017 and 2018, Sulyok et al. (2020) identified 180 samples of Turkish dried figs and reported the contaminated fig levels of AFB₁ ranged from 64 to 6220 $\mu\text{g/kg}$ in 2017, while it ran from 2 to 22300 $\mu\text{g/kg}$ in 2018. These differences between each year are influenced by many factors, especially climatic conditions such as drought. Notably, compared to the naturally occurring aflatoxin, AFM₁ is a metabolite after biotransformation. It is the first time that AFM₁ (1–2,730 $\mu\text{g/kg}$) has been reported to naturally exist in dried figs. This result suggests that AFB₁ can be transformed to AFM₁ in food.

The occurrences of AFs in different Asian countries are summarized in Table 1.

Table 1. Examples of AFs occurrence in food and feed.

Country	Type of AFs	Agricultural Product/food	Concentration rang ($\mu\text{g/kg}$)	Maximum Allowable Level ($\mu\text{g/kg}$)	Reference
China	AFB ₁	Corn in feed	221.0 (maximum) 4.0 (mean)	50	Zhao et al. (2021)
		Complete feed	59.7 (maximum) 4.1 (mean)	10	
		Complete poultry feed	57.4 (maximum) 4.9 (mean)	10	
	AFB ₁	Wheat	0.04-0.12	5	Zhao et al. (2018)
		Crackers	0.03-0.10	5	
	AFB ₁	Bean sauces (homemade)	1.26-16.41	5	Zhang et al. (2020)
		Bean sauces (Workshops)	1.35-8.67		
		Bean sauces (Factories)	ND~6.79		

Japan	AFM ₁	Ultra heat treatment milk	ND-0.0735	0.5	Guo et al. (2020)
		Pasteurized milk	ND-0.15367		
	AFB ₁	Corn	24 (Maximum)	10	
		Soybean meal	6.7 (Maximum)	10	
South Korea		Formula feed	20 (Maximum)	-	Jeong et al. (2019)
	AFs	Soybean food (meju)	0.2-48.3	15	
		Soybean paste	0.88-16.17	15	
	AFB ₁	Millet	0.4-5.6	10	
		Sorghum	0.7-1.7	10	
		Mixed Cereal	0.7-12.4	10	
Thailand	AFB ₁	Soybean food (meju)	0.6-21.4	10	Jeong et al. (2019)
		Soybean paste	1.06-15.25	10	
	AFs	Animal feeds	4-38.70	20	
		Chia seeds	0.4 - 10.99	4 (EU)	
	AFB ₁	Liver (free grazing ducks)	1.60 (average) 3.04 (maximum)	20	
		Intestine (free grazing ducks)	1.31 (average) 1.73 (maximum)	20	
		Egg yolk (free grazing ducks)	1.87 (average) 4.56 (maximum)	20	
	Pakistan	AFs	Dried apricot	0.31–11.11	Asghar et al. (2017)
			Dried mulberry	1.36–2.22	
			Dried figs (Injeer)	0.69–3.44	
		AFB ₁	Roasted peanuts	16-98	
Iran	AFM ₁	Raw milk	0.48–0.85	0.5	Taghizadeh et al. (2018)
	AFB ₁	Salty peanuts	15-85	10	
	AFB ₁	Raw peanuts	22-85	10	
	AFB ₁	Pistachio	1.14 (mean)	2	
	AFB ₁	Edible oils	0.2–0.4	30	
					Nabizadeh et al. (2018)

Turkey	AFM ₁	Pasteurized Liquid and Powdered Milk product	0.05-3.31	-	Mahmoodi Maymand et al. (2019)
	AFM ₁	Pasteurised milk	40.6-84 ng/l	500 ng/l	Pour et al. (2020)
		Sterilised milk	30.9-89.3 ng/l	500 ng/l	
		Breast milk	3.3-7.7 ng/l	500 ng/l	
	AFM ₁	Cheeses	0.05 - 0.42	0.25	Sharifzadeh et al. (2017)
	AFB ₁	Baby food	0.01-0.08	0.1	Er Demirhan & Demirhan (2021)
	AFB ₁	Raw hazelnut kernel	0.09 - 10.6	5	Kabak (2016)
		Roasted hazelnut kernels	0.17 - 9.59	5	
		Dried figs	0.1-12.5	2	
	AFs	Raw hazelnut kernel	0.09 - 11.3	10	
		Roasted hazelnut kernels	0.17 - 11.2	10	
		Dried figs	0.1 - 28.2	4	
	AFB ₁	Figs in 2017 harvest year	64 - 6220	-	Sulyok et al. (2020)
		Figs in 2018 harvest year	2 - 22,300	-	
	AFG ₁	Figs in 2017 harvest year	80 - 6630	-	
		Figs in 2018 harvest year	1 - 24,600	-	
	AFM ₁	Dried figs	1 - 2730	-	
	AFs	Chocolate products	0.15 - 2.04	-	Kabak (2019)
	AFM ₁	Raw milk	0.005 - 0.08	0.05	Eker et al. (2019)
		Ezine cheese	0.02 - 0.16	0.5	
India		Cheese halva	0.05 - 0.21	0.5	
	AFB ₁	Dairy animal concentrate feed	2.8–374.6	5 (EC Regulation)	Patyal et al. (2021)
	AFM ₁	Pasteurised milk	0.027-2.281	0.5	Sharma et al. (2020)
	AFs	Single discoloured kernels whole	7-1383400	10	Siruguri et al. (2018)

		groundnut products				
		Pool discoloured kernels whole	142-357300	10		
		groundnut products				
		Dairy animal concentrate feed	3.5–406.1	20 (FDA)		Patyal et al. (2021)
Lebanon	AFB ₁	Wheat	1.05 - 7.36	2		Joubrane et al. (2020)
Sri Lanka	AFs	Edible vegetable oils	2.25–72.70	4		Karunarathna et al. (2019)
	AFB ₁	Edible vegetable oils	1.76–60.92	2		
Indonesia	AFB ₁	Maize	0.3 - 6171	-		Nugraha et al. (2018)
		Peanut	0 - 357	-		
		Dried chilies	39.3–139.5	-		Wikandari et al. (2020)
	AFB ₂	Dried chilies	2.6–33.3	-		
Malaysia	AFB ₁	Raw peanut kernels	<LOD to 995.4	-		Norlia et al. (2018)
		Peanut-based Products	<LOD to 196.7	-		
	AFs	Raw peanut kernels	<LOD to 1021.4	15 (EU)		
		Peanut-based Products	<LOD to 247.7	10 (EU)		
Saudi Arabia	AFB ₁	Rice	0.014-0.123	-		Al-Zoreky & Saleh (2019)
	AFs	Rice	0.052–2.58	20		

ND: Not detected; LOD: Limit of detection.

2.1.2. The Contamination of OTA in Food and Feed.

OTA is a major mycotoxin produced by several fungal species of *Penicillium* and *Aspergillus* genera. The structure of OTA is a *p*-chlorophenol group containing a dihydroiso-coumarin portion bound to L-phenylalanine amide, which is known as a bioactive extrolite from secondary metabolism (Benites et al., 2017; El Khoury & Atoui, 2010). According to previous studies, there is a wide range of toxicological effects for OTA, including nephrotoxicity, teratogenicity, and immunotoxicity (Stoev, 2017; Al-Timimi & Gali, 2018; Lan et al., 2020).

In East Asia, a large range of grains and dry fruits are contaminated by OTA. Research about the OTA level in maize in China indicated that 2.5% of samples were detected to be positive for OTA in maize and maize-derived food with a mean

concentration of 10.3 $\mu\text{g/kg}$, which exceeded the maximum permitted level in China (Yang et al., 2019). A report about dry fruits showed the presence of OTA in the raisin samples, but the percentage exceeding the limit value (5 $\mu\text{g/kg}$) was low. The proportion of samples positive to OTA is below 10% (6.7%) with a concentration range of 4.6–7.4 $\mu\text{g/kg}$ (Wang et al., 2018). The effort to inhibit foods contaminated with OTA from escaping into the market contributes to the low occurrence of OTA in food commodities.

South Korea follows traditional food and prevention procedures to prevent OTA contamination, which plays an important role in avoiding food losses. The test on 100 meju samples showed half of the samples were positive for OTA with a concentration range from 0.1 $\mu\text{g/kg}$ to 193.2 $\mu\text{g/kg}$. In soybean paste samples, the percentage of samples contaminated with 0.88–26.29 $\mu\text{g/kg}$ of OTA is 48.9%. About 22.73% of 22 positive soybean pastes were contaminated with concentrations exceeding permissible limits for OTA set by the KFDA (20 $\mu\text{g/kg}$) (Jermnak et al., 2020). As traditional condiments, soybean pastes and soy sauce are popular in South Korea. Therefore, continued control of OTAs is warranted to ensure public health.

In South Asia, the OTA contamination level in various food matrices was analyzed. In 2018, 320 samples of edible nuts and dried fruits were investigated in the major cities of Punjab, Pakistan. 5% of raisin samples were analyzed to be positive for OTA with a mean concentration of 5.60 $\mu\text{g/kg}$. Only four samples of edible nuts and dried fruits were contaminated with a concentration above the maximum limit recommended by the European Union (10 $\mu\text{g/kg}$) (Iqbal et al., 2018). However, contamination of maize grains with OTA was fairly high in the maize samples in Pakistan. OTA was detected in 71% of maize samples ranging from 2.14 to 214 $\mu\text{g/kg}$ in 2020 and 61% of grain samples ranging from 1.41 to 53.9 $\mu\text{g/kg}$ in 2022 (Hassan et al., 2020; Sadeef et al., 2022).

The contamination of OTA in feed impacts animal health and triggers public health issues. In West Asia, research in Jordan indicated that about 38.5% of feed is contaminated by OTA, with an average concentration range from 2.64 $\mu\text{g/kg}$ to 3.16 $\mu\text{g/kg}$ in the poultry industry. It is worth noting that the increasing level of OTA in feed would result in greater deposition of mycotoxin in animal tissues. 66% of poultry breast samples and 100% of leg and thigh samples collected from Jordan contain OTA (Al Khalaileh, 2018). In Iran, in 2018, the risk assessment results of two mycotoxin levels in five commercial pistachio varieties indicated that the mean OTA level of 0.413 $\mu\text{g/kg}$ was lower than the maximum limit of 5 $\mu\text{g/kg}$ prescribed by EC regulations (Guo et al., 2020).

The occurrences of OTA in different Asian countries are summarized in Table 2.

Table 2. Examples of OTA occurrence in food and feed.

Country	Agricultural Product/food staff	Concentration level rang ($\mu\text{g/kg}$)	Maximum permitted	Reference
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Chapter 2

			Level (µg/kg)	
China	Raisins	4.6-7.4	5	Wang et al. (2018)
	Maize and maize-derived food	10.3 ± 5.71 (mean ± SD)	5	Yang et al. (2019)
Vietnam	Roasted coffee	0.75(mean)	5 (EU)	Wongworapat et al. (2016)
Thailand	Roasted coffee	0.17(mean)	5 (EU)	
	Instant coffee	2.19(mean)	10 (EU)	
South Korea	Meju	0.1-193.2	20	Jeong et al. (2019)
	Soybean paste	0.88-26.29	20	
Pakistan	Maize	2.14 - 214	5	ul Hassan et al. (2020)
	Maize grains	1.41 to 53.9	5	Sadeef et al. (2022)
The Hashemite Kingdom of Jordan	Raisin	5.60 (mean)	10	Iqbal et al. (2018)
	Dried apricot	3.10 (mean)	10	
	Figs	2.10 (mean)	10	
	Feed	1.72-3.70	-	Al Khalaileh (2018)
	Corn feed	1.54-3.18	5	
	Thigh and leg	1.90-2.98	-	
	Liver	4.06-7.68	-	
	Gizzard	1.89-2.26	-	
	Breast	2.81-3.31	-	
Iran	Pistachio	0.413 (mean)	5	Taghizadeh et al. (2018)
	Grape juice	0.14-2.69 µg/L	2 µg/L	Yusefi et al. (2018)
	Coffee	0.48-2.69	10	Pakshir et al. (2021)
Turkey	Chocolate products	0.18 - 0.75	-	Sharma et al. (2020)
	Packed red pepper flake	0.6-1	15	Tosun & Ozden (2016)
	Unpacked red pepper Flake	1.1-31.7	15	
	Baby food	0.06-0.07	0.5	Er Demirhan & Demirhan (2022)
Lebanon	Wheat samples collected from warehouse A	1.05-7.36	3	Siruguri et al. (2018)
	Wheat samples collected from warehouse B	1.01-7.31	3	

Cambodia	Coffee beans	0.19-1.12	-	Oeung et al. (2022)
Indonesia	Dried chilies	23.7-84.6	-	Wikandari et al. (2020)
Philippines	Coffee in the drying yard	4.17-514.46	-	Barcelo & Barcelo (2018)
	Stored green coffee beans	3.04-461.09	-	
	Roasted coffee samples	3.03-7.57	5	

SD: Standard deviation.

2.1.3. The Contamination of ZEN in Food and Feed.

ZEN is a mycotoxin produced mainly by the *Fusarium* genera, which contaminates food and feed stuff worldwide, particularly cereals like corn and wheat. There is evidence that ZEN has reproductive and developmental toxicity, carcinogenicity, genotoxicity, and immunotoxicity (Rai et al., 2020).

In East Asia, Hong et al. (2020) reported ZEN contamination in samples of cereal and their products in China. The result indicated that the ZEN positive rate was 62.6%, which ranged from 2.7 to 867.0 $\mu\text{g/kg}$, and 6.0% samples were over the limit levels. In 2018–2020, 2089 feed stuff samples and 1415 complete feed samples were collected in China for ZEN analysis. The results showed that 96.9–100% of samples were contaminated by ZEN, with the mean concentrations ranging from 48.1 to 326.8 $\mu\text{g/kg}$; 0.5% feed stuff and 1.9% complete feed samples were contaminated with ZEN, exceeding the Chinese safety standard concentration level. 2.9% of pig feed was found to be contaminated above the prescribed ZEN limit (Zhao et al., 2021). Although this result was much lower than previous data, complete feeds for pigs still had the highest occurrence of a ZEN contamination level in complete feeds. Notably, the contamination origin could be due to the contamination of the cereal component, including corn, wheat bran, and corn gluten meal. The raw materials could be seriously contaminated with more than one mycotoxin, resulting in mycotoxin contamination and exertion of synergistic toxic effects.

There were five types of cereal grain samples collected in South Korea for analyzing the level of mycotoxins in cereal grain in 2017. The contamination level of ZEN, 0.9–313 $\mu\text{g/kg}$, in sorghum samples was higher than that in the other four types of cereal grain samples. With the highest incidence (62%) for ZEN, sorghum samples contained ZEN exceeding the maximum level of 200 $\mu\text{g/kg}$ set by KDFA (Tulayakul et al., 2018). These differences may be due to different sampling areas during harvest, different weather conditions, and differences in grain varieties. Based on the highest incidence, monitoring ZEN during harvest in cereal grains (especially for sorghum) is essential to minimizing public health risks.

In Thailand, ZEN was detected in samples of seven different varieties of rice in 2019. The incidence of ZEN in rice samples was 50%. ZEN was detected in riceberry rice samples, with the highest incidence being 46.67%. Only 3.33% of jasmine rice and Japanese rice samples were contaminated by ZEN. The concentration level for ZEN in tested samples was all below maximum regulatory limits, which means the risk of exposure to ZEN is not alarming to public safety in the region ([Sinthithakul et al., 2019](#)). However, ZEN contamination in animal feeds is prevalent in Thailand. Research of mycotoxin contamination in animal feed in Thailand from 2015 to 2020 showed that ZEN had the highest contamination rate of nearly 50%, especially in rice bran, soybeans, and “unclassified ingredient” samples ([Asghar et al., 2017](#)).

The occurrences of ZEN in different Asian countries are summarized in [Table 3](#).

Table 3. Examples of ZEN occurrence in food and feed.

Country	Agricultural Product/food staff	Concentration level (µg/kg)	Maximum permitted Level (µg/kg)	Reference
China	Cereal and cereal products	2.7-867	60	Hong et al. (2020)
	Corn and its by-products (except corn bran and corn steep powder)	90.3 (mean) 822.0 (maximum)	500	Zhao et al. (2021)
	Corn bran	145.4 (mean) 742.6 (maximum)	1500	
	Soybean meal in feed	81.7 (mean) 522.0 (maximum)	1000	
	Complete feed for young pigs	81.5 (mean) 1,599.0 (maximum)	100 (for young gilts) 150 (for young pigs) 250 (Other complete feeds for pigs)	
	Complete poultry feed	108.0 (mean) 852.8 (Maximum)	500	
	Complete ruminant feed	98.2 (mean) 906.9 (Maximum)	500 (Concentrate supplement for calf, lamb and lactation period) 500 (Other complete feeds)	
Thailand	White rice	60.91 (mean)	100	Sinthithakul et al. (2019)
	Jasmine rice	60.42 (mean)	100	
	Japanese rice	58.55 (mean)	100	
	White sticky rice	92.79 (mean)	100	
	GABA	67.94 (mean)	100	

	brown rice			
	Black	54.13 (mean)	100	
	sticky rice			
	Riceberry	71.94 (mean)	100	
	rice			
	Animal feeds	25.10-879.60	100	Kananub et al. (2021)
South Korea	Brown Rice	0.4–37.6	200	Kim et al. (2017)
	Millet	0.7–61.5	200	
	Sorghum	0.9–313.0	200	
	Maize	0.9–14.7	200	
	Mixed Cereal	0.2–36.0	200	
Japan	Corn	46 (mean)	1000	Uegaki & Tsunoda (2018)

2.1.4. The Contamination of DON in Food and Feed.

Deoxynivalenol (DON) is an agriculturally significant mycotoxin mainly produced by certain *Fusarium* species, which frequently infect grains, such as corn, wheat, and rice (Sobrova et al., 2010). Exposure to DON contamination poses serious risks to public health.

In 2019, a reported case from a primary school in Zhuhai, China demonstrated that some students had nausea and vomiting after breakfast. On investigation, DON was detected in leftover raw noodles with a concentration ranging from 6856 to 11982 $\mu\text{g/kg}$. The concentration of DON in leftover cooked noodles ranged from 878.3 to 1074.2 $\mu\text{g/kg}$, which was taken by the students (Ruan et al., 2020). DON and its acetylated derivatives were detected in different cereal grains harvested widely in China. According to the results of the determination of DON in wheat samples from Northwest China in 2013, the frequency of DON contamination was 82.8%, and 10% of the DON content exceeded the Chinese regulatory limit (1000 $\mu\text{g/kg}$) (Kim et al., 2017). Another research conducted in 2020 also showed a high contamination frequency. All wheat samples were detected as positive for DON, and 99.83% of maize samples were positive for DON. The mean concentration for positive wheat samples and positive maize samples was 165.87 $\mu\text{g/kg}$ and 175.30 $\mu\text{g/kg}$, respectively (Yan et al., 2020). In the same year, rice samples from Jiangsu Province of China were collected to analyze for mycotoxins, and 30.9% of rice samples were positive for DON with a concentration range from 8.8 $\mu\text{g/kg}$ to 19145 $\mu\text{g/kg}$. In positive rice samples, the concentration of 11 samples (4.7%) contained a level of DON above the maximum regulatory level (Dong et al., 2020). In short, the proportion of samples positive to DON in cereal grains was high, but relatively few of the positive samples exceeded the regulated maximum level. DON also presents the same problem in feed. According to the research conducted by Zhao et al. (2021) DON occurred in 96.4–100% of feedstuffs and complete feeds, with the average values ranging from 458.0 $\mu\text{g/kg}$ to 1925.4 $\mu\text{g/kg}$. The maximum contamination of DON was 9186.4 $\mu\text{g/kg}$ in wheat

middlings harvested in 2018. Although only 0.1% of analyzed feed ingredients contaminated with DON exceeded China's safety standards, 8.9% of the complete pig feed samples that were contaminated with DON exceeded the Chinese safety standard concentration. It is worth noting that contamination of raw feed materials like wheat middling, which were quite heavily contaminated by DON, could lead to whole feeds being contaminated at levels beyond the limitations.

In South Korea, feed samples and feed ingredient samples were collected between 2009 and 2016 to determine DON contamination level. DON was detected in 95.3% of the compound feed samples, with a range of 0.4 to 2420.0 $\mu\text{g/kg}$. It was found in over half of the collected feed ingredient samples (64%), with a range of 0.01 to 8480.0 $\mu\text{g/kg}$. According to the guidance values of South Korea for DON management, DON should be controlled at 900 $\mu\text{g/kg}$ in supplemental and full feeds for pigs, while for the grain it should be under 10 000 $\mu\text{g/kg}$. In this study, two compound feed samples out of 160 pig feed samples exceeded the guidance value limits (Park et al., 2018).

In South East Asia, Pralatnet et al. (2016) analyzed 50 instant noodle samples and 50 bread samples for DON contamination in Thailand. The DON occurrence in instant noodles was less than that in bread. The positive rate of DON is 40%. The concentrations of DON in tested samples were all below the maximum level in regulation, which suggested a low DON exposure risk via noodles and bread in urban areas of Thailand. This result is consistent with the conclusions of previous research by Poapolathep et al. (2008)

In West Asia, 150 wheat samples from Kermanshah Province in western Iran were analyzed. The DON level for these samples ranged from 85.0 $\mu\text{g/kg}$ to 540.2 $\mu\text{g/kg}$. The concentration of DON increase during the wheat dough fermentation due to DON precursors converted to DON with the presence of yeast (Rahimi et al., 2018).

The occurrences of DON in different Asian countries are summarized in Table 4.

Table 4. Examples of DON occurrence in food and feed.

Country	Agricultural Product/food staff	Concentration level rang ($\mu\text{g/kg}$)	Maximum permitted Level ($\mu\text{g/kg}$)	Reference
China	Cereal and cereal products	6856- 11,982	1000	Ruan et al. (2020)
	Wheat	165.87 (mean)	1000	Yan et al. (2020)
	Maize	175.30 (mean)	1000	(2020)
	Rice	8.8 - 19,145	1000	Dong et al. (2020)
	Corn in feed	629.3 (mean) 3,343.6 (Maximum)	5000	Zhao et al. (2021)
	Soybean meal in feed	502.3 (mean)	5000	

		1,140.6 (Maximum)		
	Complete pig feed	661.3 (mean)	1000	
		3,712.2 (Maximum)		
	Complete poultry feed	660.8 (mean)	3000	
		2,970.1 (Maximum)		
	Complete ruminant feed	752.1 (mean)	1000 (Concentrate supplement for calf, lamb and lactation period)	
		2,613.7 (Maximum)	3000 (Other complete feeds)	
Japan	Corn	400 (mean)	1000	Uegaki & Tsunoda (2018)
South Korea	Feed material (Cereals and cereal products with the exception of maize by-products)	0.01-8480.0	10000	Park et al. (2018)
	Complementary and complete feed stuffs for pigs	231.5 (mean)	900	
Thailand	Instant noodles	89 (mean)	-	Pralatnet et al. (2016)
	Bread	4-331	-	
	Animal feeds	0.50-0.70	900	Kananub et al. (2021)
Iran	Wheat	85.0 - 540.2	-	Rahimi et al. (2018)
	Rice and bread	6.0 - 368.7	1000	Yazdanpanah et al. (2014)
	Positive puffed corn snack	60.2 - 368.7	1000	
Pakistan	Wheat and corn products	50-2050	2000	Iqbal et al. (2020)
Turkey	Baby food	6.32-37.57	200	Er Demirhan & Demirhan (2021)
	Nut-Based Products	11.81-42.09	-	Er Demirhan & Demirhan (2022)

2.1.5. The Contamination of Fumonisin in Food and Feed.

Fumonisin are global contaminants of corn and other cereals, produced by the fungus *Fusarium verticillioides* and the closely related *Fusarium proliferatum*. Fumonisin are related to several health issues all around the world, such as esophagus cancer (Kamle et al., 2019). According to the 2017 Biomin World Mycotoxin Survey, fumonisin have occurred in various parts of Asia with 85% incidence (Feijó Corrêa et al., 2018).

Maize is one of the most important staple food crops in East Asia. Some studies have reported that contamination of maize with fumonisin is frequently occurring in certain areas of China. In 2018, Li et al. (2019) detected the contents of the fumonisin B₁ (FB₁) and fumonisin B₂ (FB₂) in maize kernels. They found that a total of 57.1% of all maize samples were contaminated with fumonisin, from ND to 10.1 µg/g. A study investigated the occurrence of fumonisin in corn-based food products from Shandong Province, China. The results showed that the occurrence of FB₁ (94.4%), FB₂ (78.9%), and FB₃ (92.2%) is very frequent with mean levels of 128.2 µg/kg, 9.84 µg/kg, and 5.48 µg/kg, respectively. China has no maximum limits for fumonisin in foods or feed yet, but a few samples exceeded the maximum limits of 800 µg/kg according to European regulations, which indicates the need for more continuous and strict control (Jiang et al., 2019).

A study in Japan conducted by Uegaki and Tsunoda (Uegaki & Tsunoda, 2018) on assessing the mycotoxin concentration in Japanese domestically produced feed found that the maximum concentration of FB₁ varied in different production areas. In Kanto, the maximum concentration of FB₁ in corn feeds was 2400 µg/kg, which is higher than in other regions. In general, the difference is related to regional differences in climate, human farming, and crop transportation.

A South Korean study of the simultaneous determination of multiple mycotoxins in five commercially available grains found that the levels of FB₁ and FB₂ contamination were similar in the same grain group but varied greatly among different grains. Fumonisin detected in maize (1.9–2990 µg/kg) and sorghum (42.8–160.8 µg/kg) are higher compared to those in other types of cereal grains. However, none of the samples exceeded the maximum limit (4000 µg/kg) (Kim et al., 2017).

In South Asia, several studies in Pakistan reported the widespread presence of FB₁ in food and feed. The positive rate of FB₁ in wheat, barley, and flour samples was 90%, and that in rice samples was 42% (Iqbal et al., 2020; Majeed et al., 2018). Meanwhile, a study on feed also found a high rate of mycotoxin occurrence, and all poultry feed samples in the study were contaminated with FB₁ (Sarwat et al., 2022).

The occurrences of fumonisin in different Asian countries are summarized in Table 5.

Table 5. Examples of fumonisin occurrence in food and feed.

Chapter 2

Country	Type of fumonisins	Agricultural product/food stuff	Concentration range (µg/kg)	Maximum allowable level (µg/kg)	Reference
China	FB ₁	Maize kernel	ND-5400	-	Li et al. (2019)
	FB ₂	Maize kernel	ND-4700	-	
	FB ₁ +FB ₂	Maize kernel	ND-10100	4000(EC)	
	FB ₁	Corn-based food	4.16-1775 128.2(mean)	-	Jiang et al. (2019)
	FB ₂	Corn-based food	0.12-141.6 9.84(mean)	-	
	FB ₃	Corn-based food	0.10-70.64 5.48(mean)	-	
	FB ₁	Commercial pet foods	6.6-191.9	-	Shao et al. (2018)
Japan	FB ₁	Corn feed	ND-2400	-	Uegaki & Tsunoda (2018)
		Grass feed	ND-850	-	
South Korea	FB ₁	Brown rice	2.1–22.8	4000	Kim et al. (2017)
		Millet	2.0–32.6	4000	
		Sorghum	5.8–890.0	4000	
		Maize	3.8–2990.0	4000	
		Mixed cereal	3.1–80.1	4000	
	FB ₂	Brown rice	1.6–18.8	4000	
		Millet	1.6–31.1	4000	
		Sorghum	4.0–223.5	4000	
		Maize	1.9–620.0	4000	
		Mixed cereal	1.8–22.1	4000	
Pakistan	FB ₁	Wheat and barley products	ND-980.5 ± 211.4	200	Iqbal et al. (2020)
	FB ₁	Rice	100–400	-	Majeed et al. (2018)
	FB ₂	Rice	100–400	-	
	FB ₃	Rice	125–500	-	
	FB ₁	Poultry feed	205(median)	-	Sarwat et al. (2022)
Turkey	FB ₁	Cereal-based foods intended for infants and toddlers	42.5–100.9	200(EU)	Kirimker et al. (2020)
Indonesia and Iraq	FB ₁	Maize	20-1290	4000(EU)	Munawar et al. (2019)

2.2. Mycotoxin Contamination in Different Regions of Asia

Since different countries vary in economy, regulations, geographic location, climate, and other factors, there are different levels of mycotoxin contamination in food import and export trade. The Rapid Alert System for Food and Feed (RASFF) is the European Union's rapid early warning system, crisis management, and emergency response mechanism for food and feed. To some extent, RASFF can monitor the quality and safety situation of food exported from Asian countries to the EU. The analysis of mycotoxin contamination data from the early warning system can provide a reference for improving food safety and quality in Asian countries.

From January 2021 to March 2022, RASFF noted 290 cases of serious risk of mycotoxins in food and feed in Asia, including 11 countries: Azerbaijan, China, India, Iran, Lebanon, Pakistan, Sri Lanka, Turkey, Uzbekistan, Vietnam, and Georgia. Of these, 114 were reported to Turkey, 53 to Iran, and 34 to India. AFs and OTA are the main mycotoxins in the RASFF notification on food and feed in Asia. The category mainly includes nuts, nut products, seeds, dried fruits and vegetables, herbs, and spices. According to RASFF data, all food found contaminated belongs to developing Asian countries. Considering environmental, cultural, and economic factors in underdeveloped countries, agricultural products contaminated by mycotoxin have always been a public health problem and need keen attention.

Turkey is the country most frequently notified by RASFF, and its mycotoxin risk mainly involves AFs and OTA pollution of dried fruits and nuts. In the food category, the reported cases of dried figs account for the highest proportion, which should be highly valued. Turkey's humid maritime climate, warm and dry, coupled with long-term sunshine, is very conducive to the growth of the fig tree (*Ficus carica* L). However, subtropical and temperate climatic conditions make *F. carica* fruit easily polluted by filamentous fungi in the field growth, ripening, and drying stages (Kabak, 2016).

In Iran, 50 cases (94%) of mycotoxin warnings are related to AFs in pistachios, and the other three cases (6%) are also related to nuts and seeds. During the planting period, factors such as the area of cultivation, traditional harvesting techniques, weather conditions, and plant protection measures may have an impact on pistachio contamination by mycotoxins. During storage, water activity (a_w) and temperature are crucial for fungus growth and AF production (Liu et al., 2017).

The categories of food and feed noted by RASFF in India are more diverse, including nuts, nut products, seeds, fruits, vegetables, cereals, bakery products, feed materials, herbs, and spices, and mycotoxin contamination is 91% AFB₁ and 9% OTA. The main climate type of India is the tropical monsoon climate, which is characterized by high temperatures throughout the year, with obvious rainy and dry seasons, which is beneficial to the growth and toxin production of *A. flavus*. Many factors also have

impacts on mycotoxin contamination in India, such as unhygienic surroundings, a lack of sanitation facilities, poverty, and poor supervision of the food production chain and food system (Wenndt et al., 2021; Prakash, 2014). It is worth noting that different standards of mycotoxin contamination limits between trading countries will also have an impact on international trade. According to the latest revised food safety and standard regulations issued by the Food Safety and Standards Authority of India (FSSAI), the limited level of AFB₁ and AFs in cereal and cereal products, nuts, and oil seeds is 10 µg/kg and 15 µg/kg, respectively (FSSAI, 2022). However, the EU has stricter limits for AFs. Based on the regulatory standards for the concentration of mycotoxins in agricultural products and foodstuffs in the EU, the permission level is 2 µg/kg for AFB₁ and 4 µg/kg for total AFs, respectively (European Commission, 2006). In view of the great attention paid to mycotoxins in the import and export of food and feed, the following suggestions are helpful for reducing the impact and losses caused by mycotoxin pollution: First, fully follow the regulatory requirements of exporting countries on food and feed, with awareness about the new regulatory trends; respond in time, and gradually establish a complete regulatory response mechanism. The second is to improve the research on product quality risk characteristics, carry out risk management on products that are often noted, improve the planting environment, and increase the research investment of antifungal varieties in laboratory and practice. The third is to improve quality control. The country of origin of raw materials needs to strengthen the supervision of the production chain and product inspection, implement good production norms, and establish an effective quality management system. The processing country needs to control the procurement of raw materials, strengthen supervision in storage and transportation, and conduct inspections before export.

2.3.Prevention and Control Strategies

This systematic review of the scientific literature on mycotoxin contamination in food and feed in Asia in recent years reveals that mycotoxin contamination is widespread, especially in developing and tropical countries. Although many countries have taken active preventive measures and instituted control strategies, and most mycotoxin contamination has been controlled within the prescribed range, the economic and public health problems caused by mycotoxins in Asia still need attention and cannot be ignored.

Previous studies have shown that the contamination level of mycotoxins in food and feed is correlated with many factors, including (a) food species/feed types, such as susceptibility to mold infection, water content, and composition ratio; (b) environmental factors, such as soil, climate, temperature, and humidity; (c) food processing, such as sorting, washing, and cooking; and (d) preservatives, such as chemical agents, essential oils (EOs), and natural antioxidants (Mongkon et al., 2017).

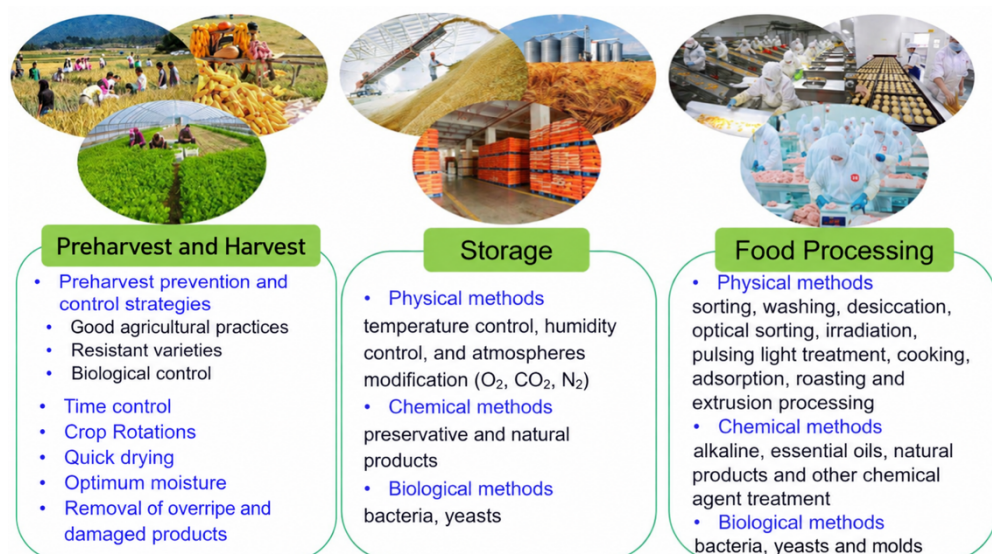


Figure 1. Mycotoxins prevention and control strategies in the process of preharvest and harvest, storage, and processing.

In order to reduce mycotoxin contamination levels, strategies from preharvest to postharvest mainly focus on inhibition of fungal contamination, such as using fungicides, controlling temperature and humidity, using preservatives, and modifying atmospheres (Zhong et al., 2018; Haque et al., 2020). At the preharvest stage, good agricultural practices, crop rotation, resistant varieties, and biological control can also help reduce fungal infection and subsequent mycotoxin production. Biological control may include the use of antagonistic microorganisms or non-toxigenic fungal strains to suppress mycotoxin-producing fungi. Biological control of mycotoxin uses a variety of microorganisms, including bacteria, yeasts, and molds, to control the growth of mycotoxin-producing fungi (Wan et al., 2020). Compared to physical and chemical treatment, biology-based detoxification methods are widely considered as environmentally friendly methods with great practical application. For example, the level of AFBs in wheat flour was significantly reduced after the introduction of lactic acid bacteria into the bread making process (Saladino et al., 2016). A study involving 63 smallholder dairy farms in Thailand was conducted to determine the contamination of AFB₁ in various dairy feeds stored under farm conditions (Mongkon et al., 2017). The result demonstrated that the concentrations of AFB₁ in all feeds increased after storage, but the increasing ratio of each feed type was different because of mechanical and chemical compositions such as moisture contents. In addition, feed storage management and environmental conditions can promote mold growth and toxin production (Mongkon et al., 2017). Although the geographical and climatic conditions of each country are different, mycotoxin contamination can be effectively controlled by adjusting feed composition, temperature, and humidity. In addition, modified atmosphere storage, such as modifying O_2 and CO_2 and the use of N_2 , has been studied

for storing wet grains, especially for animal feed (Zhong et al., 2018). It can also improve the disease resistance of products, for example, by creating varieties resistant to fungi (Haque et al., 2020). Rice is a staple food consumed in China, but due to the high temperature and humidity in southern China, infection-resistant rice breeding of mycotoxigenic fungal species could be an effective method to improve the safety of rice in China (Sun et al., 2017). Direct degradation of accumulated toxins is also a strategy for controlling mycotoxin contamination. It can be divided into three main categories: biological detoxification, mycotoxin binders, and herbal products for the amelioration of toxic effects of mycotoxins. Among them, biological detoxification, including mycotoxin modifiers (bacteria, fungi and yeast) and enzymes, was extensively studied (Wan et al., 2020).

In fact, it is difficult to completely prevent mycotoxin contamination in raw materials during preharvest, harvesting, and storage. Therefore, food processing also plays a vital role in preventing and controlling mycotoxin in food and feed. It has been reported that various strategies have been developed to reduce mycotoxin contamination levels in food processing, including physical, chemical, and biological methods (Shetty & Bhat, 1999). Physical methods of mycotoxin control can be varied, starting with simple methods, such as crop sorting and washing, food processing and cooking, etc. The advantage of these methods is their simplicity, but their ability to inhibit toxins is limited. Studies found that up to 91% of mycotoxins could be removed by simply cleaning maize grains (Shetty & Bhat, 1999; Fandohan et al., 2005). The complexity includes the application of irradiation, such as gamma (ionizing), UV, and microwave radiation, which has the advantage of having less of an effect on the morphology of food and can handle processed food such as bread. However, irradiation may also have a negative effect on crop quality, for example, by inhibiting the germination rate of barley grains (Kaushik, 2015; Milani & Maleki, 2014). Chemical treatment mainly includes alkaline and other chemical agent treatment (Liu et al., 2022). Chemical techniques can effectively destroy the structure of mycotoxins. However, since chemically treated products might involve harmful chemicals, it is necessary to evaluate the quality and safety of the products' considered biosafety (Trombete et al., 2017).

In addition to the main traditional methods, EOs and natural antioxidants have been widely studied for their role in inhibiting fungal growth and toxicity in food due to their high efficiency, safety, and wide availability (Friedman & Rasooly, 2013; Li et al., 2021; Lv et al., 2018; Wang et al., 2019a; Wang et al., 2019b). EOs are widely used in the food industry as food preservatives. The ability of EOs to inhibit fungal activity has been demonstrated in many different models. For example, *Zingiber zerumbet* L. essential oil-based chitosan nanoemulsion against AFB₁ (Deepika et al., 2021) and sodium alginate-cinnamon essential oil control fungal growth and OTA production (Kapetanakou et al., 2019). In terms of specific applications, EOs and their nanoformulations are being widely studied and have good application prospects in food storage (Das et al., 2021; Deepika et al., 2021; Chaudhari et al., 2021). At present, there are abundant research achievements on natural antioxidants, such as all-trans- β -

carotene and other carotenoids extracted from mango peels (Pilar Sanchez-Camargo et al., 2019), piperine extracted from black peppers and long peppers (Caceres et al., 2017), etc. The natural antioxidants extracted from these agricultural and sideline products are recognized to be safe and feasible in sufficient solvents.

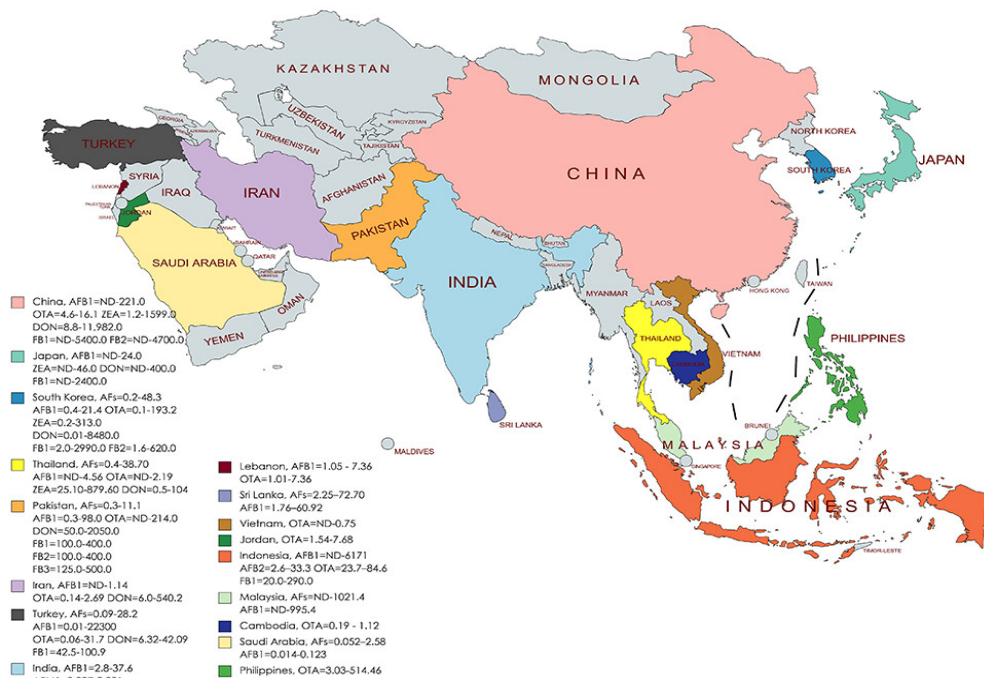


Figure 2. Overall range of mycotoxin contamination levels in Asian countries from 2016 to 2022 ($\mu\text{g/kg}$). Data were compiled from the studies summarized in Tables 1–5 (Sun et al., 2023)

Food safety is intricately linked to people's convention, health, and economic activities. This Review summarizes the current status of major mycotoxin contamination in food and feed in Asia. The overall range of mycotoxin contamination levels in Asian countries from 2016 to 2022 ($\mu\text{g/kg}$) is shown in Figure 2. Mycotoxin inevitably exists widely in all kinds of food and feed commodities, causing huge losses to food safety and economic trade. It can be seen from RASFF notification that specific products are susceptible to mycotoxin contamination, and some developing countries have a greater burden of mycotoxin prevention due to many factors such as geographical location and their own economic situation. There are obvious differences in the supervision, prevention, and control of mycotoxins among different Asian countries. Most Asian countries have strict control regulations, while some countries have poor regulations and high exposure risks. Developed countries in Asia should continue to maintain strict monitoring and establish an effective control system for the whole food production chain, while developing countries should strengthen the prevention and control strategies for mycotoxins.

Although several mycotoxins represent major food and feed safety concerns in Asia, aflatoxins remain particularly relevant because of their high toxicity, strict regulation, and strong association with *A. flavus* contamination in major agricultural commodities such as maize and peanuts. This provides the rationale for focusing the experimental part of this thesis on early detection and risk monitoring of *A. flavus* and AFB₁.

Summary

The widespread occurrence of mycotoxins across agricultural commodities demonstrates that contamination remains a persistent challenge throughout the food and feed supply chain. It emphasizes the need to move beyond contamination control alone and toward earlier recognition of the biological and biochemical changes that precede severe deterioration. In postharvest systems, fungal development and product deterioration are dynamic processes that may begin before visible spoilage or measurable accumulation of mycotoxins becomes evident. Consequently, the identification of sensitive and non-destructive indicators capable of reflecting these early changes is an important prerequisite for preventive monitoring and timely risk management.

VOCs provide a promising means of capturing such changes because their production is closely associated with plant metabolism, microbial activity, lipid oxidation, enzymatic reactions, and other deterioration-related processes. Alterations in volatile profiles may therefore provide an early chemical signature of changes occurring within stored agricultural products. Building on the contamination and control framework developed in Chapter 2, Chapter 3 shifts the focus from the occurrence and management of mycotoxin hazards to the biochemical signals accompanying postharvest deterioration. It examines deterioration-associated VOCs in agricultural products, their origins and analytical characterization, and their potential as non-destructive indicators for early quality and safety monitoring. This conceptual transition provides the basis for the subsequent experimental investigation of VOC-based early detection and mycotoxin risk assessment in this thesis.

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Chapter 3

Postharvest agricultural product deterioration signature volatile organic compounds

This chapter is adapted from the published article:

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Introduction

Postharvest deterioration refers to the degradation in the quality of agricultural products after harvest, caused by factors such as improper handling, suboptimal storage conditions, and delays in processing (Nath et al., 2024). It results in quantitative losses due to a decrease in edible food mass, and qualitative losses related to reduced nutritional value, texture, color, and other sensory attributes, even though the food may still be technically edible. Preventing postharvest deterioration is essential for reducing postharvest losses (PHL) (Stathers et al., 2020). Effective management offers wide-ranging benefits, including optimizing food availability, reducing economic costs, conserving resources, preserving nutrients, preventing foodborne illness, extending shelf life, and minimizing waste. Adopting good postharvest practices is therefore key to minimizing food losses and ensuring the maximal utilization of agricultural products (Zhang et al., 2022).

Volatile organic compounds (VOCs) have emerged as powerful biomarkers for monitoring postharvest quality and detecting early signs of spoilage in agricultural commodities (Bonah et al., 2020; Giménez-Campillo et al., 2025). VOCs are low-molecular-weight compounds emitted as a result of metabolic processes in plant tissues and microorganisms. Their profiles change significantly in response to physiological stress, microbial activity, enzymatic degradation, and chemical oxidation—making them sensitive and non-invasive indicators of product freshness and integrity (Tiwari et al., 2020).

VOC-based sensing mechanisms primarily operate through chemical or physical transduction of VOC interactions into measurable electrical, optical, or spectral signals (Chen et al., 2022; Gu et al., 2020). In recent years, material innovation, system integration, and intelligent functionality are the keys to advancing the next generation of gas sensors toward high sensitivity, low power consumption, wearability, and smart applications (Zhu et al., 2022). Advances in nanomaterials and microfabrication have further enhanced the resolution, portability, and cost-efficiency of these detection platforms (Kwon et al., 2025). The rationale for employing VOCs as biomarkers lies in their early and often compound-specific emission patterns that precede visible spoilage. Unlike conventional microbiological or physicochemical tests, VOC-based methods offer rapid, real-time, and potentially non-destructive monitoring. In addition, the integration of VOC sensing technologies with smart agriculture frameworks has gained momentum. Innovations include the development of portable e-nose devices (Vinicius da Silva Ferreira et al., 2023), artificial intelligence(AI)-enhanced sensor arrays (Zhang et al., 2024), IoT-enabled monitoring platforms (Navarro et al., 2020), and machine learning algorithms capable of decoding complex VOC signatures (Ma et al., 2023) to detect contamination or classify spoilage stages. These multidisciplinary advances are moving the field toward real-time, on-site, and automated postharvest quality control systems.

This review provides a comprehensive summary of VOC detection methods used to monitor postharvest deterioration of agricultural products over the past decades. It examines and evaluates various technologies, highlighting their advantages and limitations (Figure 3). Additionally, it discusses the integration of VOC detection into smart systems for real-time surveillance and timely intervention. The review emphasizes the importance of addressing both economic and food safety risks posed by postharvest spoilage and outlines future research directions, including potential innovations and improvements in VOC-based detection strategies.

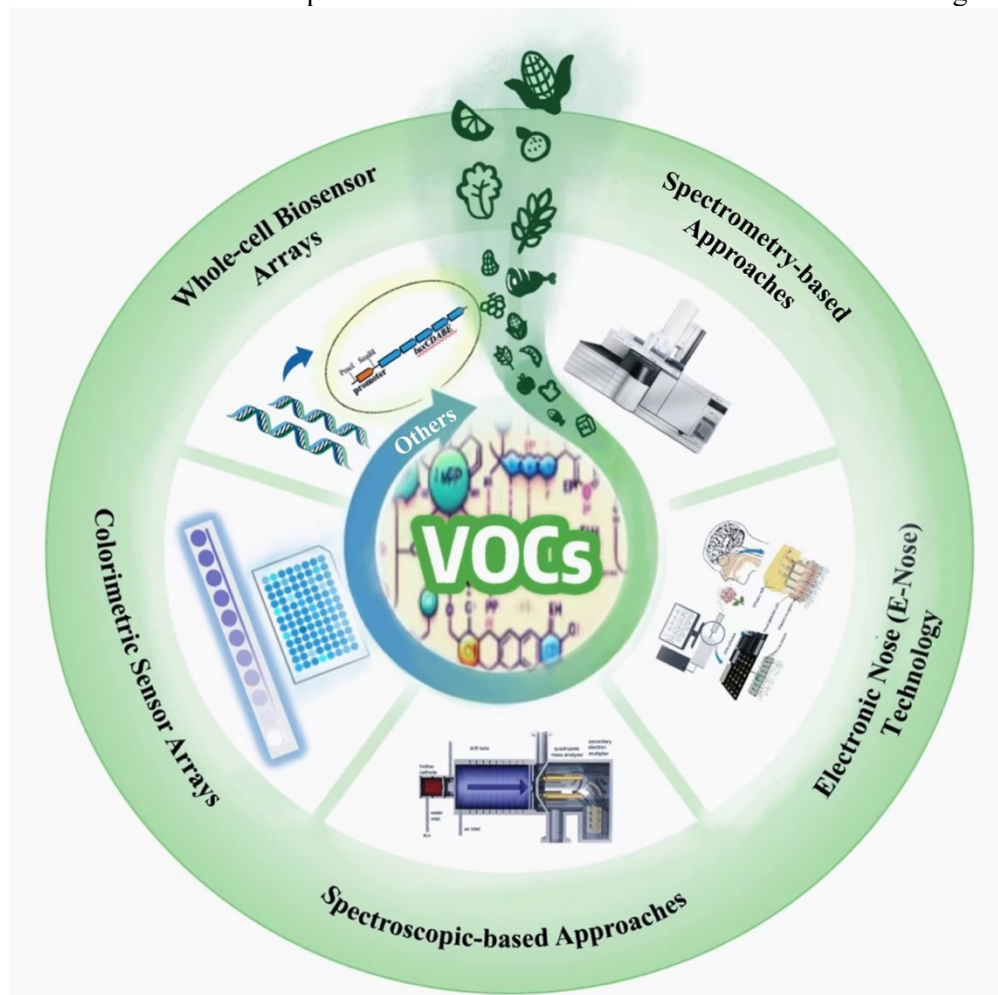


Figure 3. Scheme of postharvest deterioration detection technique based on VOCs.

3.1. Volatile Organic Compounds in Postharvest Deterioration

3.1.1. Major types and sources of VOCs in agricultural products

Agricultural products contain a wide variety of VOCs that play a significant role in shaping the aroma, flavor, and overall quality of these products. Consequently, they can serve as valuable physiological indicators for assessing the quality of agricultural products. Monitoring and evaluating the quality status of agricultural products can be achieved by analyzing the types and quantities of VOCs present. Common VOCs found in agricultural products include terpenes/terpenoids, ketones and aldehydes, ester compounds, alcohols, sulfurs, acids, nitrogen compounds, etc. As described in Table 6, Terpenes/terpenoids are the most abundant group of secondary plant metabolites mainly found in fruits, vegetables, and herbs, such as limonene in berries contributes to their fresh and zesty aromas (Gu et al., 2022). Linalool in basil gives them their unique herb scents (Walters et al., 2020). Ketones, aldehyde compounds, alcohol compounds, and ester compounds are common in fruits and grains, such as hexanal can contribute to the fresh aroma of strawberries, and ethyl acetate in apples contributes to the sweet aroma (Yang et al., 2021). Acid compounds such as acetic acid can be found in fruits and vegetables (Gu et al., 2022). Sulfur compounds like cysteine sulfoxides are prevalent in pungent vegetables such as garlic (Kovacevic et al., 2023). These VOCs can be considered as key biomarkers for monitoring the quality of agricultural products during storage. Detecting and analyzing these compounds allows for early intervention to prevent spoilage, thereby ensuring the quality and safety of food products (Tiwari et al., 2020). Notably, the complex aroma of an agricultural product is often the result of a diverse mixture of VOCs, influenced by a complex interplay of factors such as variety, ripeness, damage, storage conditions, and microbial contamination.

Table 6. Major types of VOCs associated with different agricultural products.

Types of VOCs	Products	Major biomarkers	Reference
Terpenes/Terpenoids	Fruit and vegetables	Limonen, Linalool	(Gu et al., 2022; Walters et al., 2020)
Ketones and Aldehydes	Grains and fruits	Hexanal	(Yang et al., 2021)
Ester Compounds	Grain, fruits, legumes	Ethyl Acetate	(Li et al., 2021; Wang et al., 2021)
Alcohols	Grains and fruits	3-Methyl-1-butanol, Ethanol	(Boriesson., 1999)
Sulfurs	Garlic	Cysteine sulfoxides	(Kovacevic et al., 2023)
Acids	Fruit, fermented foods	Acetic acid	(Gu et al., 2022)

VOCs in agricultural products can be classified into two subtypes based on their production and response to various factors: constitutive and induced. Constitutive VOCs are naturally and continuously produced while induced VOCs are generated as a response to a wide range of environmental stresses. Constitutive VOCs are naturally

produced, and typically released with time. [Yang et al. \(2021\)](#) summarized the profile of VOCs change in apples that aldehyde was the main volatile component in the early stage, and then the alcohol content began to increase significantly, and the ester content increased with the decrease of aldehyde. [Amundsen et al. \(2023\)](#) observed that Lingonberries exhibited a significant decrease in the total concentration of phenolic compounds across five ripening stages. In contrast to constitutive VOCs, induced VOCs are produced in response to various abiotic and biotic stresses ([Tiwari et al., 2020](#)). Postharvest abiotic stress involves the temperature, relative humidity, oxygen levels, damage during transport, etc. Among the VOCs, terpenes, nitrogen-containing compounds, and phenolic compounds exhibit the most substantial susceptibility to abiotic stressors ([Nagalingam et al., 2023](#)). Moreover, when agricultural products are infected by bacteria and fungi, viruses, insects, or parasites, it can lead to biotic stress that generates VOCs as by-products of metabolism changes. These VOCs are closely linked to spoilage and fermentation, often resulting in noticeable changes to the flavor and aroma of the product.

3.1.2. Metabolic Pathways Leading to VOCs Emission

As a consequence of agricultural products' interaction with biotic and abiotic factors, the VOCs are biosynthesized through interconnected pathways regulated by complex mechanisms. Recent advances in omics technology over the past years have led to a better understanding of plant VOCs biosynthesis ([Picazo-Aragones et al., 2020](#)). The biosynthesis of VOCs is closely linked to primary metabolism and the availability of energy sources. They can be roughly categorized into three classes based on their biosynthetic origin, including terpenoids, phenylpropanoids/benzenoids, fatty acid derivatives, and amino acid derivatives, along with some species- or genus-specific compounds that may not fit into these major classes. [Dudareva et al. \(2013\)](#) illustrate the interconnected biosynthetic pathways of VOCs with primary catabolic intermediates like acetyl-CoA, pyruvate, phosphoenolpyruvate (PEP), and erythrose-4-phosphate (E4P) as precursors ([Figure S1](#)). C₁₈ fatty acids, including linoleic acid and linolenic acid, are catalyzed by lipoxygenases and generate 9-hydroperoxy and 13-hydroperoxy derivatives of fatty acids. These intermediates turn into fatty acid derivatives, including methyl jasmonate and green leaf volatiles ([Gu et al., 2022](#)). The lipoxygenase pathway also can synthesize oxylipins, isoprene, carotenoid derivatives, indoles, phenolics, methyl salicylate, and aromatic VOCs. Sesquiterpenes, another group of VOCs, are synthesized through the cytosol mevalonate pathway (MVA), with acetyl-CoA as a key component, resulting in various intermediate metabolites ([Dudareva et al., 2013](#)). The methylerythritol phosphate (MEP) pathway occurs exclusively in plastids, and uses pyruvate as a precursor to create two common five-carbon (C₅) intermediates, isopentenyl diphosphate (IPP) and its allylic isomer, dimethylallyl diphosphate (DMAPP), leading to the synthesis of geranyl diphosphate (GPP) ([Picazo-Aragones et al., 2020](#)). In higher plants, the MVA and MEP pathways are interconnected through metabolic crosstalk ([Bick & Lange, 2003](#)). Geranyl diphosphate is converted to geranylgeranyl diphosphate (GGPP) with the help of the GGPase enzyme,

ultimately producing volatile carotenoids. Geranylgeranyl diphosphate (GGPP) is the recently discovered precursor of sesquiterpenes (Sun et al., 2016). Monoterpenes, diterpenes, and hemiterpenes are also produced through the carotenoid biosynthesis intermediates. These 3-Deoxy-d-arabinoheptulosonate 7-phosphate (DAHP) pathway-based VOCs can be classified into different classes, including terpenoids, benzenoids, phenylpropanoids, derivatives of amino acids, and fatty acids. Among these classes, terpenoids are the largest followed by benzenoids and phenylpropanoids of aromatic origin (Tiwari et al., 2020). The advancements in the biosynthesis, ecological roles, and applications of plant VOCs have been summarized by Maffei et al. (2011).

While much attention has been given to the role of VOCs during plant growth, their role in postharvest deterioration is now attracting increasing research interest. Since the volatilomics can characterize the physiological state of agricultural products before visible changes occur, it holds significant potential for early monitoring of deterioration, particularly in early mold detection (Nordström et al., 2020; Jiang et al., 2023).

3.2. Detection Techniques for VOC-based Spoilage

3.2.1. Spectrometry-based Approaches

Spectrometry-based techniques are among the most widely used and valuable methods for establishing unique VOC profiles in food safety, fraud detection, freshness assessment, and quality evaluation (Chen et al., 2022). The identification of signature VOC compounds in deteriorated agricultural products for early pathogens monitoring based on spectrometry approaches is summarized in Table 7.

Gas chromatography-mass spectrometry (GC-MS) has been widely applied in food volatilomics for the evaluation of food quality. GC-MS separates compounds in a sample based on their volatilities and interactions with the stationary phase of the GC column and identifies compounds based on the mass-to-charge ratio and their specific fragmentation (Wu et al., 2023). It is particularly advantageous when analyzing complex mixtures and when the precise identification of individual components is essential. Its sensitivity generally allows for detection at the ppb level. However, the quantitative performance is highly dependent on the sample preparation strategy, some targets can be as low as ppt level after using solid-phase microextraction (SPME) and other enrichment methods. GC-MS has been successfully applied to the analysis of flavor profiles and spoilage markers in a wide variety of foods, including fruits, juices, meats, and cereals, with most target VOCs were present at concentrations above the ppb level (Kataoka, et al, 2000; Baimatova & Gionfriddo, 2025). However, traditional GC-MS systems are typically bulky and are confined to laboratory settings, limiting their use for continuous and onsite monitoring in storage facilities or during shipment. Although advancements in device miniaturization have led to the development of portable GC-MS for onsite detection, its application in real-time monitoring is still lacking (Leary et al., 2023). Another

mass spectrometry-based analytical technique, proton transfer reaction mass spectrometry (PTR-MS) is often more compact and can be designed for portability, making them better suited for on-site and field applications, such as postharvest storage air monitoring. PTR-MS is an online measurement based on the reaction of volatile molecules with H_3O^+ ions followed by subsequent detection of protonated molecules with a mass spectrometer (Weraduwaage et al., 2022). For instance, PTR-MS is particularly useful for monitoring real-time VOC changes, such as flavor release during ripening, and for rapidly distinguishing microbial volatile metabolites, with reported sensitivities in the sub-ppb range. In contrast, GC-MS provides chromatographic separation and therefore offers stronger capability for resolving and identifying closely related compounds (Schuhfried et al., 2017; Mazzucotelli et al., 2022). However, direct-inlet mass spectrometry approaches such as PTR-MS may have limitations in compound-level identification, especially when isobaric or structurally similar compounds are present, because the absence of chromatographic separation can make signal assignment difficult. High-resolution mass analyzers, including time-of-flight (TOF), Orbitrap, and Fourier transform ion cyclotron resonance (FT-ICR) systems, can improve mass accuracy and support broader VOC profiling (Li et al., 2020; Malik et al., 2024; Kuchikata et al., 2024). In particular, TOF-based systems are valuable for rapid spectral acquisition and time-resolved VOC monitoring when combined with appropriate sampling and data-processing strategies. Therefore, integrating real-time direct-inlet MS techniques with chromatographic GC-MS analysis provides a complementary strategy for postharvest agricultural product VOC detection and lays a stronger foundation for monitoring and early-warning applications.

Here are some other notable hybrid techniques, including gas chromatography-ion mobility spectrometry (GC-IMS), selected ion flow tube-mass spectrometry (SIFT-MS), gas chromatography-electron capture detector (GC-ECD), gas chromatography-atomic emission detection (GC-AED), membrane introduction mass spectrometry (MIMS), etc. (Erler et al., 2020; Han & Lim, 2024). GC-IMS combines the separation power of gas chromatography with the rapid, high-sensitivity ion migration analysis of ion mobility spectrometry to identify and analyze chemical substances. The limits of detection (LOD) for GC-IMS are $>0.1 \text{ ng g}^{-1}$, which is generally lower in sensitivity compared to GC-MS. However, GC-IMS offers clear advantages in rapid screening and early-warning applications, particularly in agricultural settings where medium to high concentration composite gas pattern recognition is required. Therefore, it is more suitable for early warning food deterioration processes or scenarios requiring large-sample screening, such as sorting lines and storage monitoring. In recent year, it has been widely used for detecting volatiles in fruits and grains (Erler et al., 2020). For example, the typical concentration range of volatiles in pears is 0.1 to 20,000 ng g^{-1} , including compounds such as (E)-2-hexen-1-ol, (E)-2-heptenal, benzaldehyde, 6-methyl-5-hepten-2-one, (E)-2-octenal, and acetophenone (Giménez-Campillo et al., 2025). GC-ECD detects molecules that can capture electrons, particularly monitor halogenated VOCs. It is widely applied in

pesticide residue analysis, though it is less commonly used in quality monitoring (Han & Lim, 2024). MIMS is another direct MS analysis that requires VOCs to pass through a semi-permeable membrane into a mass spectrometer. This technique excels in continuous VOC monitoring and can be designed for portability (Richards et al., 2018). Emerging X-ray-based techniques are increasingly being investigated for structural analysis of VOCs, utilizing methods such as X-ray fluorescence (XRF) to detect atomic-level alterations linked to spoilage indicators. However, their broader application remains constrained due to high equipment costs and complex instrumentation requirements (Feng et al., 2021).

Table 7. Signature VOCs emitted by deteriorated agricultural products for early pathogen monitoring based on spectrometric approaches^a.

Foodstuff	Pathogens	Approaches	Signature VOC compounds	Pattern recognition	Approaches	References
Fragrant rice	<i>Aspergillus niger</i> , <i>Penicillium janthinellum</i> , <i>Penicillium alternatum</i> , <i>Penicillium funiculosum</i> , <i>Aspergillus flavus</i> and <i>Aspergillus parasitica</i>	GC-IMS	Alcohols, aldehydes, ketones, and esters	PCA	GC-IMS	(Chen et al., 2022)
Xiang Ling walnut	<i>Aspergillus flavus</i>	GC-IMS	Ethyl acetate-D, 3-methyl-2-butanol, and cyclohexanone	PCA	GC-IMS	(Wang et al., 2021)
Citrus (Citrus reticulata Blanco)	<i>Penicillium italicum</i> , <i>Penicillium digitatum</i> and <i>Lasiodiplodia theobromae</i>	GC-MS	dihy-drocarvone, Z-carveol, cis-beta-terpineol, cis-limonene 1,2-epoxide, D-carvone, 3-methyl-2-buten-1-ol, styrene and methyl (2-methyl-3-butene-2-yl) ether, and alpha-gujajene	PCA and PLS-DA	GC-MS	(Wu et al., 2023)
Fuji apples	<i>Penicillium expansum</i>	GC-MS	(E)-hex-2-enal, 1-methoxy-3-methylbenzene, methyl heptanoate, diethyl carbonate, ethyl 2-phenylacetate, propyl octanoate, and ethyl decanoate	PCA	GC-MS	(Kim et al., 2018)

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	<i>Botryosphaeria dothidea</i>		(E)-hex-3-enyl acetate, 1-methyl-4-propan-2-ylbenzene, 2-phenylethanol, α -terpinene, and α -terpinolene			
	<i>Alternaria alternata</i>		phenylmethanol, 2-ethylhexan-1-ol, and acetophenone			
Wheat Kernels	<i>Fusarium graminearum</i>	GC-MS	<i>F. graminearum</i> PH-1 infestation: 5-pentyl-cyclohexa-1,3-diene, 3-hexanone, and 1,3-octadiene. Zearalenone production: 6-butyl-1,4-cycloheptadiene, hexahydro-3-methylenebenzofuran-2(3H)-one, and (E,E)-3,5-octadien-2-one	PCA and PLS-DA	GC-MS	(Ji et al., 2022)
Maize kernels	<i>Aspergillus flavus</i>	HS-GC-IMS	<i>Aspergillus flavus</i> : ethyl acetate-D and 3-hydroxybutan-2-one-D. AFB ₁ production: (E)-2-octenal-M, benzene acetaldehyde, (E)-hept-2-enal-M, 2-heptanone-D, and 2-pentyl furan	PCA	HS-GC-IMS	(Li et al., 2021)
Grape (Cabernet Sauvignon and Petit Manseng)	<i>Botrytis cinerea</i> , <i>Coniothyrium diplodilla</i> (Speg.) Sacc., <i>Colletotrichum</i> sp.	GC-IMS	Hexanal and (E)-2-hexenal	PCA, NAA	GC-IMS	(Li et al., 2022)
Strawberry	<i>Botrytis cinerea</i>	HS-MCC-IMS HS-SPME fast GC-MS	3-methylbutanal, cis-4-decenal, 2-methyl-1-butanol, 2-methyl-1-propanol, 1-octen-3-one and 1-octen-3-ol	Multivariate data analysis	HS-MCC-IMS HS-SPME fast GC-MS	(Vandendriessche et al., 2012)

^a PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; NAA, nearest axis aligned; HS-MCC-IMS, headspace-multi-capillary column-ion mobility spectrometry; HS-SPME, headspace solid-phase microextraction.

3.2.2. Electronic Nose (E-Nose) Technology

With the development of electronic technology, an online detection method E-nose based on a semi-selective sensor array and pattern recognition is increasingly

avored for rapid VOC detection (Gu et al., 2020). Among the most widely used are metal oxide semiconductor (MOS) sensors, which detect VOCs based on changes in electrical resistance upon gas adsorption onto a metal oxide surface. In addition to MOS, other types including metal oxide semiconductor field-effect transistors (MOSFET), piezoelectric crystal (PC), quartz crystal microbalance (QCM), electrochemical sensors, and surface acoustic wave (SAW) (Ali et al., 2023). MOSFETs offer enhanced sensitivity and rapid signal transduction by detecting gas interactions at the gate region of the transistor. PC and QCM sensors function by measuring frequency shifts resulting from the adsorption of VOCs onto the crystal surface, providing real-time detection (Fan et al., 2023). Electrochemical sensors operate based on redox reactions at an electrode and are particularly useful for detecting specific gas species with high selectivity. SAW sensors, which detect variations in acoustic wave propagation caused by gas adsorption, are noted for their excellent selectivity (Wu et al., 2023). Among all these sensor types, MOS sensors remain the most widely adopted in agricultural contexts due to their high sensitivity, low cost, and suitability for high-volume applications. Their LOD is typically ≥ 1 ppm, depending on the specific VOCs, usage scenarios, and sensor types. MOS sensors are sensitive to a wide range of agricultural VOCs and are particularly suitable for trend analysis of high-level or composite gases in greenhouse and storage monitoring. Other sensors, such as QCM and SAW, offer a comparable application range to MOS but, with the use of diversified coatings, can achieve broader VOC detection capabilities (Zytek et al., 2023; Ali et al., 2023)

As a representative example of an artificial olfactory system, E-nose is superior to many conventional methods by its noninvasive, fast, and user-friendly features, and has been applied to detect pathogen contamination in food in many recent works (Table 8). At present, commercially available E-nose uses pre-existing data (collected in previous experiments) and a trained model (from machine learning or statistical analysis) to recognize different VOC patterns, which can effectively be used to identify different VOC in food samples (Nouri et al., 2020). Some food contaminated by microorganisms metabolizes signature VOC compounds, which offer unique odor fingerprints that can be used for pathogen early identification and monitoring (Giungato et al., 2018; Gu et al., 2019). A comprehensive description of the application of E-nose for pathogen early monitoring has been thoroughly discussed by Bonah et al. (2020).

Although E-nose allows for non-invasive detection, its application in agriculture is limited by challenges like sensitivity to temperature and humidity, difficulty in distinguishing volatile compounds and concentrations, and high costs for commercial use. Comprehensive reviews of the advantages and disadvantages of e-nose-based approaches have been discussed in detail in numerous research works (Ali et al., 2023; Shi et al., 2017).

Table 8. Recent studies on food pathogens detection based on key VOCs components by Enose^b.

Sample	Pathogen	Signature VOC compounds	Pattern recognition	Reference
Brown rice grain	<i>Aspergillus sp.</i>	Octane, 2,2,4,6,6-pentamethylheptane, decane, dodecane, toluene, ethanol, 1-pentanol, 1-hexanol, 1-octen-3-ol, 2-heptanone and 2-pentylfuran	PCA, LDA, SVM	(Jiarpinijnun et al., 2020)
Grains	<i>Aspergillus</i>	1-octanol and tetradecane	PCA	(Gu et al., 2019)
Apples	<i>Penicillium expansum</i>	Nitrogen oxides, Broad methane, Sulfur-containing organics, Aromatics, organic sulfides	PCA, PCA-DA, LDA, PLS-DA, KNN	(Guo et al., 2020)
Japonica rice (<i>Oryza sativa</i> subspecies. Japonica)	Fungal	1-octen-3-ol and 3-octanone	PCA	(Zhang, 2022)
Apple	<i>Staphylococcus</i> , <i>Salmonella</i> , <i>Shigella</i>	Acetone, ethanol, methanol, acetaldehyde, ammonia and propanol	PCA, HCA	(Ezhilan et al., 2018)
Peaches	<i>Botrytis cinerea</i> , <i>Monilinia fruticola</i> and <i>Rhizopus stolonifer</i>	Terpenes (e.g., β -myrcene and α -pinene) and aromatic compounds	PCA, PLSR	(Liu et al., 2018)
Peanut	<i>Aspergillus sp.</i> (<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i> , and <i>Aspergillus ochraceus</i>)	Amines, ammonia, alcohols and ketones	PC- LDA, PLSR	(Shen et al., 2018)

^b PCA, principal component analysis; LDA, linear discriminant analysis; SVM, support vector machine; PCA-DA, principal component analysis followed by discriminant analysis; HCA, hierarchical cluster analysis; PLS-DA, partial least squares discriminant analysis; KNN, K-nearest neighbor; PLSR, partial least squares regression.

3.2.3. Spectroscopic-based Approaches

Spectroscopic techniques detect VOCs by examining the interactions between electromagnetic radiation and VOC molecules. These methods have garnered significant attention in food research due to their inherent advantages, including high biosafety, unique molecular fingerprinting capabilities, and strong penetration depth. This category encompasses a wide array of techniques such as infrared (IR) spectroscopy, Raman spectroscopy, near-infrared (NIR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, fourier transform infrared spectroscopy (FTIR), photoionization detection (PID), laser-induced fluorescence (LIF), cavity ring-down spectroscopy (CRDS), terahertz imaging, hyperspectral reflectance analysis, luminescence-based detection, and colorimetric imaging. Each method offers unique advantages: IR, Raman, and NIR spectroscopy provide molecular vibrational information that supports functional group identification, with IR and Raman offering more distinct fingerprinting capabilities, while NIR excels in rapid and non-

destructive quantification (Niklas et al., 2021). In practical applications, Raman spectroscopy is more suitable for high-moisture samples (such as fruits and vegetables) due to its insensitivity to water. However, its key limitation is an extremely low scattering cross section, making trace gas detection with low LOD challenging. UV-Vis spectroscopy excels in analyzing conjugated compounds and pigments, suitable for rapid screening; FTIR offers fast, non-destructive analysis with high sensitivity, selectivity, and resolution, achieving detection limits as low as ppb levels. However, its high cost, susceptibility to matrix interference, and bulky equipment restrict its use in field applications (Zhou et al., 2022; Liu et al., 2023; Epping & Koch, 2023). PID supports real-time monitoring and offers a strong balance between sensitivity and selectivity, especially effective for field applications (Rezende et al., 2019). CRDS achieves ultra-sensitive gas-phase measurements by extending optical path lengths; and hyperspectral imaging enables spatially resolved VOC detection in heterogeneous food matrices (Kwaśny & Bombalska, 2023). Terahertz imaging, though less mature, is gaining attention for its ability to penetrate packaging materials and detect early spoilage signals at the molecular level (Hindle et al., 2018; Abina et al., 2023).

Several hybrid sensing strategies have been developed by integrating complementary mechanisms. A notable example is the AI-enhanced ion mobility and mid-infrared spectroscopy (IMMS), which synergistically combines multi-dimensional signal features to achieve high-accuracy VOC identification (Zhu et al., 2023). Another emerging approach leverages guided-mode resonance (GMR) structures coupled with infrared spectroscopy to improve detection sensitivity (Tantiwanichapan et al., 2023). The GMR concept, applicable to both optical fiber and on-chip platforms, offers enhanced light-matter interaction. For instance, a graphene oxide-coated, nanopatterned fiber-tip GMR sensor has demonstrated selective and sensitive detection of ethylene and methanol, highlighting the potential of this integrated photonic design (Tabassum et al., 2017). Recent studies on agriculture product pathogens detection based on key VOCs by spectroscopic approaches were concluded in Table 9.

Table 9. Recent studies on food pathogen detection based on key VOC components using E-nose technology^c.

Sample	Pathogen	Approaches	Signature VOC compounds	Reference
Peanuts	<i>Aspergillus spp.</i>	NIR	-	(Shen et al., 2018)
Crop	-	GMR	Ethylene and methanol	(Tabassum et al., 2017)
Agriculture products	-	FTIR	Acetone, ethanol, and isoprene	(Zhou et al., 2022)
Rice	-	LOPGP-FTIR	-	(Liu et al., 2023)
-	-	IMMS	Isopropyl alcohol	(Zhu et al., 2023)

^c NIR, near Infrared; GMR, guided-mode resonance; FTIR, fourier transform infrared spectroscopy; LOPGP-FTIR, long optical path gas phase fourier transform infrared spectroscopy. IMMS, ion mobility and mid-infrared spectroscopy

3.2.4. Other Sensor Arrays and Nanotechnology-Based Approaches

Some other approaches rely on reactive interactions (chemical or biological) to detect VOCs. Similar to the E-nose, the colorimetric sensor is another main branch of olfactory visualization sensors. Colorimetric sensor arrays are optical arrays based on chemical-response colorants such as dyes and nano-porous pigments, depending on the color changes produced by the reaction between color-sensitive materials and VOCs, from where the odor information can be converted into digital images thus qualitatively or quantitatively analyzing the target attributes (Wang et al., 2021). The colorimetric sensor has been successfully applied to many fields of food and agriculture for many years, including food freshness, shelf-life monitoring, and quality analysis such as tea quality analysis (Li et al., 2023), and food varieties distinction such as rice varieties discrimination (Arslan et al., 2022). The colorimetric sensor has recently been used in wheat mildew monitoring and has shown great potential as a noninvasive “odor visualization” monitoring technique (Liu et al., 2023). Previous studies have shown that the colorimetric sensor can maintain its stability and sensitivity at different ambient temperatures and humidity, which makes up for the defects of MOS-type E-nose (Arslan et al., 2022). However, different dyes often react to the interaction of the same VOCs in the mixture, resulting in the deficiency of the existing colorimetric sensor in quantifying VOCs (Duan et al., 2021). Many previous studies worked on the detection and monitoring of mildew wheat by the colorimetric-based sensor array (Lin et al., 2023; Wang et al., 2021). Compared with the traditional detection methods, the colorimetric sensor makes it easier to achieve miniaturization and portability. At present, qualitative portable arrays have been tried to be used in food packaging and commercialized in the detection of food freshness. Guo et al. (2020) combined cross-reactive colorimetric barcode combinatorics and deep convolutional neural networks to monitor meat freshness by the colorimetric portable sensor array. The portable prediction platforms can also be applied to detect metabolic

VOCs of food spoilage. Drawing on its data processing tools, it can help the development of grain and oil mildew sensors.

The whole-cell biosensor based on synthetic biology is an emerging food safety rapid on-site detection technology. Whole-cell biosensors use live cells as sensing elements such as transcription factors and riboswitches and reporting components such as fluorescence and gases, etc. (Chen et al., 2023). The sensing and reporting elements are coupled by gene expression regulation and coupling to form a simple gene circuit, converting information into a recognizable signal to detect the target substance. Compared with other early detection techniques, whole-cell biosensors exhibit strong anti-interference capability due to the relative stability of the living cell environment. At the same time, there is no need for complex sample pretreatment. The biosensor mass production speed is fast with a low manufacturing cost and is portable and user-friendly. These significant advantages give whole-cell biosensors great application potential and market value in field-operable real-time food early monitoring applications (Chalupowicz et al., 2020). However, their sensitivity is generally moderate, typically at the ppm level. Reports indicate that the lowest LOD for three infection-derived VOC markers—nonanal, 3-methyl-1-butanol, and 1-octen-3-ol—are 0.17 ppm, 2.03 ppm, and 2.09 ppm, respectively (Ma et al., 2020). As an onsite monitoring tool, whole-cell biosensors rely on biological activity and thus have relatively short shelf-life. Because of the risk of cell inactivation, it mostly serves research purposes yet and faces the problem of storage in practical applications in agriculture (Ma et al., 2023). Thus, sustained efforts are still needed to improve the capacity of whole-cell biosensors that are currently tested at the laboratory level.

Other innovative sensors include chemi resistors based on the change in electrical resistance, electrochemical sensors through electrochemical reactions, piezoelectric sensors based on quartz crystal vibration, conductometric sensors based on conducting polymers or nanomaterials, etc. (Moon et al., 2022; Ba Hashwan et al., 2023). They are often more suited for portable, real-time, and field applications, providing a versatile and cost-effective alternative for VOC detection. Recent studies on food pathogens detection based on key VOCs by relative interactions were concluded in Table 10.

Table 10. Recent studies on food pathogens detection based on other sensor arrays and nanotechnology-based approaches^d.

Sample	Pathogen	Sensor type	Signature VOC compounds	Reference
Wheat	<i>Aspergillus flavus</i>	Colorimetric sensor	1-Octen-3-ol	(Duan et al., 2021; Lin et al., 2023; Wang et al., 2021)
Shelled peanuts and maize kernels	<i>Aspergillus flavus</i> strain ACCC 32656	Whole-cell biosensor	2-acetyltoluene, sulfurous acid, 2-ethylhexyl hexyl ester, ethyl propionate, 1-methyl-1 H-pyrrole, 3,5-heptadiyn-2-one, and hexanal	(Ma et al., 2023)
Citrus fruit	<i>Penicillium digitatum</i>	Whole-cell biosensor	Limonene	(Chalupowicz et al., 2020)
Potato tubers	<i>Pectobacterium</i>	Whole-cell biosensor	1-octanol, phenylethyl alcohol, 2-ethyl hexanol, nonanal, and 1-octen-3-ol.	(Veltman et al., 2022)

^d MOF, metal-organic framework.

To facilitate the selection of appropriate VOC detection techniques for various postharvest and food safety applications, it is essential to evaluate the strengths and limitations of each approach systematically. While each method—ranging from spectrometry-based systems and E-nose configurations to spectroscopic and emerging biosensor platforms—offers unique advantages in terms of sensitivity, specificity, or field deployability, they also come with trade-offs involving cost, scalability, and environmental robustness. Therefore, a comprehensive comparison of these methods with respect to critical performance indicators, including sensitivity, specificity, cost, scalability, application scope, and known limitations, is summarized in Table 11 to support technology matching in practical agricultural contexts.

Table 11. Comparison chart of main VOC detection techniques.

Technique		Limits of detection (LOD)*	Sensitivity	Specificity / Resolution	Cost	Scalability / Portability	Limitations	Applicability in agricultural scenarios	Reference
Spectrometry-based Approaches	GC-MS	ppt-ppb level (depend on sample preparation techniques)	High	High (m/z-based compound identification)	High	Low (Normally lab-based, portable device exist)	Bulky equipment, not suited for real-time or field use	Suitable for low concentrations of metabolites or disease signaling molecules in most agricultural products.	(Kataoka et al, 2000; Baimatova & Gionfriddo, 2025)
	PTR-MS	0.1-0.5 ppb	Very High	Moderate	High	Moderate (Commercialized compact and semi-portable devices.)	Limited mass resolution, expensive, matrix interference	Suitable for on-site and field applications, such as postharvest storage air monitoring.	(Schuhfried et al., 2017; Mazzucotelli et al., 2022).
	GC-IMS	0.1 ppb	High	Moderate	High	Low	High cost, low portability	Suitable for rapid screening and early warning	(Giménez-Campillo et al., 2025)
	MIMS	≥1 ppb	Moderate	Moderate	Moderate	Moderate	Membrane fouling, semi-selective, requires calibration	Suitable for realtime monitoring of VOC in the ambient air	(Ketola et al., 2002; (Richards et al., 2018).
	X-ray-based techniques	1-100 ppm	Low	Low	High	Low	High equipment costs, complex instrumentation requirements, not suitable for trace concentrations	Suitable for nondestructive detection of food, such as spoilage markers and high-concentration pesticide residue	(Feng et al., 2021).
Electronic Nose (E-Nose) Technology	E-nose (MOS)	≥1 ppm	Moderate to High	Moderate	Low	High	Cross-sensitivity to humidity/temperature, drift over time	Sensitive to diverse agricultural VOCs and suitable for trend analysis of high-level or composite gases in	(Ali et al., 2023)

								greenhouse and storage monitoring.	
	E-nose (SAW/QCM)	≥1 ppm	Moderate to High	High	Moderate	High	Requires controlled conditions, limited robustness outdoors	Comparable to MOS in application range, but with diversified coatings for broader VOC detection.	
Spectroscopic-based Approaches	IR/Raman/NIR Spectroscopy	ppm level	Moderate to High	Moderate to High	Moderate to High	Moderate to High	Expensive, sensitive to matrix interference	Suitable for high-moisture samples (such as fruits and vegetables) Detecting trace VOCs is difficult.	(Niklas et al, 2021; Zhou et al., 2022; Liu et al., 2023; Epping & Koch, 2023)
	UV-Vis Spectroscopy	ppm level	Moderate	Moderate	Moderate	Moderate	Limited to chromophoric compounds, low spatial resolution	Suitable for common VOCs rapid screening.	
	FTIR	ppb level	Moderate to High	High	High	Moderate	Expensive, sensitive to matrix interference, limit application in the field	Infrared absorption is effective for crop metabolite detection but fails to detect homonuclear gases like N ₂ , O ₂ , and H ₂ . Limit field applicability.	
	Photoionization detection	ppb level (0.6 ppm for toluene)	High	Low to moderate	Low	High	Cannot distinguish individual VOC species without a pre-separation step (e.g., with GC).	Suitable for detecting phenolic or terpene VOCs.	(Rezende et al., 2019)
	CRDS	ppt level	Very High	High	High	Low	Complex setup, expensive optics, lab-restricted	Suitable for trace signal.	(Kwaśny & Bombalska, 2023).
	Terahertz Imaging	≥1 ppm	Moderate	Moderate	High	Low	High cost, limited availability, sensitive to matrix interference,	Suitable for agricultural product quality inspection, detecting volatile gases (such as H ₂ S, methanethiol,	(Hindle et al., 2018; Abina et al., 2023).

Chapter 3

Other Sensor Arrays and Nanotechnology-Based Approaches							immature technology	ethanol, ammonia, etc.) in packaged foods (e.g., salmon).	
	Hybrid sensing strategies (IMMS)	ppb level	High	High	High	Moderate	Cost significantly higher than traditional single sensor	Multi-mode joint detection is suitable for complex scenarios.	(Zhu et al., 2023)
	Colorimetric Sensor	ppm level	Moderate	Moderate	Low	High	Semi-quantitative, dye cross-reactivity, limited specificity	Detects spoilage markers in agri-foods (e.g., wheat mildew), or enables real-time VOC monitoring in packaging for freshness assessment.	(Li et al., 2023; Liu et al., 2023)
	Whole-cell Biosensor	ppm level (nonanal, 3-methyl-1-butanol, and 1-octen-3-ol was 0.17-, 2.03-, and 2.09-ppm)	Moderate	High (target-dependent)	Low	High	Short shelf-life, cell stability issues, lab-stage maturity	Detect VOCs or pathogen metabolites released during infections to achieve non-invasive early diagnosis	(Ma et al., 2020; Veltman et al., 2022)

*The scope of LOD depends on the specific VOC and usage scenarios. The data in the table is a typical range. Please refer to the reference for details

3.3. Practical applications: VOC-based Detection of Agricultural Product Deterioration

3.3.1. Fruits and Vegetables

Some studies have evaluated different novel VOC-based detection technologies, which can be effectively applied in the early monitoring of agricultural product deterioration. In studies on fruit and vegetable freshness, [Voss et al. \(2019\)](#) presented a promising prototype of an E-nose to perform the VOCs emitted in the peach growth cycle including the postharvest stage, demonstrating a high accuracy for the reduced data set for 7 sensors. [Zhang et al. \(2022\)](#) conducted a correlation analysis of quality changes and shelf-life of postharvest apples based on E-nose and GC-MS, the prediction model offered an effective forecasting of apple shelf life at different temperatures. [Ifmalinda \(2022\)](#) showed the potential application of e-nose to identify mechanically damaged avocados. Similar applications have also shown great potential in fresh-cut vegetables, such as characterization spoilage markers in broccoli ([Chen et al., 2019](#)), green bell pepper ([Chen et al., 2018](#)), and iceberg lettuce ([Ioannidis et al., 2018](#)). In addition to predicting freshness and damage, VOC-based detection is also suitable for determining postharvest diseases. [Hagbin et al., \(2022\)](#) assessed an experimental E-nose system and machine learning for early detection and monitoring of *Botrytis cinerea* in Hayward kiwifruit based on odor-extracted information, demonstrating the effectiveness of the radial basis function neural network trained with CFS-selected features in achieving highly accurate classification. In previous research, E-nose has also been used in the detection and discrimination of common fungal pathogens in peaches ([Liu et al., 2018](#)), apples ([Guo et al., 2020](#)), and pomegranates ([Nouri et al., 2020](#)). Fruits and vegetables generally have more prominent VOC markers, such as terpenes/terpenoids, ketones, esters, and alcohol ([Gu et al., 2022](#); [Walters et al., 2020](#)). These VOC compounds are key contributors to fruits and vegetables' fruity and aromatic characteristics, while grains and legumes produce very few esters naturally, with their VOC profiles dominated by aldehydes, alcohols, and other compounds.

3.3.2. Grains and Legumes

Considering the quality of grains and legumes in the postharvest stage, sensors have been applied more to evaluate early mold contamination and predict the disease. [Jiarpinijnun et al. \(2020\)](#) visualized VOCs profiles of fungal infection during storage of Jasmine brown rice by E-nose coupled with chemometrics. [Lin et al. \(2019\)](#) presented a novel colorimetric sensor based on nanoscaled chemo dyes that can detect inert VOCs during the mildewing process of stored wheat. The infection of the wheat sample with *F. graminearum* also showed a specific relationship between the composition of fungal flora and other VOCs such as 5-pentyl-cyclohexa-1,3-diene, 3-hexanone, and 1,3-octadiene ([Ji et al., 2022](#)). [Li et al. \(2021\)](#) have shown that potential biomarkers specific to *A. flavus* contamination in maize kernels, and potential VOCs

correlated with the level of mycotoxin AFB₁. Grains and legumes typically have higher levels of aldehydes due to lipid oxidation, making aldehydes strong markers of rancidity and spoilage in these foods, such as hexanal in peanuts and maize (Ma et al., 2023). In addition, alcohol production is a major feature in grain spoilage, while only minimal alcohol formation during storage in legumes. Thus, alcohols are more likely to be found in grains as signature markers. Much previous research reported different colorimetric sensors to monitor key VOCs marker 1-octen-3-ol that is emitted after mold infection in wheat (Duan et al., 2021; Lin et al., 2023; Wang et al., 2021).

3.4.Challenges and Limitations

3.4.1.Complexity and Diversity of Volatilomics

The differences in the types and concentrations of VOCs can vary widely based on factors like the source (e.g., sample variety), location (e.g., storage conditions, environmental factors), treatment (e.g., processing, handling, chemical treatments), microbial activity (e.g., bacteria and fungi), and time (e.g., aging, ripeness, and maturity). VOCs profiles vary among different types of agricultural products because of the different plant's metabolic processes, composition, and structural characteristics. In recent studies, approximately 360 VOCs have been identified for strawberry aromas, and 200 VOCs have been identified in raspberries (Song et al., 2016). This complexity makes it difficult to detect, identify, and quantify all relevant VOCs accurately, especially when they are present at trace levels. Analyzing this variability is crucial for quality control, food safety, and flavor and aroma assessments. It can also help identify factors that affect the shelf life and overall quality of these products.

3.4.2.Interference from External Factors

The interference of external factors in detection technologies is a critical challenge in terms of the variety of VOCs composition. The first is the interference of external odors. Since detecting deterioration through VOCs in agricultural products is an indirect method, it requires sampling and analyzing the VOCs emitted by the sample. This process can be easily influenced by other VOCs present in the environment. Such as residues of pesticides and fertilizers, and interfering gas in the air may be mixed with target VOCs, leading to misleading detection results. For example, high temperatures may increase volatile release, changes in humidity may affect the response of sensors, and atmosphere control may influence plant metabolism and microorganism VOCs emissions (Cellini et al., 2021). Especially in open agricultural settings, changes in meteorological conditions can impact the diffusion and concentration of volatile compounds in the storage. The composition of VOCs in agricultural products is also highly influenced by environmental stresses, which produce induced VOCs in response to various biotic and abiotic stresses. As mentioned above, the stressors mainly include microorganism infection, pest attacks, temperature and humidity variations, and other environmental stressors (Tiwari et al., 2020). These may lead to changes in the type and concentration of VOCs produced

by agricultural products, thus interfering with the detection results (Murali-Baskaran et al., 2022).

3.4.3. Standardization and Validation of Detection Methods

Standardization and validation are crucial for ensuring accuracy and reproducibility in volatilomics. However, the lack of standardized protocols for VOC sample collection, processing, and analysis leads to inconsistent results across studies, affecting reliability. Different extraction methods, like GC-MS for specific components and the E-nose for overall patterns, can produce varying VOC profiles. For instance, none of the hundreds of identified VOCs in strawberries were consistently reported across studies (Ulrich et al., 2018). To improve consistency, combining multiple technologies and establishing standard samples and procedures is recommended. Validation involves assessing calibration, accuracy, precision, sensitivity, and stability to ensure reliable results. Calibration curves and field tests help correct for instrument drift and environmental interference. In field conditions, environmental factors like temperature, humidity, and airflow can affect the instrument's readings. Calibration in these conditions ensures that measurements are accurate in the real world. The field test can help researchers correct errors caused by instrument drift, external factor interference, or sensitivity changes (Hong et al., 2023).

Overall, to address the challenge of accurate, reliable, and reproducible VOC detection in field sampling, it is essential to regularly calibrate instruments using reference standards and perform multiple sampling trials to ensure reproducibility. Standardizing VOC extraction and collection methods can minimize variability, while comparing results with laboratory standards helps verify accuracy. Sensitivity should be assessed by testing detection limits and accounting for environmental background noise. Controlling environmental factors, using field blanks, and cross-validating with multiple detection technologies can further enhance reliability. Monitoring and correcting instrument drift, performing on-site field tests, and implementing standardized reporting protocols are also critical to improving data consistency and comparability across studies.

3.5. Integration into Smart Monitoring Systems

3.5.1. Internet of Things (IoT) Applications in Postharvest Monitoring

IoT technologies and intelligent sensing involve connecting devices through networks to achieve interconnectivity and intelligent control (Figure S2). These devices are usually equipped with sensors, software, and other technologies to collect and exchange data, thereby improving efficiency and accuracy by data aggregation, real-Time feedback, redundancy identification, and remote updates (Lutz & Coradi, 2022). At present, there have been a large number of applications of the IoT in agriculture, such as in the aspect of smart farming, precision agriculture, resource management, soil management, pest and disease monitoring, etc. (Navarro et al.,

2020). In addition to monitoring agriculture products in the field, they are becoming an indispensable practice of postharvest agricultural monitoring, such as environment management that can remotely control the storage environments, disease prediction that can early monitor pest or microorganism contamination. Sanjeevi et al. (2020) introduced a postharvest hierarchical model based on ontology, facilitating the IoT-driven prevention of postharvest losses and the accurate differentiation of healthy sekai-ichi apples. Similarly, a real-time IoT-based monitoring system continuously tracks temperature, humidity, luminosity, and gas concentrations in cold storage, automatically alerting personnel when conditions exceed safe thresholds (Afreen & Bajwa, 2021). The IoT-based notification system can integrate cloud data to enable unified management of farms and granaries with similar storage environments within the same region. When IoT sensors detect signs of spoilage in any granary, real-time data, such as temperature fluctuations or volatile organic compound levels, are uploaded to the cloud and used to alert other granaries with similar environmental conditions. This allows for quicker responses and prevention of environmental changes or anomalies, thereby enhancing overall management efficiency and ensuring food security.

Smart monitoring systems leverage agricultural data to build AI models, with real-time data collection and automation key to advancing the industry. These systems offer benefits in postharvest agriculture, such as disease detection and efficient data analysis. However, challenges such as external interference affecting data accuracy and increased risks from technological complexity, privacy, and security require robust intrusion detection systems and IoT-specific architectures to effectively mitigate these security risks (Qaddos et al., 2024).

3.5.2. Artificial Intelligence (AI) and Machine Learning for Data Analysis

Artificial intelligence, particularly machine learning, has become indispensable in transforming raw sensor data into actionable insights for postharvest quality control. AI algorithms enable the system to automatically learn from multidimensional environmental and volatile compound data, allowing for classification, forecasting, and pattern recognition without the need for manual rule-based programming (Lutz & Coradi, 2022). Pre-processing techniques—such as baseline correction, normalization, and data compression—are typically employed to reduce noise and standardize sensor responses, enhancing the reliability of subsequent analysis (Andre et al., 2022).

Typically, VOC information is processed using two primary categories of algorithms: statistical models and intelligent model analysis (Kim et al., 2022). Statistical methods, including principal component analysis (PCA), linear discriminant analysis (LDA), and support vector machines (SVM), are commonly applied for dimensionality reduction and visualization of sensor response patterns. These methods are computationally efficient, highly interpretable, and particularly effective for small datasets where linear relationships exist between features and

classes. They are commonly used for exploratory data analysis, visual separation of classes, and quality classification under well-controlled experimental conditions. However, their capacity to model nonlinear interactions is limited, and their performance may degrade in the presence of noise or complex feature structures (Moshayedi et al., 2023). In contrast, intelligent methods such as artificial neural networks (ANN), multilayer perceptron (MLP), k-nearest neighbors (kNN), and tree-based methods like decision tree (DT), random forest (RF) and XGBoost offer powerful alternatives. These intelligent algorithms are particularly advantageous when dealing with large-scale, high-dimensional datasets or in dynamic sensing environments where adaptability and high prediction accuracy are required (Kim et al., 2022; Wang et al., 2022). Nevertheless, these algorithms are often criticized for their black-box nature, their susceptibility to overfitting when training data is limited (Chowdhury et al., 2022; Christmann et al., 2022). In real-time applications, latency and computational overhead present further constraints, requiring lightweight or optimized architectures to support responsive deployment in embedded systems (Li et al., 2023; Assimakopoulos et al., 2024). Advanced models designed to address challenges involve deep learning architectures such as convolutional neural networks (CNNs) and long short-term memory (LSTM), explainable AI (XAI), as well as evolutionary approaches like AdaBoost and genetic algorithms, all of which offer enhanced capability in handling complex, nonlinear, and high-dimensional odor datasets (Nataraian et al., 2024; Wang & Liu, 2024).

Algorithm selection should be guided by data scale, task complexity, interpretability, and available resources. Statistical models (e.g., PCA, LDA, SVM) are suitable for small, interpretable tasks, while intelligent models (e.g., RF, XGBoost, CNNs) perform better in high-dimensional, real-time settings. Hybrid approaches combining both are increasingly favored for balancing accuracy and transparency. For applications demanding traceability, integrating XAI is recommended. Additionally, auto-machine learning offers a promising solution to automate model selection and optimization, lowering deployment barriers. In practice, different machine learning models have proven particularly effective in tasks such as AI-enhanced mid-infrared gas spectroscopy to track Isopropyl alcohol biomarker (Zhu et al., 2023), detecting fungal infections like gray mold in stored kiwifruit (Haghbin et al., 2022), and classifying produce based on ripeness or microbial spoilage VOCs (Ma et al., 2023; Voss et al., 2019). Furthermore, feature extraction algorithms can automatically identify the most relevant volatile signatures from massive datasets, and multimodal fusion techniques integrate data from multiple sensing platforms can improve predictive accuracy (Lee, 2023). Future research should emphasize interpretable, resource-efficient AI architectures that can adapt to dynamic environmental conditions while ensuring scalability, traceability, and robustness in real-world deployments.

3.5.3. Real-time Monitoring and Intelligent Decision Support

Building on the synergy between IoT infrastructure and AI algorithms, real-time monitoring systems are capable of dynamically evaluating the freshness and safety of stored agricultural products. These systems utilize VOC fingerprint sensing and environmental data streams—such as temperature, humidity, and gas composition—which are uploaded to cloud servers and interpreted by trained AI models. The resulting outputs inform intelligent alerts, actionable recommendations, or automatic control responses aimed at preserving quality and reducing spoilage losses (Qaddos et al., 2024). For example, the integration of AI and IoT has enabled the development of systems that predict AFB₁ contamination based on environmental trends (Moshayedi et al., 2023) and others that classify grain quality or monitor oxidative stress responses in crops (Ebrahimi et al., 2014; Lew et al., 2020). The continuous tracking of VOCs and instant feedback to users allows for a proactive rather than reactive approach to postharvest management.

As these smart systems evolve, they are expected to not only support food supply chain resilience but also drive sustainability, cost-efficiency, and eco-friendliness through precise environmental control and waste reduction. Future development will likely focus on adaptive AI models, secure data architectures, and low-power edge computing to further enhance responsiveness, scalability, and integration.

3.6. Development Trends and Future Prospects

3.6.1. Current Development Trends of Detection Technologies

The current technological landscape of VOCs detection mainly includes a combination of conventional detection technologies with miniaturization technology. Many sensor technologies have been applied to VOCs detection in agricultural product deterioration, such as E-noses and gas sensors. There are also many notable recent advancements based on VOCs detection such as IoT Integration, sensor miniaturization, microfabrication and nanotechnology, self-powered sensing systems, AI-enabled real-time monitoring, selective sensors, microelectromechanical systems, printed and flexible electronics technologies, microfluidic systems, smartphone-based gas detection, and wireless communication (Epping & Koch, 2023). Advances in these systems continue to drive innovation in the field of VOCs detection, thereby the device is becoming more compact, intelligent, and portable while maintaining high analytical performance.

The main challenges of these advances lie in the manufacture of appropriate electronic components (acquisition and transmission modules), signal processing, and software for data collection, such as all major components of the GC system have been reduced. In these cases, instrument sensitivity can be adjusted by modifying the input flow rate. Conducting environmental air analysis or headspace sampling in odorless bags or gas canisters is the simplest option, minimizing the possibility of obtaining artificial results. However, when these options are impractical (e.g., when gas samples are too small or the content of target compounds is trace), the use of

VOCs adsorbent materials to concentrate VOCs samples can be considered (Cellini et al., 2021). You et al. (2020) reported a portable gas chromatograph featuring a carbon nanotube sponge preconcentrator, offering improved sensitivity and detection limits for trace VOCs in air samples. Dey et al. (2021) introduced a NiO/ZnO-based p–n junction single-diode device capable of selectively sensing multiple VOCs simultaneously by adjusting the bias voltage. Huang et al. (2021) developed a battery-powered portable E-nose system using multiple metal oxide gas sensors and machine learning algorithms to detect and classify VOCs in wine. While most current commercial E-nose devices rely on MOS-type gas sensor arrays, ongoing research is exploring alternative materials, such as two-dimensional transition metal carbides/nitrides (MXenes), which are gaining attention for improving gas sensing sensitivity (Chen et al., 2020). The development of VOCs collection and device miniaturization has greatly advanced VOCs measurements, proving particularly useful for indoor storage assessments and field studies.

3.6.2. Development of Portable and On-site Detection Systems

Portable and on-site detection enables real-time analysis directly at the point of storage or processing of agricultural products, eliminating delays associated with transporting samples to centralized laboratories. Recent advancements have demonstrated the potential for visualizing VOC fingerprint differentials using portable platforms, such as two-dimensional code-configured E-nose systems. For example, M Conrado et al. (2021) developed a simple, non-invasive, paper-based optoelectronic nose arranged in a QR code format to assess olive oil aroma. Similarly, Chang et al. (2018) introduced a photonic crystal-based sensor fabricated via a “nanoscale easy tear (NET)” process, inspired by the concept of tearable packaging. This compact, low-cost sensor enables effective on-site monitoring through colorimetric responses.

Integration with smartphones further enhances the usability of these systems by leveraging existing hardware for data visualization, user interface, and wireless communication. Li et al. (2019) developed a smartphone-based platform for plant disease diagnosis using a disposable colorimetric sensor array capable of detecting leaf volatiles at ppm levels within one minute. Lew et al. (2020) presented a Raspberry Pi system with a charge-coupled device camera that replicates smartphone imaging functionality for real-time and low-cost VOC monitoring.

3.6.3. Development of Multi-parameter Monitoring

Spoilage is a complex process influenced by various factors, and a comprehensive assessment requires consideration of multiple parameters simultaneously (Snyder et al., 2024). In addition to VOCs analysis, assessing different odor characteristics, monitoring the pH and acidity of agricultural products, conducting microbiological and texture analyses, temperature and humidity monitoring, conductivity measurement, water activity determination, etc., are also crucial for understanding chemical changes and the growth of

spoilage microorganisms in agricultural products (Fan et al., 2023). A comprehensive assessment enables a more nuanced understanding of the factors influencing product quality and facilitates timely interventions to mitigate spoilage risks (Figure 4).

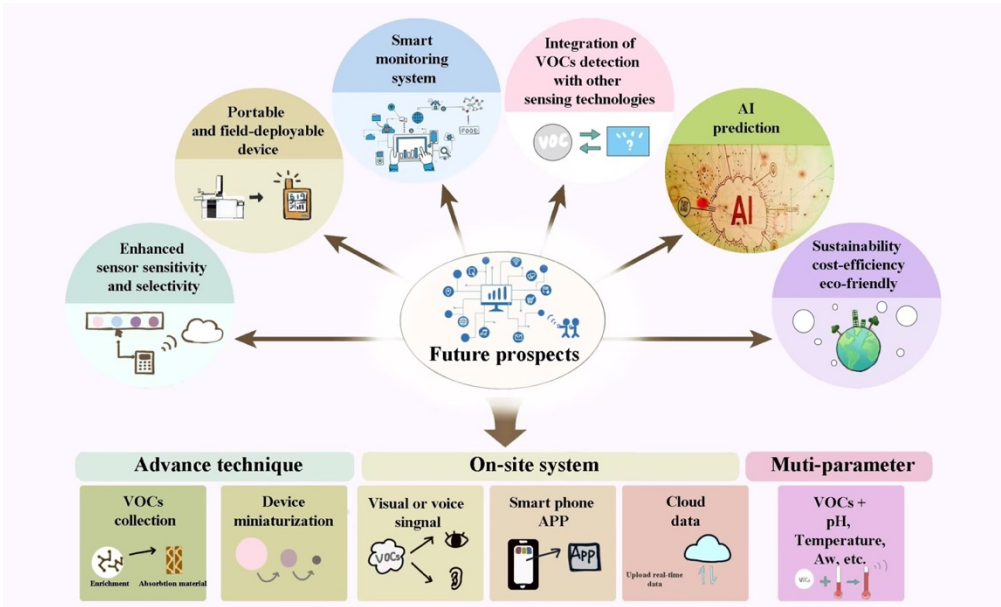


Figure 4. Future prospects and research directions of postharvest agricultural product deterioration detection based on VOCs.

3.7. Conclusion

VOCs represent sensitive and dynamic chemical markers for the early detection of spoilage and quality degradation in postharvest agricultural products. This review summarizes key VOC signatures associated with deterioration and evaluates recent advances in detection technologies, including spectrometry, spectroscopic methods, E-noses, and sensor arrays. We present a comparative evaluation of their performance, LOD, applicability, and constraints in agricultural scenarios, as well as emerging trends, highlighting their potential to enhance real-time decision-making in storage, transportation, and shelf-life management. While early VOC detection holds clear promise for reducing food loss and safeguarding consumer health, several challenges remain. These include the diverse and variable metabolic profiles of VOCs, susceptibility to environmental interference, and the lack of standardized protocols for method validation. Furthermore, high instrumentation costs continue to hinder large-scale deployment, highlighting the need for scalable, cost-effective solutions to facilitate broader adoption in the field.

Summary

Chapter 3 established the conceptual and analytical basis for using VOCs as indicators of postharvest deterioration in agricultural products. The VOCs change provide a chemical representation of the evolving physiological and microbiological status of stored commodities and support the potential of VOC-based approaches for rapid and non-destructive monitoring.

However, translating this general potential into an effective early-warning strategy requires several questions to be addressed experimentally. In particular, VOC profiles are influenced by multiple factors, including the commodity matrix, microbial species, stage of deterioration, and analytical platform. Moreover, many deterioration-associated volatile changes become more pronounced as spoilage progresses, whereas practical risk management would benefit most from detecting fungal activity during the earliest stages of colonization, before obvious visual symptoms or substantial mycotoxin accumulation occur. The identification of reproducible early volatile signatures therefore requires not only sensitive chemical characterization but also the integration of complementary analytical approaches capable of capturing different dimensions of the volatilome.

Based on the framework established in Chapter 3, Chapter 4 addresses these questions using *A.flavus* infection of maize as a defined experimental model. Maize represents a particularly relevant commodity because of its susceptibility to *A. flavus* colonization and the associated risk of aflatoxin B₁ contamination. The study specifically focuses on the asymptomatic period during the first days following inoculation, when biochemical changes may already be underway despite the absence of visible deterioration. To obtain a more comprehensive representation of these early volatile changes, E-nose, GC-IMS, GC-MS, and GC×GC-MS are integrated across three maize cultivars. This multi-platform design combines rapid pattern recognition with increasingly detailed chemical characterization and enables the temporal evolution of infection-associated VOCs to be examined from complementary analytical perspectives.

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Chapter 4

Integrated Multi-Platform Volatile Profiling for Early Detection of Asymptomatic *Aspergillus flavus* Infection in Maize Kernels from Three Cultivars

Introduction

Aspergillus flavus is a filamentous fungus that poses significant concerns in agricultural systems due to its ability to produce aflatoxins - potent mycotoxins with carcinogenic, hepatotoxic, and immunosuppressive effects. Maize is one of the most vulnerable staple crops and a major source of human and animal exposure, exacerbated by climate change, poor agronomic practices, and inadequate storage. Globally, aflatoxin contamination leads to an estimated USD 6–18 billion in annual economic losses (Yohannis et al., 2025). In China, significant levels of aflatoxin contamination have been reported in maize products (36.51 µg/kg) because of environment conditions in certain regions such as Zhejiang and Jiangsu Province (Fang et al., 2022; Hao et al., 2022). In Europe, climate-driven increases in temperature and drought stress are transforming aflatoxin contamination from a postharvest quality issue into a food safety risk in maize production systems, necessitating a shift from conventional postharvest testing to early-warning strategies that integrate ecological risk modeling with non-invasive sensor technologies based on volatile and plant stress signatures (Bereziartua et al., 2025).

One of the most critical bottlenecks in managing *A. flavus* contamination is the lack of reliable tools for early-stage detection. Conventional analytical approaches, including fungal isolation, microscopy, and aflatoxin quantification, are typically destructive, labor-intensive, and time-consuming (Sun et al., 2025). More importantly, these methods usually detect infection only after visible mold growth or substantial toxin accumulation has already occurred, when intervention options are limited. Consequently, there is a pressing demand for rapid, non-destructive, and real-time monitoring techniques capable of detecting *A. flavus* infection during the asymptomatic phase.

Volatile organic compounds (VOCs) have emerged as powerful indicators of food quality and microbial activity (Liu et al., 2024). VOCs are low-molecular-weight secondary metabolites emitted by plants and microorganisms, and their composition is often highly species- and stress-specific, effectively serving as chemical fingerprints of plant–pathogen interactions. The VOCs profile of a biological system is highly dynamic and responds sensitively to biotic stresses such as fungal invasion. Recent studies have demonstrated the feasibility of using VOCs for the early detection of fungal contamination. For instance, electronic nose (E-nose) systems have successfully discriminated rice kernels infected with *Aspergillus* spp. from healthy ones based on changes in VOC patterns (Gu et al., 2019). Using gas chromatography–ion mobility spectrometry (GC-IMS), Qin et al. (2024) reported that maize kernels emitted distinct ketones, esters, and aromatic aldehydes as early as 18 h after *A. flavus* inoculation. Similarly, Nordström et al. (2022) applied GC-MS combined with machine learning to identify *Fusarium circinatum* infections in pine seedlings prior to the appearance of visible symptoms. Collectively, these studies underline the strong potential of VOC-based approaches for non-invasive, real-time diagnosis of fungal infections.

Despite recent advances, the temporal dynamics of VOCs emissions during the earliest stages of *A. flavus* infection—particularly within the first few days post-inoculation(dpi)—remain insufficiently resolved, and most existing studies rely on single analytical platforms, thereby limiting chemical coverage and diagnostic robustness. For effective biosafety surveillance in maize supply chains, VOCs biomarkers must therefore enable reliable, non-invasive discrimination of infected kernels across genotypes prior to the onset of visible symptoms and aflatoxin biosynthesis, which typically occurs at around 3 dpi.

In this study, we aim to address these gaps and provide potential VOC signatures that serve as non-invasive biomarkers for early fungal detection by conducting a multi-platform analysis of VOC emissions from maize kernels inoculated with *A. flavus* during the first four days of infection. By combining E-nose, GC-IMS, GC-MS and GC×GC-MS, we seek to construct a robust volatile fingerprint of early fungal colonization. Our objective is to: (1) comprehensively study the aroma profile of three widely cultivated maize cultivars in China, infected with *A. flavus*, using a combination of three analytical platforms, (2) test whether *A. flavus* infection in maize can be detected through VOCs before the appearance of visible disease symptoms, and (3) identify key volatile markers that can serve as early indicators of *A. flavus* infection in different maize cultivars, thereby providing a scientific basis for the development of rapid, non-destructive diagnostic tools to support early warning systems in maize storage and processing.

4.1. Materials and Methods

4.1.1. Maize Cultivars and Fungal Inoculation

The *A. flavus* NRRL3357 wild-type strain was kindly provided by Prof. Wenbing Yin (Institute of Microbiology, Chinese Academy of Sciences, Beijing, China). Fungal development was observed after 7 days of cultivation at 37 °C in the dark. Conidia were harvested with 0.1% Tween-80 and counted with hemocytometer.

Three widely cultivated maize cultivars in China, including Zhengdan958 (ZD), Ludan513 (LD), and Wuyue78 (WY), were kindly provided by the Shandong Academy of Agricultural Sciences, Shandong, China. The surface sterilization of maize was performed by rinsing with 80% ethanol, followed by washing with distilled water, immersion in a 5% NaClO solution for 10 to 15 minutes, three subsequent washes with distilled water, and air-drying for 30 minutes. Once sterilized, the water activity (a_w) was adjusted to 0.92 a_w . Each maize variety was divided into four treatment groups: CK (control), 1 dpi, 2 dpi, and 3 dpi. Each treatment group was comprised of three biological replicate plates (Petri dishes), each containing 5 g of maize kernels. A conidium suspension of *A. flavus* (1×10^6 spores/mL) was inoculated with 500 μ L into each Petri dish of the infected groups (1, 2, 3 dpi), while the control group (CK) received an equal volume of sterile inoculum. All dishes were incubated at 28 °C.

4.1.2.E-nose Analysis

Samples (5 g each) were placed into 20 mL vials and equilibrated at 25 °C for 30 minutes before being analyzed using a PEN3 E-nose system (Airsense Analytics, Schwerin, Germany), which is equipped with 10 metal oxide semiconductor (MOS) sensors. The self-cleaning and detection times were set to 180 s and 60 s, respectively. For data analysis, the sensor signal values between 48 s and 52 s were selected. The details of the 10 MOS sensors used are provided in [Supplementary Table 1](#). Prior to analysis, the raw sensor signals were subjected to preprocessing, including normalization to standardize the data range and baseline correction to eliminate any drift or fluctuations in the sensor response, ensuring accurate and consistent measurements.

4.1.3.GC–IMS Analysis

Volatile compounds in maize kernels at different stages of infection were analyzed using a GC–IMS flavor analyzer (FlavourSpec®, Dortmund, Germany). For each analysis, 5 g of maize kernels was placed in a 20 mL headspace vial and incubated under the specified conditions. After incubation, the headspace gas was withdrawn using a syringe maintained at 65 °C and introduced into the GC–IMS instrument. Following a 15-minute incubation at 60 °C, 500 µL of the headspace volatile were injected using splitless mode desorption. Chromatographic separation was carried out using a FS-SE-54-CB-1 capillary column (15 m×0.53 mm i.d., 1.0 µm film thickness; CS-Chromatographie Service GmbH, Germany), maintained at 60 °C, with nitrogen as the carrier gas. The carrier gas flow ramped from 2 mL/min for 2 min to 20 mL/min within 8 min, reaching 100 mL/min after 10 min, and finally 150 mL/min at 10 min, all under isothermal conditions. The entire analysis process took 30 min ([Li et al., 2021](#)). GC-IMS data were acquired in positive ion mode and processed using LAV software. Volatile compounds were tentatively identified by comparing their retention indices and drift times with reference values in the GC×IMS Library Search database (G.A.S., Dortmund, Germany), which incorporates the NIST 2014 retention index database and the G.A.S. IMS drift-time library.

4.1.4.Headspace solid phase microextraction (HS-SPME) and GC–MS Analysis

Maize samples (5 g) inoculated with *A. flavus* were prepared as mentioned in Section 2.1. The samples were incubated at 28 °C for 0, 1, 2, and 3 days, and then transferred into 20 mL sealed headspace vials. Prior to analysis, 1-pentanol was used as the internal standard. A 1-pentanol stock solution prepared in chloroform was added to each 20 mL headspace vial to obtain a final concentration of 10 ppm. The choice of the SPME fiber coating and GC–MS analysis procedure was based on [Georgiadou et al. \(2015\)](#) and [Ma et al. \(2023\)](#) with a slight modification. After equilibration at 40 °C for 30 min under agitation at 250 rpm, VOCs were extracted from the vial headspace at 40 °C for 60 min using a 1 cm 50/30 µm DVB/CAR/PDMS StableFlex SPME fiber (Supelco, Sigma-Aldrich, Bellefonte, PA, USA). Subsequently, the analytes adsorbed onto the fiber were thermally desorbed in the

injector port of a TRACE 1310 gas chromatograph (Thermo Scientific, Waltham, MA, USA) at 250 °C for 5 min under splitless conditions using an HP-5 capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Agilent Technologies, Santa Clara, CA, USA). The GC oven temperature was initially held at 40 °C for 3 min, then increased to 160 °C at a rate of 3 °C/min and held for 2 min, followed by a further increase to 220 °C at 8 °C/min, with a final hold of 3 min. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The GC effluent was introduced into a TSQ 9000 triple quadrupole mass spectrometer (Thermo Scientific, Waltham, MA, USA). Mass spectral data were collected in full-scan mode using electron ionization (EI) at 70 eV over the mass range of m/z 35–350 for non-targeted analysis of VOCs. A C7–C30 n-alkane mixture (Sigma-Aldrich/Supelco) was analyzed between samples for retention index calibration. VOCs were tentatively identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) 2014 and 2017 mass spectral libraries. The tentative assignments were further supported by comparison of the calculated linear RI with reference values from the NIST Chemistry WebBook and relevant literature. Only compounds with a spectral match score greater than 80% and an RI deviation within ± 30 were retained. The peak area of each volatile compound was normalized against the internal standard and expressed as a relative abundance for comparative analysis.

4.1.5. GC×GC–MS analysis

An exploratory GC×GC–MS analysis was conducted to further examine the short-term dynamics of volatile changes during early *A. flavus* infection. Maize kernel samples collected at 1, 2, and 3 days post-inoculation (dpi) were analyzed, and mold-inoculated samples were compared with the corresponding control (CK) samples at each time point. This analysis was used as an exploratory differential-screening approach to identify volatile features that differed between *A. flavus*-inoculated maize and the corresponding control samples at each early time point, rather than as a fully targeted quantification method.

Volatile compounds were analyzed using a Shimadzu GCMS-QP2020 NX system (Shimadzu, Japan), comprising a GC-2030 gas chromatograph coupled to a single-quadrupole mass spectrometer detector. Sample introduction was performed using a Centri® autosampler (Markes International Ltd., UK). Compounds were collected on Tenax GR/Carbopack B sorbent tubes (C2-AAXX-5124, Markes International, UK) under a closed-loop dynamic headspace sampling configuration. Briefly, maize kernels were placed in a sealed sampling box at 28 °C. The sorbent tube was connected to the inlet side of a portable air-sampling pump, while the outlet side of the pump was connected to activated charcoal; both the sorbent tube and the charcoal outlet were positioned inside the sampling box. During sampling, the headspace air was actively drawn through the tube at a flow of 2L/min, and a total volume of 20 L was collected for each sample. Tubes were purged at room temperature for 5 min and subsequently thermally desorbed at 270 °C for 10 min at a flow rate of 50 mL/min for both steps. Volatiles desorbed during the purge or the thermal desorption steps were focused onto a general-purpose trap (U-T12ME-2S,

Markes International Ltd., UK) maintained at $-10\text{ }^{\circ}\text{C}$, followed by rapid desorption and injection into the GC $\times$ GC–MS system for 3 min at $300\text{ }^{\circ}\text{C}$ using a split ratio of 1:41. Comprehensive two-dimensional gas chromatography separation was achieved using an Insight flow modulator (SepSolve Analytical, UK) equipped with a $50\text{ }\mu\text{L}$ sample loop and a modulation period of 2 s. The first-dimension column was a SH-I-624Sil-MS capillary column ($20\text{ m} \times 0.18\text{ mm} \times 1.0\text{ }\mu\text{m}$; Shimadzu, Japan) operated at a constant flow rate of 0.5 mL/min . The second-dimension column was a StabilWax column ($3\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$; Supelco, USA) operated at constant flow rate of 15 mL/min . A bleed line consisting of a $3.4\text{ m} \times 0.10\text{ mm}$ capillary was used. The second-dimension effluent was split using a splitter comprising a $1.1\text{ m} \times 0.18\text{ mm}$ capillary directed to the MS and a $0.7\text{ m} \times 0.18\text{ mm}$ capillary directed to the FID, corresponding to approximately 55% and 45% of the flow to the MS and FID, respectively. The GC oven temperature program was as follows: initial temperature of $35\text{ }^{\circ}\text{C}$ held for 1 min, increased to $260\text{ }^{\circ}\text{C}$ at $6\text{ }^{\circ}\text{C/min}$, and held for 5 min. Mass spectrometric detection was performed in electron ionization (EI) mode at 70 eV with a scan range of m/z 35–350 and an acquisition frequency of 33 Hz. The ion source and transfer line temperatures were set at 200 and $250\text{ }^{\circ}\text{C}$, respectively.

Data acquisition was performed using GCMS Solution software version 4.53 (Shimadzu, Japan). Raw data were converted into AIA format and further processed using ChromSpace software version 2.1.7 (SepSolve Analytical Ltd., UK) for visualization of the two-dimensional chromatograms, manual peak inspection, peak integration, and export of the peak table. For differential screening, the chromatographic peak profiles of the *A. flavus*-inoculated samples at 1, 2, and 3 days post-inoculation were compared with those of the corresponding control samples at each time point. Compounds showing differences in peak presence or peak intensity between the inoculated and control samples were selected as candidate differential volatile compounds. Tentative compound identification was conducted using the NIST17 mass spectral library with a similarity match threshold of $\geq 85\%$, combined with linear retention index (LRI) matching within ± 20 units. A C7–C30 n-alkane standard mixture was analyzed under the same chromatographic conditions for LRI calculation.

4.1.6. Statistical Analysis

All experiments were performed with three biological replicates. Principal component analysis (PCA), hierarchical clustering, and boxplot visualization were used to assess overall variation and differences in VOC profiles among groups. Orthogonal partial least squares-discriminant analysis (OPLS-DA), variable importance in projection (VIP) analysis, support vector machine (SVM), and random forest were further applied as exploratory approaches to investigate group separation patterns and to screen potential discriminatory VOC markers. Two-way analysis of variance (ANOVA) was performed in Python using pandas (v2.2.3) and statsmodels (v0.14.6), and the effect-size heatmap was generated using matplotlib (v3.10.8). Additional analyses were performed using R (version 4.2.1; R Foundation for Statistical Computing, Vienna, Austria) in RStudio (version 2022.07.2; Posit Software,

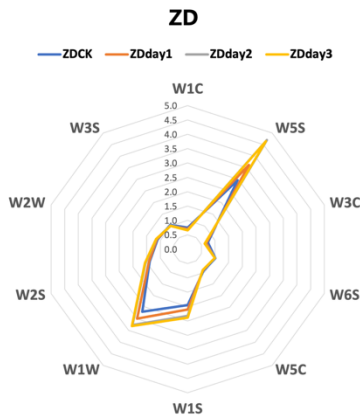
PBC, Boston, MA, USA), SIMCA 14.1 (MKS Data Analytics Solutions, Umeå, Sweden), and Wekemo Bioincloud ([Gao et al., 2024](#)).

4.2. Results and Discussion

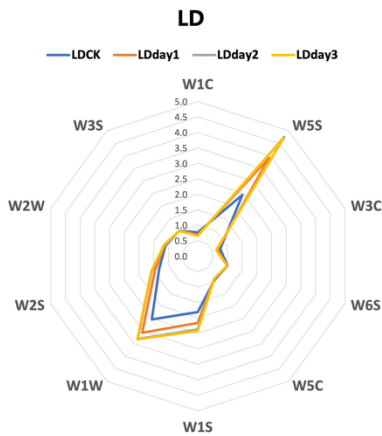
4.2.1. E-Nose Analysis and PCA Classification

In the field of spoilage detection, E-nose systems enable non-invasive, high-throughput quality assessment by dynamically monitoring real-time variations in VOCs released during food decomposition ([Ali et al., 2023](#); [Li et al., 2024](#)). To visually compare the dynamic VOCs responses across the three maize cultivars during the early infection stage, radar maps were generated based on E-nose sensor signals collected from samples at 0 (CK), 1, 2, and 3 dpi. The results of the E-nose radar map are shown in [Fig. 5a-c](#), which indicated the infection status and infection time had a stronger influence on sensor responses than maize variety. Across the three cultivars, the most pronounced changes were observed in the W5S (nitrogen oxide compounds) and W1W (terpenes and organosulfur compounds) sensor arrays, whereas the responses of W1S (broad methane compounds) and W2S (broad alcohol compounds) were more moderate. The remaining sensors W3C (ammonia/aromatic compounds), W6S (hydrogen derivatives), W1C (aromatic compounds), W3S (methane/aliphatic compounds), W2W (sulfur-containing/chlorinated compounds), and W5C (hydrocarbons/aromatic derivatives), showed comparatively limited variation among groups. These results indicate that *A. flavus* infection altered the overall volatile characteristics of maize kernels in a broadly similar manner across cultivars.

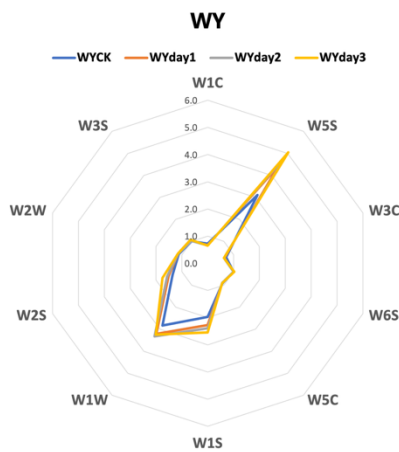
a.



b.



c.



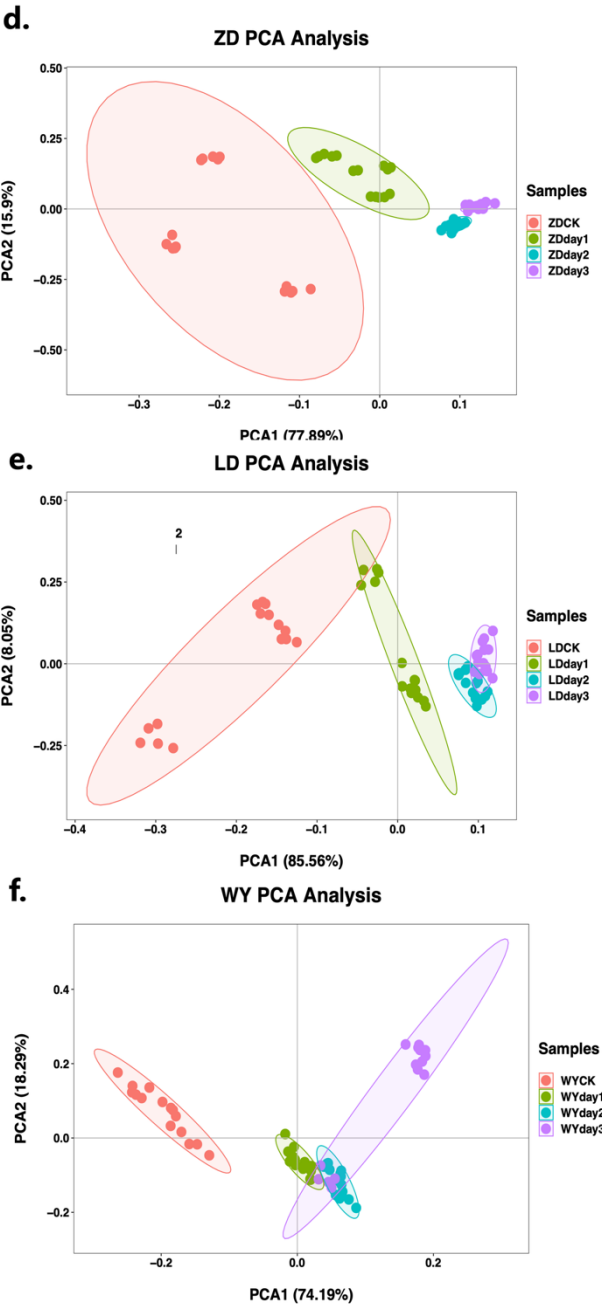


Figure 5. Electronic nose (e-nose) analysis of three maize cultivars (ZD, LD, and WY) at different stages (CK and days 1–3): (a–c) Radar plots illustrating the response patterns of sensor arrays; (d–f) Principal Component Analysis (PCA) score plots showing the clustering and separation of samples.

The elevated levels of nitrogen oxide compounds during *A. flavus* infection may result from fungal metabolic activities (Yang et al., 2024), residual nitric oxide signaling within the kernels, and nitrogen metabolism disorders caused by cellular degradation (Delledonne et al., 1998; Sarma et al., 2025). Although maize kernels are postharvest products, stress-induced biochemical changes such as amino acid catabolism can still occur. Meanwhile, the elevation of terpenes likely reflects the release of endogenous volatiles due to tissue disruption, along with terpenoid production by the fungus itself (Chen et al., 2011). Organosulfur compounds are mainly generated through the degradation of sulfur-containing amino acids and microbial sulfur metabolism during infection (Rausch & Wachter, 2005). These compounds possess antimicrobial and antioxidant properties, indicating a complex interaction between host degradation processes and fungal metabolic adaptation. Overall, these findings highlight the complex interplay between pathogen metabolism and host physiological responses, providing valuable insights into the early biochemical markers of fungal contamination in maize.

To comprehensively capture the variations in volatile profiles during *A. flavus* infection, PCA analysis was applied (Gu et al., 2019). As shown in Fig. 5d–f, the first two principal components explained 93.79%, 93.61%, and 92.48% of the total variance for ZD, LD, and WY samples, respectively, exceeding the commonly accepted threshold of 85% (Cheli et al., 2009; Jiarpinijnun et al., 2020). In all three cultivars, the control samples were clearly separated from infected samples, while the infected groups showed time-dependent clustering patterns with partial proximity between some adjacent time points. The three biological replicates within each group clustered closely, indicating good repeatability of the E-nose measurements.

To elucidate the specific volatile compound differences between uninfected maize and samples from 1–3 dpi, detailed volatile profiling was conducted using GC-IMS and GC-MS techniques.

4.2.2. GC-IMS Analysis

GC-IMS was used to investigate dynamic VOC changes associated with early *A. flavus* infection in the three maize cultivars (Li et al., 2021; Qin et al., 2024). A total of 52 VOCs were detected, including 8 unidentified compounds, 3 monomers, and 2 dimers (Table S2). The detected VOCs were mainly assigned to alcohols, aldehydes, ketones, esters, furans, hydrocarbons/aromatic compounds, and a small number of sulfur-containing compounds. These compound classes were also those most clearly associated with differences between control and infected samples. Previous culture-based and review studies have reported a broad diversity of VOCs associated with *A. flavus*, including alcohols, aldehydes, hydrocarbons, furans, esters, and terpenes (Josselin et al., 2021, 2022; Lucca et al., 2010; Moore & Lloyd, 2023; Sun et al., 2014,

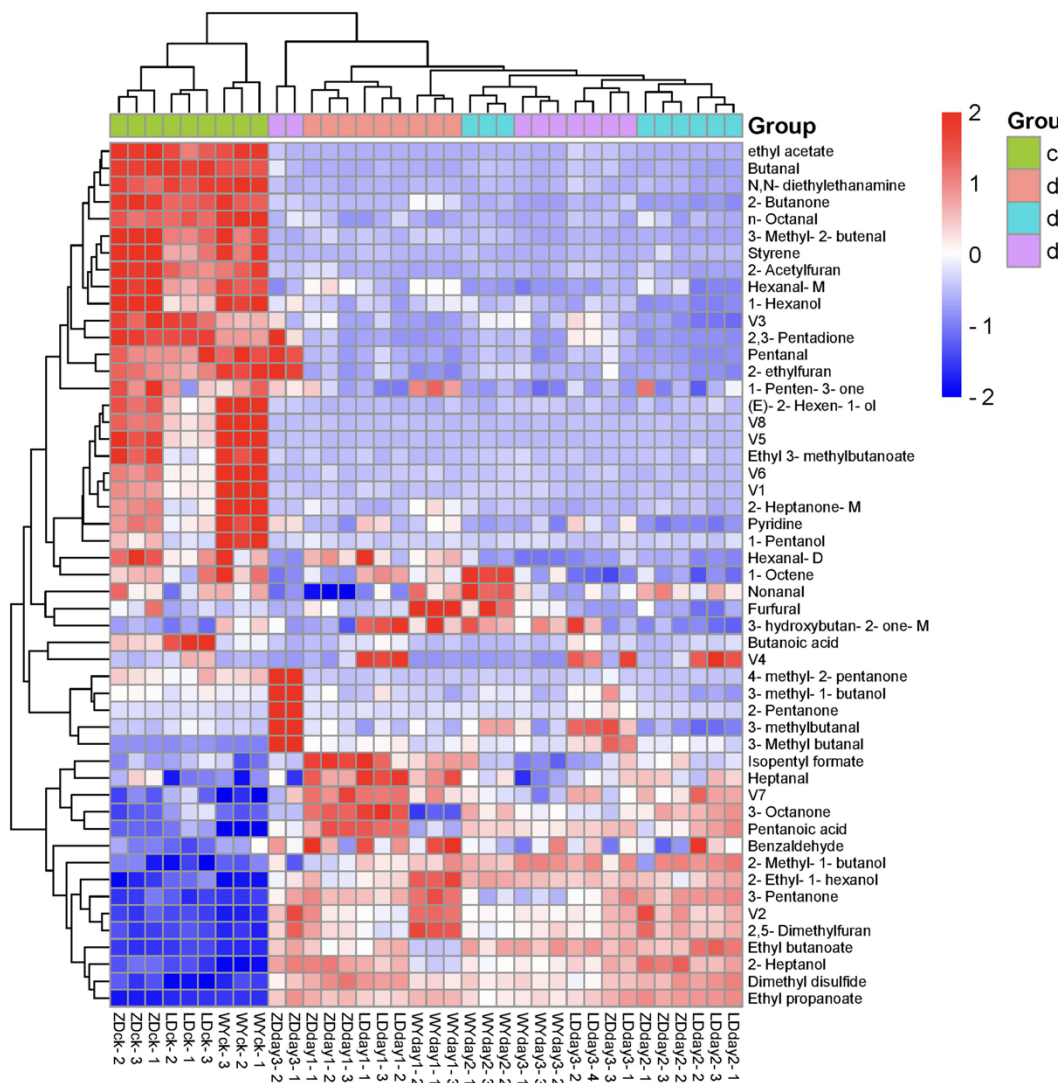
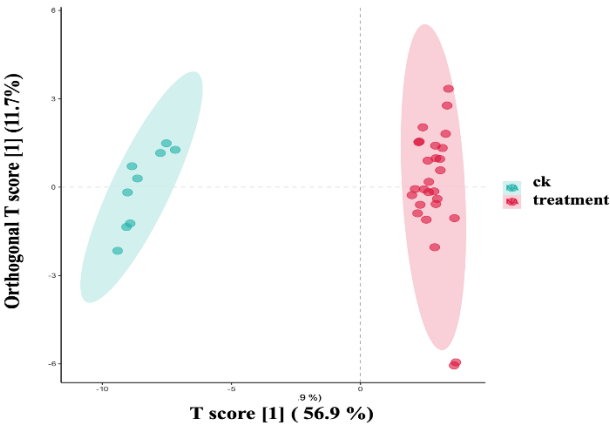


Figure 6. Clustering heatmap of volatile organic compounds (VOCs) in three maize cultivars (ZD, LD, and WY) at various stages of mold growth (CK and days 1–3) based on GC-IMS data.

a.



b.

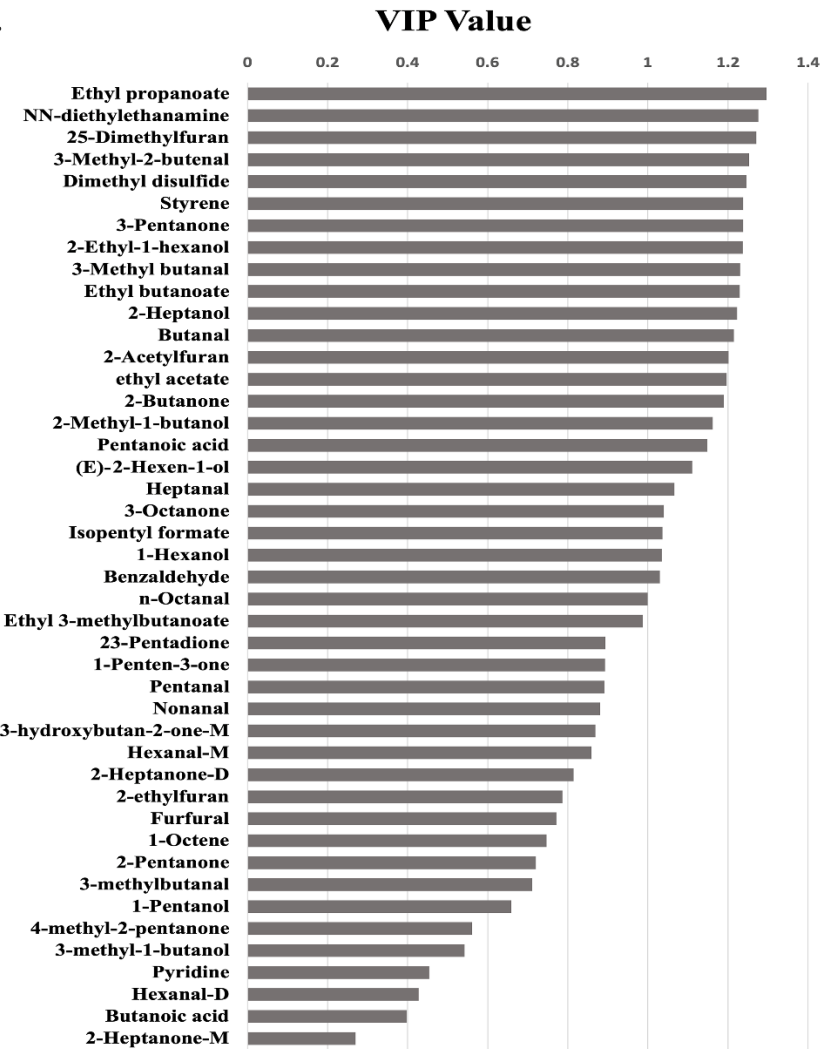


Figure 8. Identification and screening of differential volatile metabolites between mold-infected and control maize samples by GC-IMS. (a) OPLS-DA score plot showing group separation; (b) VIP plot indicating the contribution of key volatile compounds to the discrimination.

OPLS-DA analysis demonstrated strong separation between infected and control samples for all maize varieties, and VIPs identified 23 signature early-stage fungal biomarkers ($VIP > 1$) (Fig. 8 a, b). Two-way ANOVA effect-size analysis showed that the day factor exerted the strongest influence on most GC-IMS VOCs, whereas cultivar and cultivar - day interaction effects were more compound-dependent (Fig. S3). Candidate VOC markers from the GC-IMS dataset were selected based on a combination of VIP ranking, the significance and effect size of the day factor in two-way ANOVA, consistency of temporal variation across the three maize cultivars, and clear separation patterns observed in the heatmap. Using these criteria, a set of upregulated markers ethyl propanoate, dimethyl disulfide, 2,5-dimethylfuran, 3-pentanone, ethyl butanoate, 2-ethyl-1-hexanol, and 2-heptanol were prioritized as representative candidate markers of asymptomatic *A. flavus* infection. These compounds showed relatively stronger abundance changes in infected samples, particularly at later infection stages, and were associated with ester, furan, sulfur-containing, and ketone metabolic responses, respectively (Lucca et al., 2010; Qin et al., 2024). Ethyl propanoate has been proposed as a pre-mold stage marker (Ma et al., 2023), while 2,5-dimethylfuran was detected from day 2 of *A. flavus* NRRL-5565 growth on cracked corn. And, dimethyl disulfide was found from *A. flavus* 1000E grown under identical conditions but absent on PDA (Lucca et al., 2010). Although it was minimal in laboratory cultures (Josselin et al., 2022), ketone production was strongly induced in infected maize tissues, with short-chain ketones such as 2-butanone and 3-pentanone representing reliable fungal stress indicators (Qin et al., 2024). The emission of these VOCs across genetically distinct maize cultivars suggests the activation of a conserved biochemical defense response to *A. flavus* invasion, possibly involving the interplay between host oxidative stress and fungal detoxification pathways, which may contribute to lipid peroxidation and the generation of characteristic volatiles such as short-chain ketones and sulfur-containing compounds (Fountain et al., 2016; Lanubile et al., 2017; Li et al., 2022; Soni et al., 2020; Sun et al., 2024). These metabolic shifts may reflect both fungal adaptation to host-derived reactive oxygen species and the degradation of host cell membranes under biotic stress, highlighting the potential of these VOCs as indicators of fungal contamination.

While highly sensitive and informative for dynamic profiling, the tentative identifications provided by GC-IMS necessitate confirmation by a definitive analytical technique. Therefore, to more reliably identify and characterize the key discriminatory VOCs, GC-MS was further employed.

4.2.3. GC-MS Analysis

To further characterize the molecular composition of VOCs associated with early *A. flavus* infection, HS-SPME-GC-MS was performed on maize samples collected at 1-3 dpi. A total of 36 VOCs were consistently tentatively identified across the dataset (Fig. 9a), including 10 esters, 8 aromatic hydrocarbons, 4 aldehydes, 3 terpenes, 3 alcohols, 2 ketones, 1 nitrogen-containing compound, and 5 compounds classified as others (Table S3). These results indicate that esters and aromatic hydrocarbons accounted for the largest proportion of the annotated VOCs detected by GC-MS.

The heatmap in Fig. 9b revealed that a subset of VOCs increased in abundance in infected samples, with several compounds showing relatively stronger responses at day 2; however, the temporal trajectories were compound-dependent rather than uniformly progressive across all VOCs.

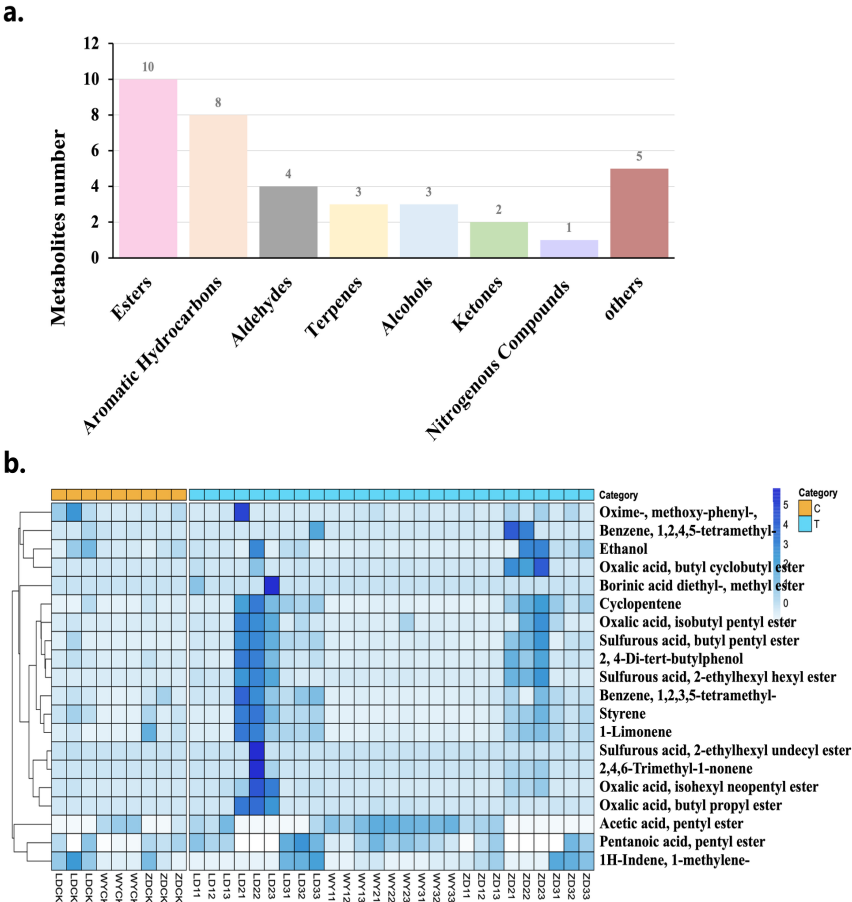


Figure 9. Comparative and dynamic analysis of identified volatile metabolites in maize during early infection stages (CK and days 1–3) using GC-MS. (a) Distribution of volatile organic compounds (VOCs) chemical classes; (b) Heatmap and hierarchical clustering analysis showing the dynamic changes during early mold stage.

To further explore temporal changes in volatile metabolites following fungal infection, support vector machine (SVM) and random forest (RF) analyses were applied as exploratory tools for feature ranking and preliminary assessment of class-separating patterns. In the SVM ranking results (Fig. 10a), decanal, isolongifolene, pentanoic acid butyl ester, cinnamyl alcohol, and several ester-related compounds showed relatively high importance scores, indicating that they contributed substantially to sample discrimination within the present dataset. ROC analysis suggested good classification performance for the SVM models across different variable subsets (Fig. 10b), with the best AUC values observed when larger numbers of variables were included. These results indicate that the GC-MS feature matrix contained sufficient discriminatory information to distinguish infected from control samples within this dataset. Based on the SVM ranking results, the top 15 volatile metabolites were retained as candidate markers for subsequent screening.

The feature importance results generated by the RF model (Fig. 10c) showed partial consistency with those obtained by SVM. Isolongifolene, cinnamyl alcohol, pentanoic acid butyl ester, and butylbenzene were among the variables with relatively high importance, whereas some compounds such as octanal and limonene showed greater prominence in RF than in SVM. This difference likely reflects the distinct sensitivities of the two algorithms to feature interactions and nonlinearity. By integrating the top-ranked features from both methods, seven overlapping candidate VOCs were prioritized for further interpretation: butylbenzene, pentyl pentanoate, decanal, ethyl propanoate, cinnamyl alcohol, isolongifolene, and 2,3-dihydrofuran (Fig. 10d). Boxplots of these compounds (Fig. S4) showed time-dependent abundance changes associated with infection.

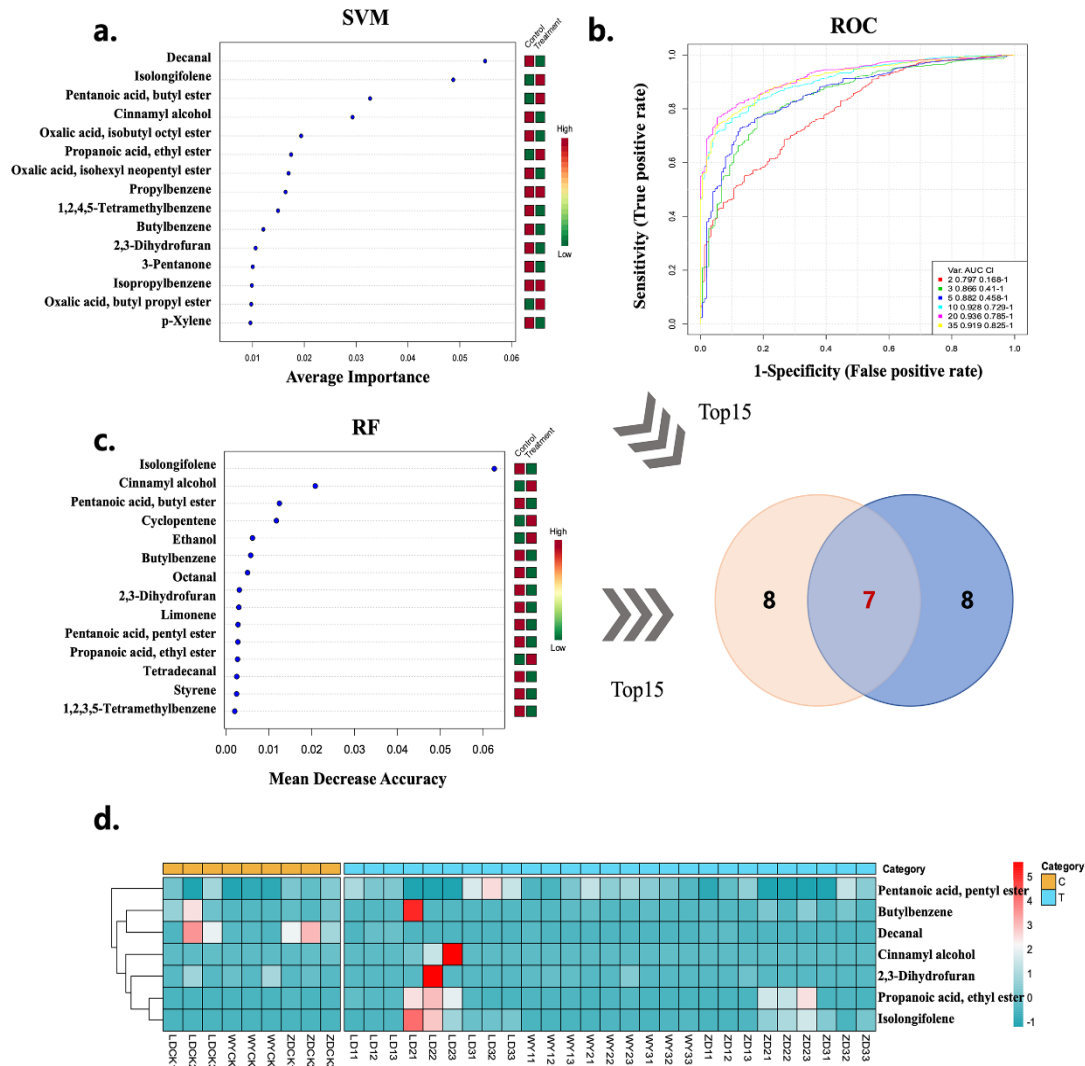


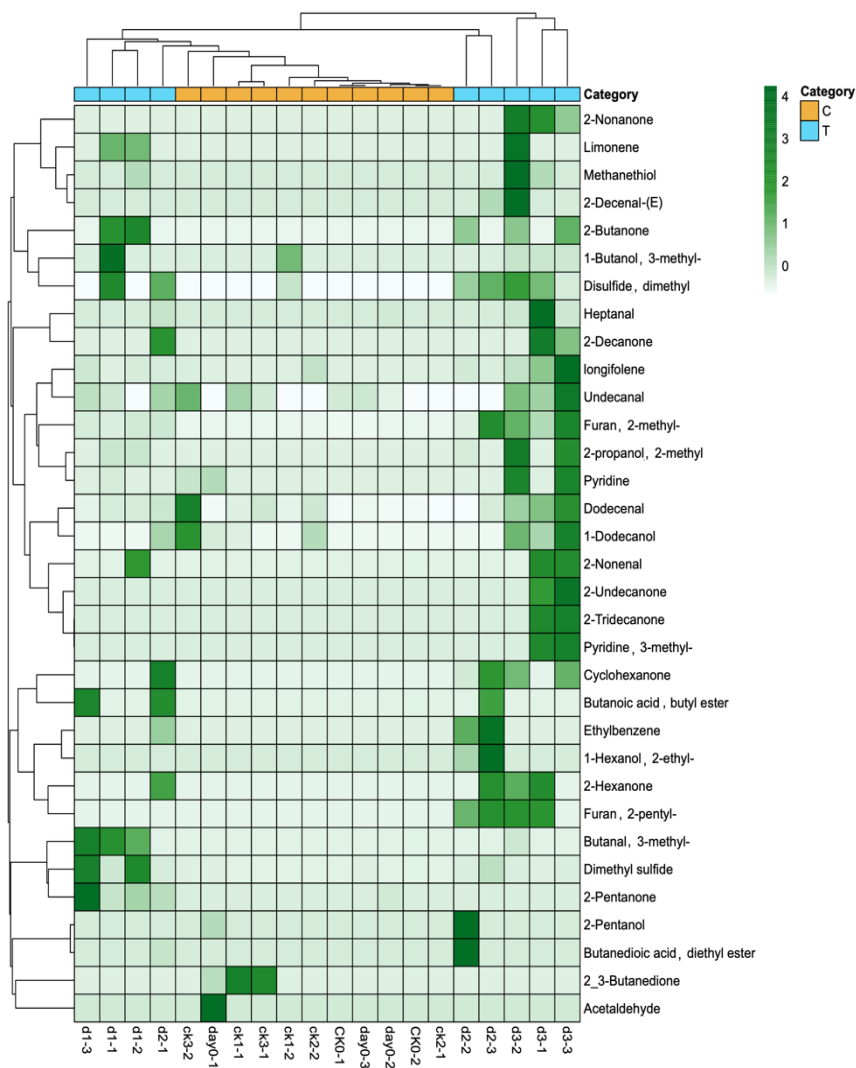
Figure 10. Exploratory machine-learning analysis of GC–MS-derived VOC features associated with early *A. flavus* infection in maize. (a) Ranking of the top 15 VOC features based on average importance in the support vector machine (SVM) model. The adjacent color tiles indicate the relative abundance trend of each VOC in control and infected samples. (b) Receiver operating characteristic (ROC) curves of the SVM models constructed with different numbers of variables. (c) Ranking of the top 15 VOC features based on mean decrease accuracy in the random forest (RF) model. The adjacent color tiles indicate the relative abundance trend of each VOC in control and infected samples. (d) Heatmap and hierarchical clustering analysis of the seven overlapping candidate VOCs across maize samples during early *A. flavus* infection.

Comprehensive analysis of both models revealed that ester compounds, aldehydes, and terpenes play important roles in the response of maize to early *A. flavus* infection. Previous studies have emphasized that aflatoxin biosynthesis in *A. flavus* is tightly linked to the fungal intracellular redox state, which is consistent with the mechanisms underlying the production of characteristic VOCs observed in this study, such as lipid peroxidation-derived aldehydes, abnormal accumulation of ester substances and antioxidant-responsive terpenoid compounds (Zeringue, et al., 1996; Fountain et al., 2014). The marked accumulation of specific esters (e.g., pentyl pentanoate and ethyl propanoate), suggests a pronounced restructuring of ester metabolism, likely arising from the processes of lipoperoxidation in the seed and acetyl-CoA accumulation under toxin-induced stress conditions (Ding et al., 2023; Scarpari et al., 2014). The lipid-based signaling network, such as the 9-lipoxygenase pathway, drives the accumulation of lipid peroxidation-derived aldehydes and reshapes terpenoid volatile production during early fungal colonization, linking oxidative lipid metabolism to host volatile reprogramming (Battilani et al., 2018). Collectively, these VOCs represent metabolic outputs of oxidative stress, energy imbalance, and defense activation triggered by early *A. flavus* infection. These results support the use of these seven VOCs as candidate discriminatory markers of early *A. flavus* infection, although further validation is still required.

4.2.4. GC×GC–MS analysis of dynamic differential volatiles during the first three days of A. flavus infection

To further complement the GC-MS-based characterization of early volatile changes, an GC×GC-MS analysis was performed to compare mold-inoculated maize with the corresponding CK samples at 1, 2, and 3 dpi. After mass spectral and LRI matching, followed by manual exclusion of low-quality or implausible assignments, 33 candidate differential volatile compounds were retained across the three time points (Table S4).

a.



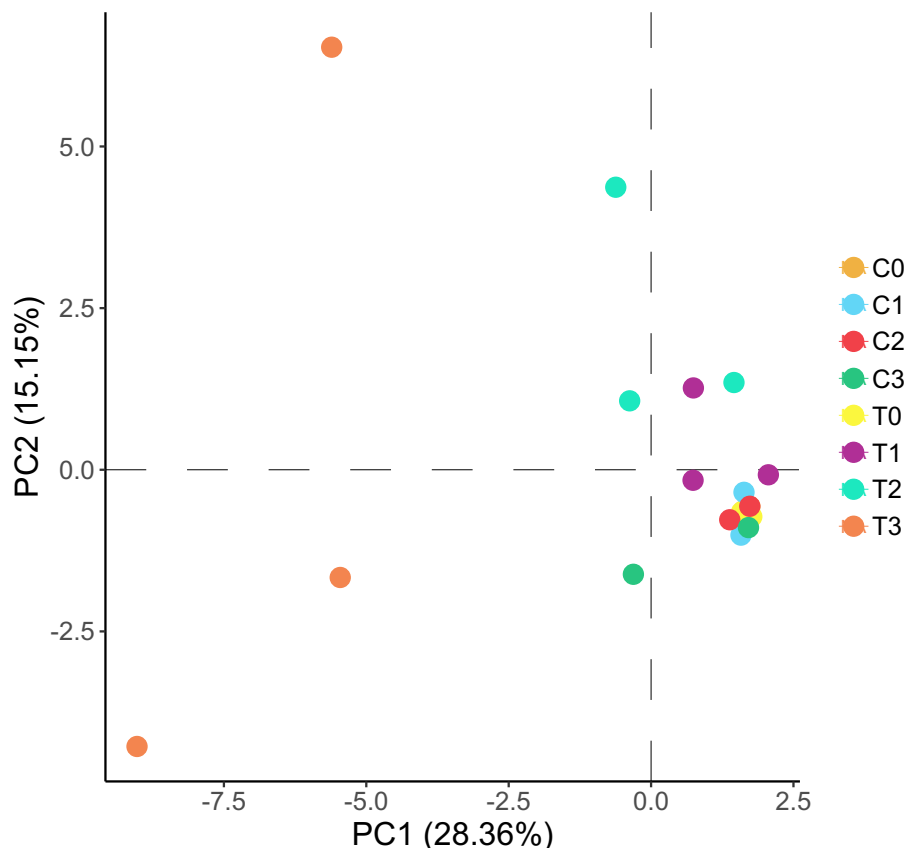


Figure 11. Temporal variation of differential VOCs in maize during *Aspergillus flavus* infection revealed by GC×GC–MS. (a) Hierarchical clustering heatmap of differential VOCs detected in control (C) and *A. flavus*-inoculated (T) maize samples during 0–3 days post-inoculation. (b) Principal component analysis (PCA) score plot showing the temporal distribution of control (C0–C3) and inoculated (T0–T3) maize samples.

Hierarchical clustering and heatmap analysis (Fig. 11) showed that the early response at 1 dpi was mainly characterized by changes in relatively low-molecular-weight VOCs, including 3-methylbutanal, 2-butanone, 2-pentanone, 3-methyl-1-butanol, dimethyl sulfide, and dimethyl disulfide, indicating that early fungal development was accompanied by the release of small and highly volatile metabolites. At 2 dpi, the VOC profile became more diversified, with increased responses from several chemical classes, including ketones, esters, furans, alcohols, and aromatic compounds. By 3 dpi, the volatile pattern changed more markedly, with the accumulation of a broader range of medium- and higher-molecular-weight compounds, particularly heptanal, (E)-2-decenal, 2-nonanone, 2-decanone, undecanal,

dodecenal, 2-undecanone, and 2-tridecanone. This shift suggests that the metabolic processes associated with fungal colonization became increasingly complex as infection progressed. This temporal progression was further supported by PCA. The first two principal components explained 43.51% of the total variance (PC1, 28.36%; PC2, 15.15%). Samples collected at the 1 dpi showed considerable overlap with the corresponding controls, while the separation became more evident as incubation progressed, with 2 dpi samples showing increased dispersion and 3 dpi samples displaying the clearest deviation from the control and earlier treatment groups. The pronounced separation of 3 dpi was consistent with the heatmap, in which multiple VOCs exhibited substantially increased abundance.

Overall, the GC×GC–MS results indicated a progressive expansion in both the number and chemical diversity of differential VOCs from 1 to 3 dpi (Figure S5). These observations provided additional temporal evidence that measurable and stage-dependent VOC perturbations had already emerged within the first three days after inoculation, in agreement with the early-warning potential indicated by the GC-MS platform (Table 12).

Table 12. Summary of differential volatile compounds detected by GC×GC–MS between *A. flavus*-inoculated maize and the corresponding CK samples at 1, 2, and 3 dpi

Time point	1 dpi	2 dpi	3 dpi
Representative compound classes	Low-molecular-weight aldehydes, ketones, alcohols, and sulfur compounds	Ketones, esters, furans, alcohols, and aromatic compounds	Medium-/long-chain aldehydes and ketones, alcohols, pyridine derivatives, and terpenoids
Representative compounds	Butanal, 3-methyl-; 2-Butanone; 2-Pentanone; Dimethyl sulfide; 1-Butanol, 3-methyl-	Cyclohexanone; Butanoic acid, butyl ester; Furan, 2-pentyl-; 1-Hexanol, 2-ethyl-; Ethylbenzene	2-Nonanone; 2-Decenal, (E)-; Heptanal; 2-Decanone; 2-Undecanone; 2-Tridecanone
Main implication	Predominance of small, highly volatile metabolites during the early response	Diversification of the VOC profile during infection progression	Pronounced shift toward higher-molecular-weight carbonyl compounds at the advanced stage

4.2.5. Integration and Comparison of VOC Profiles across Four Platforms

The combined interpretation of E-nose, GC–IMS, GC–MS, and GC×GC–MS results revealed a coherent and complementary depiction of volatile metabolic alterations induced by early *A. flavus* infection in maize kernels. E-nose analysis captured global infection-driven changes and enabled rapid discrimination of infected samples, but lacked molecular specificity. GC–IMS provided time-resolved VOC fingerprints, highlighting a pronounced metabolic shift during the first days after inoculation. GC–MS provided compound-level information and prioritized candidate VOC markers associated with early infection. In parallel, exploratory GC×GC–MS analysis added a differential screening perspective by comparing inoculated and CK samples at each time point, showing a progressive expansion in both the number and chemical diversity of differential VOCs from 1 to 3 dpi. Together, these results indicate that early *A. flavus* infection produces a staged volatile response that can be detected before visible spoilage becomes evident (Zhu et al., 2024; Jiang et al., 2024).

4.3. Conclusion

This multi-platform investigation elucidated the volatile fingerprint of maize during the early phase of *A. flavus* infection. By integrating E-nose sensing with GC–IMS and GC–MS, and further complementing these results with exploratory GC×GC–MS differential screening, this chapter showed that infected maize kernels could be distinguished from controls before visible symptoms developed. A panel of candidate VOCs was identified that differentiated infected from non-infected maize across cultivars and infection time points. Multivariate analyses highlighted a core VOC set—ethyl propanoate, dimethyl disulfide, 2,5-dimethylfuran, 3-pentanone, ethyl butanoate, 2-ethyl-1-hexanol, 2-heptanol, butylbenzene, pentyl pentanoate, decanal, cinnamyl alcohol, isolongifolene, and 2,3-dihydrofuran—as promising cross-cultivar candidate indicators of infection prior to the appearance of visible symptoms or substantial aflatoxin accumulation. GC×GC–MS revealed a time-dependent shift from early changes in small, highly volatile metabolites to greater chemical diversity and accumulation of higher-molecular-weight carbonyl compounds at later infection stages. These findings support the potential of VOC profiling for rapid, non-destructive early warning of *A. flavus* contamination in stored maize. Further validation using larger sample sets and naturally contaminated maize is required before practical application.

Summary

Chapter 4 demonstrated that early *Aspergillus flavus* infection in maize is accompanied by detectable alterations in volatile profiles before the appearance of visible symptoms. This study characterized the temporal evolution of infection-associated VOCs and identified a set of candidate volatile markers with potential value for early fungal detection. These findings provide experimental support for the concept that metabolic changes associated with fungal colonization can serve as early

Chapter 4

indicators of contamination. Translating these biological responses into simpler and more readily measurable signals represents an important next step toward practical surveillance. Whole-cell biosensors offer such an opportunity because living reporter cells can integrate complex chemical stimuli into measurable biological responses, while data-driven modelling can further translate these multidimensional signals into quantitative estimates of contamination status and mycotoxin risk.

Chapter 5 extends the focus from identifying volatile signatures of fungal infection to predicting both the stage of mold contamination and the production of AFB₁. By combining a whole-cell biosensor array with regression-based modelling, this chapter investigates whether biosensor response patterns can be used to quantitatively estimate mold progression and AFB₁ levels in contaminated maize and peanut kernels. The inclusion of two agricultural commodities further allows the applicability of this sensing and prediction strategy to be evaluated across different food matrices. In this way, Chapter 5 advances the thesis from early recognition of fungal colonization toward quantitative assessment of mold development and mycotoxin risk, representing a further step toward rapid, practical, and predictive monitoring of food-safety hazards.

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Chapter 5

A novel approach for predicting aflatoxin B₁ production using regression models and whole-cell biosensors in moldy maize and peanut kernels

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Introduction

Mold contamination, particularly the presence of mycotoxins, poses a significant threat to the food safety and quality of grain and oil products, impacting both exporters and manufacturers worldwide (Jiarpinijnun et al., 2020). Considering the vast scale of global cereal production, estimated at 2,854 million tonnes with stocks of 894 million tonnes in 2024 (FAO, 2024), safeguarding this supply is critical. However, this production is challenged by the pervasive issue of mycotoxins, which affects approximately 25% of food crops worldwide, highlighting the urgent need for effective monitoring and management strategies. Among the various mycotoxins, aflatoxin B₁ (AFB₁), produced by the pathogen *Aspergillus flavus*, stands out as a particularly concerning human carcinogen (Eskola et al., 2020). This fungus threatens crops like peanuts and maize and proliferates under diverse environmental conditions, causing substantial economic losses. The ability of *A. flavus* to produce AFB₁ under various conditions makes contamination pervasive and difficult to prevent, further underscoring the need for robust strategies against mold contamination to safeguard the global food supply.

The stark economic consequences of mycotoxin contamination, such as the high rejection rates of agricultural crop shipments and exacerbated food and feed loss, motivate the present research. Between January 2021 and March 2022, the Rapid Alert System for Food and Feed (RASFF) reported 290 serious cases of mycotoxin risks in food and feed in Asia, with the shipments predominantly rejected for exceeding the EU limit of 10 µg/kg for AFB₁ in feed (Sun et al., 2023). Consequently, the development of early detection approaches and risk prediction models to identify effective intervention strategies has garnered significant attention (Stutt et al., 2023). Accurate, rapid, and early detection of mold infection during storage is crucial for implementing timely measures to reduce food losses and establish predictive infection models, thereby mitigating the economic and health impacts of mycotoxins.

Conventional detection methods, such as visual examination, culturing and plating methods, and isozyme analysis, which rely on morphological, microbiological, and biochemical identifications, have long been the foundation of mold detection (Ray et al., 2017). However, these methods are often slow and lack specificity. More advanced laboratory techniques, including DNA-based methods like PCR (Abd El-Aziz et al., 2021; Garcia-Lopez et al., 2021; Li et al., 2019) and antigen-based assays like enzyme-linked immunosorbent assay (ELISA) (Wang et al., 2017), offer high sensitivity and specificity. Nevertheless, they are labor-intensive, time-consuming, and not suitable for real-time or large-scale monitoring (Gong et al., 2021). This has driven interest in non-invasive approaches. In contrast, these alternatives that leverage spectroscopic-based methods, electronic noses (E-noses), and colorimetric sensors, that are integrated with machine learning models, have emerged as promising alternatives. Machine-learning models can analyze the data collected by these sensors, enabling continuous monitoring of crops. This integration effectively overcomes the labor-intensive and time-consuming issues of lab-based methods and the lack of real-

time monitoring ability of traditional methods (Guo et al., 2020; Lin et al., 2019). These innovative strategies address the key limitations of traditional and lab-based methods, offering more efficient and scalable solutions crucial for timely intervention and safeguarding the food supply chain.

Recent advancements have highlighted the potential of volatile organic compounds (VOCs) emitted from infected crops as valuable indicators of mold presence, with specific VOCs identified as biomarkers for *A. flavus* infection in crops such as maize and peanuts. These VOCs enable the development of sensors for early detection, thus offering a promising approach for preventing crop spoilage. However, conventional detection methods, including gas chromatography and E-noses, often face challenges in terms of portability, high costs, and the need for specialized technical expertise (Ali et al., 2023). Given the limitations of conventional detection methods, researchers have turned their attention to alternative solutions, among which whole-cell biosensors show great promise. To overcome these limitations, the integration of whole-cell biosensors presents a cost-effective and adaptable solution for monitoring *A. flavus* infections. These biosensors, which can be engineered to selectively detect specific VOCs, offer the advantage of being incorporated into portable devices and integrated into smart monitoring systems, making them suitable for on-site, real-time monitoring and decision-making (Chalupowicz et al., 2020).

Recent studies have shown that whole-cell biosensors can predict early spoilage events in fungi-infected postharvest commodities, such as maize, peanuts, potatoes, and grape berries, by sensing headspace VOCs, demonstrating their viability for practical agricultural applications (Ma et al., 2023a, 2023b; Veltman et al., 2023). These biosensors are developed by identifying key sensing promoters through transcriptomic analysis of liquid cultures of free-living *E. coli* cells exposed to infection-related VOC signatures, followed by immobilization of the cells in calcium alginate microbeads, which promotes biofilm formation (Smidsrød & Skjåk-Bræk, 1990). Coupling these biosensors with various machine-learning classification models, including Neural Networks (NN), Support Vector Machines (SVM), and Random Forests (RF), has shown high prediction accuracy (Ma et al., 2023a, 2023b). However, the characteristic VOCs associated with spoilage are often context-dependent, varying with factors such as substrates, storage conditions, and microbial species. This variation in complex VOC profiles limits the ability of biosensors to reflect the spoilage process. (Fan et al., 2023). Furthermore, using liquid cultures of free-living *E. coli* cells to identify potential promoters does not replicate the real conditions of immobilized cells in a biofilm state. Additionally, machine-learning classification models provide a binary "yes" or "no" signal, which fails to capture the degree of mold contamination or AFB₁ production.

To overcome the limitations of our previous binary classification approach—specifically, its reliance on non-specific promoters and lack of quantitative detail—we propose a transcriptome-guided RNA-seq strategy. This method will identify highly responsive promoters in sodium calcium-immobilized *E. coli*, mimicking their

state in real applications, when triggered by VOCs from *A. flavus*-infected agricultural substrates. We will integrate these promoters into a whole-cell biosensor array, utilizing calcium alginate immobilization to enhance cell stability and gas diffusion for improved VOC sensing. This array will be paired with advanced ensemble regression and classification machine learning models, such as CatBoost, RandomForest, XGBoost, Support-Vector Machines (SVM), Sparse Partial Least Squares Discriminant Analysis (SPLSDA) and Stochastic Gradient Boosting (SGB) (Dorogush et al., 2018; Lawrence et al., 2004; Nielsen, 2016), to enable dynamic, time-resolved monitoring of fungal contamination across multiple time points. The prediction accuracy will show strong correlation with total mold count and AFB₁ levels. This integrated biosensor-machine learning system offers a promising strategy for reliable, early fungal contamination monitoring in agricultural diagnostics and food safety.

5.1. Materials and Methods

5.1.1. Strains and Materials

Two aflatoxigenic strains of *A. flavus* were used in this study: the reference strain NRRL 3357 was stored in our lab, and a second strain was isolated from peanut soil and maintained in our laboratory. Both strains were cultured on potato dextrose agar (PDA) for routine propagation. For plasmid propagation and whole-cell bioreporter construction, *E. coli* DH5 α and BL21 strains, procured from Ruibio Biotech in Beijing, China, were employed and cultivated in Luria-Bertani (LB) medium supplemented with 100 μ g/mL ampicillin from Solarbio in Beijing, China, at 37 °C with agitation at 180 rpm. The parental plasmid pGEN-luxCDABE, essential for the construction of bioluminescent bacteria, was generously provided by Harry Mobley (Addgene plasmid # 44918). Shelled peanuts and maize kernels were purchased from a local supermarket in Beijing, China. Restriction enzymes PmeI and SnaBI for plasmid double digestion and T4 DNA ligase for ligation were obtained from New England Biolabs (Beijing, China). The 2 $\times$ Tag PCR Mix was purchased from Tiangen Biotech (Beijing, China), and primers for promoter cloning were synthesized by Sangon Biotech (Beijing, China). For DNA purification, the HiPure Gel Pure DNA Mini Kit was sourced from Magen Biotechnology (Guangzhou, China). Sodium alginate powder was procured from Solarbio Life Sciences (Beijing, China). Lastly, flat bottom 96-well white and transparent polystyrene microplates were acquired from Corning (Shanghai, China) for experimental assays.

5.1.2. Infection of Peanuts and Maize by *A. flavus*

Before *A. flavus* infection, the water activity of peanut and maize kernels was adjusted to ~0.90. Five grams of kernels were surface-disinfected by immersion in 80 % (v/v) ethanol for 10 s, rinsed with distilled water, soaked in 5% (v/v) sodium hypochlorite for 15 min, rinsed three times, and air-dried for 30 min. The kernels were then inoculated with 500 μ L of 1×10^6 conidia/mL suspension, placed on Petri dishes, and incubated at 28 °C for symptom monitoring or VOCs analysis with the whole-cell biosensor array.

5.1.3. Bacterial Cultivation, Immobilization, and Exposure to Volatile Compounds

The *E. coli* strain BL21 was initially cultivated in LB medium overnight, followed by a subculture into fresh LB medium for a further 3 hours at 37 °C with constant agitation at 220 rpm. The cells were encapsulated in 1.5% calcium alginate by mixing the suspension with alginate and extruding the mixture dropwise into 0.1 M CaCl₂, as previously described (Ma et al., 2023). The resulting beads were placed in a controlled chamber and exposed for 2 h to VOCs emitted from corn and peanut kernels inoculated for 2 days (Fig. 12a). After exposure, the beads were flash-frozen in liquid nitrogen to preserve cell integrity.

5.1.4. Liberating Immobilized E. coli Cells From Calcium Alginate Beads for RNA Extraction

E. coli cells immobilized within calcium alginate beads were first ground to a fine powder using a mortar and pestle, with the aid of liquid nitrogen. Following grinding, the powdered beads containing *E. coli* cells were liberated using a chemical dissolution method. Specifically, the beads were treated with a solution of 0.1 M ethylene diamine tetraacetic acid (EDTA) (Chueh et al., 2010) and 0.2 M sodium citrate (Wang et al., 2021) for a duration of 10 minutes to dissolve the calcium alginate matrix. This treatment facilitated the release of the bacterial cells while maintaining their integrity. Following the dissolution, the mixture was filtered through sterilized filter paper to remove any undissolved alginate pellets, ensuring a clean separation of the bacterial cells from the matrix debris. The filtrate, enriched with liberated *E. coli* cells, was subjected to centrifugation to pellet the bacterial cells. The supernatant was discarded, and the bacterial cell pellet was carefully collected for subsequent RNA extraction procedures.

5.1.5. mRNA sequencing and bioinformatic analysis

Total RNA was extracted, according to the protocol provided in SV Total RNA Isolation System (Promega, Madison, Wisconsin, USA), followed by ribosomal RNA depletion using MicroExpress kit (Ambion, Thermo Fisher Scientific) to acquire purified mRNA. Subsequently, fragmentation buffer was added to randomly break the obtained mRNA into short fragments, and a library was constructed according to the strand-specific library preparation method (Parkhomchuk et al., 2009). After the library quality check, different libraries were pooled based on the required effective concentration and target sequencing data volume, and then proceeded to Illumina sequencing by Novogene (Beijing, China).

The raw sequencing data were subjected to stringent quality control using FastQC v0.11.9 and Fastp v0.23.4 to remove contaminants and low-quality reads, ensuring that only high-confidence data proceeded to downstream analyses. The filtered reads were aligned to the *E. coli* K-12 MG1655 reference genome (BioProject: PRJEB37847) using the Bowtie2 v2.5.1 software with default alignment parameters, which offers ultrafast and memory-efficient alignment with support for gapped, local, and paired-end alignment modes. This makes Bowtie2 particularly suitable for aligning reads to long reference sequences and provides superior performance in terms of speed and memory usage compared to other tools like BWA. Post-alignment, gene-level quantification of mapped reads was conducted using the featureCounts function within the Subread package v2.0.8. FeatureCounts is highly accurate and efficient in summarizing mapped reads at the gene or transcript level, and it provides flexibility in handling multi-mapping reads and various annotation formats. It is also significantly faster and more memory-efficient than alternatives like htseq-count. For differential expression analysis, DESeq2 v1.34.0 was employed to identify differentially expressed genes (DEGs) between the experimental and control conditions, applying a threshold of adjusted p-value < 0.05 and absolute value of $\log_2$ fold change being greater than 1 as criteria for significance. To further explore the functional implications of the DEGs, gene ontology (GO) enrichment analysis was performed using the clusterProfiler package v4.4.0.

5.1.6. Construction of Promoter-Reporter Plasmids

Promoters of target genes were obtained from the BioCyc database (<https://biocyc.org/>). Each promoter region, defined as approximately 300 bp pairs flanking either side of the core promoter sequence, was amplified via PCR using specific primers designed for each sequence (Table S5, Supplementary Information). The amplified promoter fragments were inserted upstream of the luciferase operon in the pGEN-luxCDABE plasmid to create recombinant reporter plasmids. Plasmid construction involved the double digestion of both the purified promoter amplicons and the pGEN-luxCDABE vector with PmeI and SnaBI restriction enzymes. Digested products were purified and subsequently ligated using T4 DNA ligase. The resulting recombinant plasmids were transformed into *E. coli* DH5 α competent cells following the transformation protocol previously established (Ma et al., 2023). Transformants were selected on LB agar plates supplemented with 100 $\mu\text{g/mL}$ ampicillin. Positive transformants were confirmed by colony PCR and sequencing, and bioluminescent activity was assessed using a microplate reader to ensure proper expression of the luciferase operon.

5.1.7. Immobilization of Bioluminescent Cells and Assembly of Whole-Cell Biosensor Array

Each confirmed bioluminescent *E. coli* strain carrying a distinct promoter–reporter construct, was immobilized in calcium alginate as described in Section 2.3. The immobilized cells were then dispensed into the wells of a 96-well microplate, producing a biosensor array for simultaneous multiplex detection.

5.1.8. Exposure to VOCs and Bioluminescence Measurement

The whole-cell biosensor array monitored volatile emissions from *A. flavus*–infected peanut and maize kernels at 0, 1, 2, 3, 4, 5, 6, and 7 days post-inoculation, as described in Section 2.3. The 96-well plates were arranged in a sealed chamber to facilitate headspace exposure to VOCs without direct contact between the samples and biosensor cells.

Bioluminescence signals were recorded at 0, 1, 2, 3, 4, and 5 hours after exposure using a microplate reader. These measurements captured the dynamic response of each promoter over time. Multiple independent experiments were conducted, resulting in 258 valid observations per *A. flavus* strain.

5.1.9. Calculation of Induction Factor

To identify the most responsive luminescent whole-cell biosensors to VOCs emitted by *A. flavus*–infected peanut and maize kernels, the induction factor (IF) was employed as a key metric for comparative analysis.

The induction factor was calculated in two main steps for each whole-cell biosensor across all tested time points: The bioluminescence value obtained from the biosensors exposed to VOCs from infected samples was divided by the bioluminescence value from the same biosensors exposed to control (uninfected) samples. This ratio provided a measure of the relative response of the biosensor to infection-induced VOCs at each time point.

$$(1) \quad \text{Ratio} = \frac{\text{Bioluminescence (infected sample)}}{\text{Bioluminescence (control sample)}}$$

The induction factor (IF) was defined as the highest ratio recorded for each biosensor across all measurement times, reflecting the peak bioluminescent response to VOC exposure. IF was calculated as follows:

$$(2) \quad \text{IF} = \max \{ \text{Ratio at 0, 1, 2, 3, 4, 5 h} \}.$$

These IF values were then used as input features for the regression models and to compare biosensor performance across treatments. To select the most responsive luminescent cells, the calculated induction factors for all biosensors

were compared. Biosensors with higher induction factors were considered more sensitive and effective in detecting VOCs from *A. flavus*-infected samples. This approach facilitated the identification of optimal whole-cell biosensors for subsequent analysis and potential applications in the detection of fungal contamination in food products.

5.1.10. Analysis of AFB₁ production

AFB₁ was quantified using an established HPLC method previously applied and validated in our laboratory, based on the Chinese national standard GB 5009.22–2016 with minor modifications (Yang et al., 2020). AFB₁ in peanuts and maize samples was extracted and purified with the ToxinFast immunoaffinity column according to the protocol (Huaan Magnech, Beijing, China). Waters Alliance 2690 high-performance liquid chromatography (Waters, United States) with a reversed-phase C18 column (4.6 mm × 250 mm, 5 µm) was used to analyze the concentration of AFB₁. A photochemical derivator was added for the detection of AFB₁. The mobile phase of AFB₁ was 70% methanol. The excitation and emission wavelengths of AFB₁ determination were 360 nm and 440 nm. The mobile phase flow rate was 1 mL/min. A five-point calibration curve was additionally constructed using AFB₁ standard solutions at concentrations of 0.25, 0.50, 1.00, 1.50, and 2.00 ppm. A linear relationship was obtained between AFB₁ concentration and chromatographic peak area, with the regression equation $y = 551.24x - 10.34$ and $R^2 = 0.9924$. Samples with high AFB₁ concentrations exceeding the calibration range were diluted 100-fold prior to HPLC analysis, and the final concentrations were corrected according to the dilution factor.

5.1.11. Development of Machine-Learning Regression Models for Infection Staging and AFB₁ Estimation

In this study, data for constructing and validating predictive regression models were generated using two distinct aflatoxigenic *A. flavus* strains, cultured under controlled laboratory conditions. A total of 258 valid observations were collected from multiple independent experimental replicates for each *A. flavus* strain, with each observation comprising bioluminescence intensity measurements from a panel of whole-cell biosensors and corresponding biological parameters. For model development, data obtained from the first strain were randomly partitioned into a training set (60 %) and an internal testing set (40 %) using a stratified sampling approach. The entire dataset from the second strain was subsequently employed as an independent external validation set to rigorously assess model generalizability across diverse biological backgrounds. Two continuous response variables were defined for prediction: (i) infection stage, quantified as days post-inoculation (dpi; range: 0–7), and (ii) AFB₁ concentration. The primary input feature set consisted of bioluminescence intensities derived from eight promoter-responsive elements (*dkgB*,

yiaG, *hipB*, *yeeN*, *ygcF*, *frC*, *elfA*, and *evgA*), which were initially identified through comprehensive transcriptomic screening. Additionally, for comparative analysis and to evaluate the predictive capacity of established biosensor components, bioluminescence intensities from fourteen previously characterized promoters (*pspA*, *soxS*, *grpE*, *dnaK*, *fabA*, *glgS*, *katG*, *leuA*, *ompF*, *oxyR*, *recA*, *spy*, *uvrA*, *yibT*), originally utilized in a whole-cell biosensor array for classification of early mold contamination in maize and peanut kernels (Ma et al., 2023), were also incorporated as input features for constructing parallel regression models.

All raw signals were z-score-standardized prior to modelling. To enlarge the training space while minimizing class imbalance, a hybrid data-augmentation pipeline was implemented: (i) interpolation-based synthesis inspired by SMOTE, in which new samples were generated along the line connecting two random neighbours using interpolation factors of 0.2–0.8; and (ii) additive Gaussian noise with a standard deviation equal to 1–2% of the original feature or target deviation. Aberrant observations were discarded according to the three-sigma (3σ) criterion. Feature engineering followed a three-step strategy: (i) removal of highly collinear descriptors (Pearson $r > 0.90$), (ii) principal-component analysis (PCA) retaining components that jointly explained $\geq 95\%$ of cumulative variance, and (iii) ranking of the remaining variables by model-specific importance scores to identify the most influential predictors.

Six supervised regressors were benchmarked: Random Forest (RandomForest), Extreme Gradient Boosting (XGBoost), CatBoost, SGB, SVM, and SPLSDA. Hyper-parameter optimization was performed with Bayesian optimization wrapped in a 10-fold cross-validation framework and was driven by maximization of the coefficient of determination (R^2), while mean absolute error (MAE) and root-mean-square error (RMSE) were monitored as secondary objectives. The tuned search spaces included: RandomForest—number of variables tried at each split (*mtry*), minimum terminal-node size, and splitting rule; XGBoost—learning rate (*eta*), maximum tree depth, minimum child weight, subsampling ratios (*subsample*, *colsample_bytree*), and regularization term (*gamma*); CatBoost—learning rate, tree depth, L2 leaf regularization, and random-strength parameter; SGB—number of trees, interaction depth, shrinkage rate, subsampling fraction, and minimum observations per terminal node; SPLSDA—number of latent components (*K*) and sparsity parameter (*eta*); SVM—penalty constant (*C*), kernel width (γ), and insensitivity parameter (ϵ).

Model performance was first assessed on the held-out internal test subset, and subsequently on the external strain dataset, using R^2 , MAE and RMSE.

Variable importance was quantified by each algorithm's relative-influence metric and visualized as horizontal bar charts of the top 20 contributors (scaled 0–100%). All resampling and external-validation statistics were summarized across ten independent runs and displayed as box-and-whisker plots. The entire workflow was executed in R v4.4.1 with the following core packages: ranger (RandomForest), xgboost (XGBoost), catboost (CatBoost), gbm (SGB), kernlab (SVM), spls (SPLSDA), rBayesianOptimization (hyper-parameter tuning), caret (resampling orchestration), ggplot2 (visualization), and dplyr (data wrangling).

5.2. Results and Discussion

5.2.1. Differential Gene Expression Analysis of Calcium Alginate-immobilized *E. coli* Cells in Response to Headspace VOCs of *A. flavus*-infected Maize and Peanuts

To identify the promoters that serve as responsive elements in whole-cell bioreporters, we subjected calcium-alginate immobilized *E. coli* cells to the VOCs emitted by *A. flavus*-infected peanut and maize kernels (Fig. 12a). Calcium alginate offers significant advantages over agar and other hydrogels for immobilizing bacterial cells as gas sensing elements, including mechanically robust, high stability, tunability for gas diffusion, biocompatibility, and practical ease of use (Duarte et al., 2013; Voo et al., 2016). Its selection was informed by its suitability for our needs, as evidenced by our optimization work (Ma et al., 2020), where we fine-tuned its parameters to enhance VOCs sensing capability. These attributes make it a preferred choice for applications requiring efficient gas exchange and cell functionality, supported by extensive research into its properties and applications (Duarte et al., 2013; López-Menchero et al., 2021). The porous structure of the calcium alginate microbeads facilitated VOCs diffusion, allowing them to act as signaling molecules or stressors, thereby inducing changes in gene expression in encapsulated *E. coli* cells (Veltman et al., 2023).

Differential gene expression analysis revealed distinct transcriptional profiles in calcium alginate-immobilized *E. coli* cells exposed to VOCs from the infected substrates (Fig. 12a). Exposure to VOCs from *A. flavus*-infected peanuts upregulated 272 genes, whereas only 8 genes were upregulated in response to VOCs from infected maize. Similarly, the number of downregulated genes differed significantly, with 82 genes affected in the peanut condition versus 60 genes in the maize condition (Fig. 12b). These results suggest that the VOC mixtures emitted by infected peanuts elicit a stronger transcriptional response in *E. coli* compared to those from maize. This

disparity could be attributed to differences in the composition, concentration, or bioactivity of VOCs produced during *A. flavus* infection of the two substrates (Josselin et al., 2021).

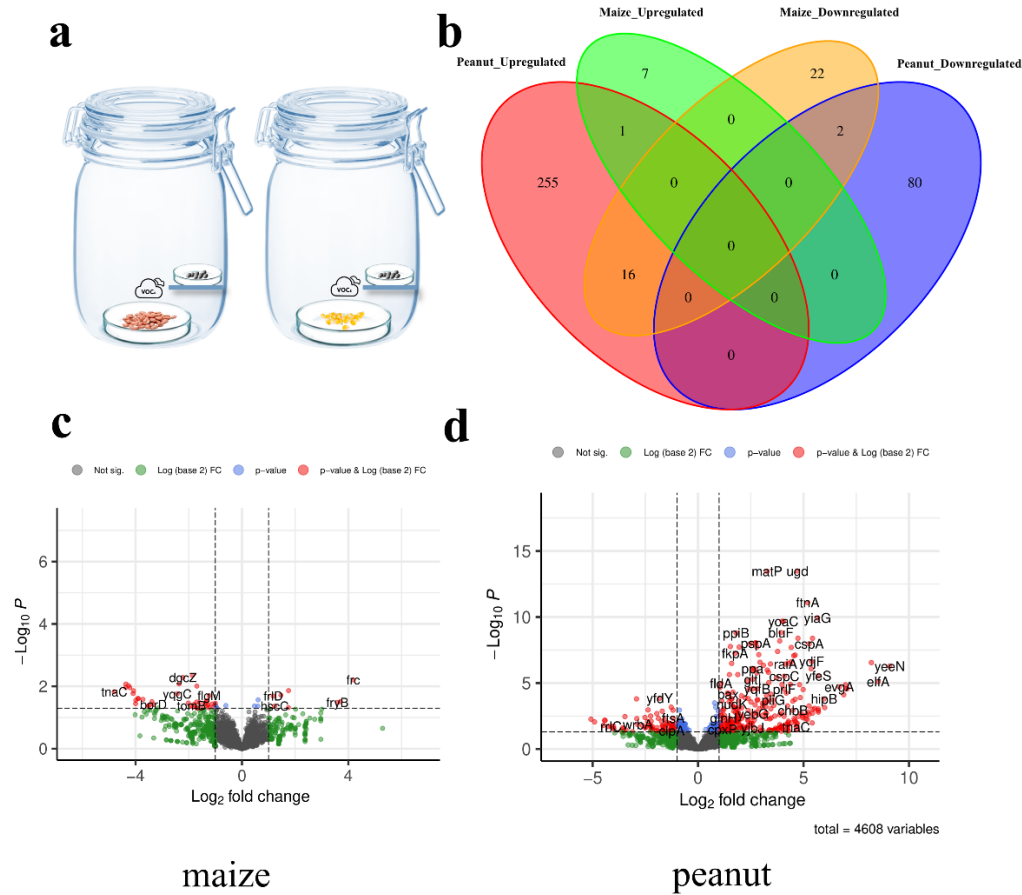


Figure 12. Analysis of gene expression of immobilized *E. coli* in calcium alginate beads in response to the VOCs from *A. flavus* infected maize and peanuts. (a) Schematic representation of immobilized *E. coli* within calcium alginate beads co-cultured with *A. flavus*-infected maize and peanuts within a sealed environment. (b) Venn diagram illustrating the overlap and uniqueness of differentially expressed genes in *E. coli* in response to VOCs emitted by *A. flavus*-infected maize and peanuts. The sets are defined as follows: Maize_Upregulated (upregulated genes in response to VOCs from infected maize), Maize_Downregulated (downregulated genes in response to VOCs from infected maize), Peanut_Upregulated (upregulated genes in response to VOCs from infected peanut), and Peanut_Downregulated (downregulated genes in response to VOCs from infected peanut). (c) Volcano plot depicting the differential expression of genes in maize. (d) Volcano plot illustrating the differential expression of genes

in peanuts. Note: the criteria for DEGs are defined by an absolute $\log_2FC$ greater than 1 and an adjusted p-value less than 0.05.

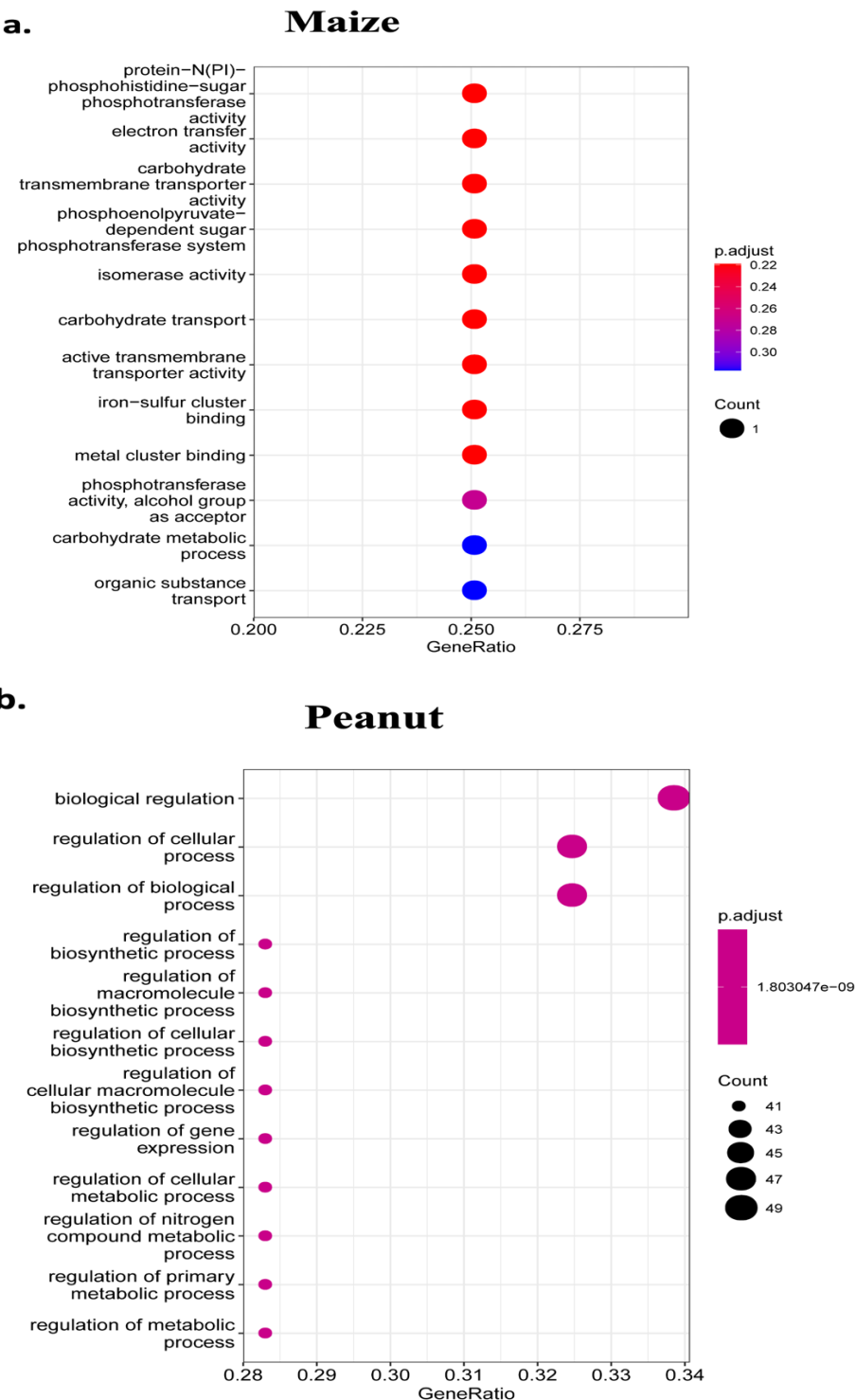
Volcano plot analyses provided further insights into the transcriptional changes induced by the VOCs (Fig. 12c and 12d). In the maize condition, the most significantly upregulated genes included *frc* (involved in the cellular response to acidic pH), *fryB* and *frlD* (both involved in phosphorylation), and *hscC* (associated with stress response). Downregulated genes included *tnaC*, *borD*, *yqgC*, *dgcZ*, *tomB*, and *flgM*. In contrast, VOCs from infected peanuts resulted in a significantly higher number of upregulated genes, including *yeeN* (regulation of DNA-templated transcription), *elfA* (biofilm formation), and *evgA* (phosphorelay signal transduction). Fewer downregulated genes were observed, notably *rrlC* and *wrbA*.

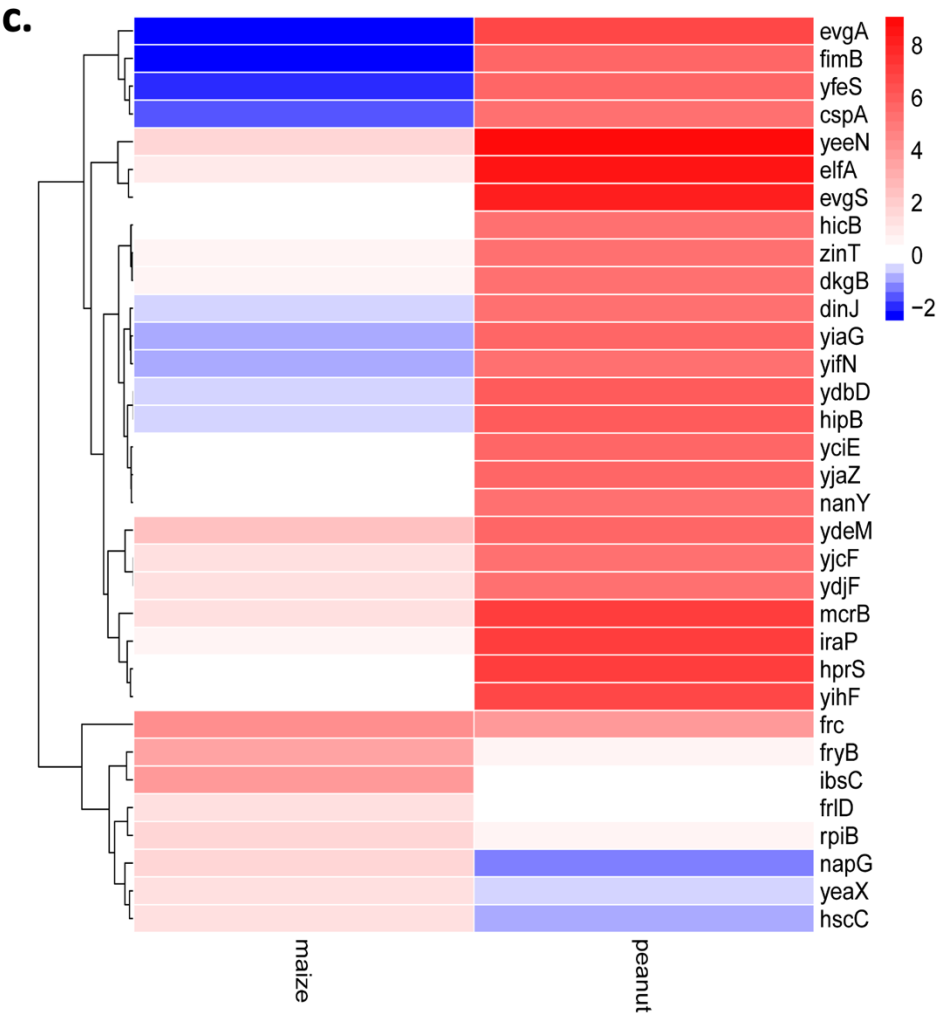
The variability in the number of DEGs between conditions underscores the distinct impacts of VOCs from the two substrates. While prior studies reported greater diversity of VOCs emitted by *A. flavus*-infected maize (55 VOCs) (Li et al., 2021) compared to peanuts (31 VOCs) (Ma et al., 2023), these findings are highly dependent on experimental conditions, including different strains and VOCs detection methodology (e.g., GC-MS or GC-IMS). Moreover, VOCs from infected peanuts may possess unique signaling molecules or stress-inducing compounds that provoke a stronger transcriptional response in *E. coli*. More importantly, the distinct transcriptional profiles observed in *E. coli* exposed to VOCs from *A. flavus*-infected peanuts and maize highlight the potential of using the upregulated promoters in *E. coli* as sensing elements for detecting spoilage in stored agricultural products. Previous studies have demonstrated that specific promoters in *E. coli* can respond dynamically to environmental conditions, including VOCs, and their activity can be harnessed for biosensor development (Yung et al., 2016). Calcium alginate immobilization provides a protective niche for *E. coli*, enhancing its stability, longevity and gas diffusion, which is critical for practical applications in monitoring stored agricultural products (Veltman et al., 2023). Our approach leverages the natural transcriptional responses of calcium alginate-immobilized *E. coli* cells to VOCs, providing a promising avenue for developing biosensors that can non-invasively monitor spoilage in stored peanuts and maize kernels.

5.2.2. Identification and Characterization of Upregulated Genes in Response to VOCs

Following differential gene expression analysis of *E. coli* exposed to the headspace VOCs (HVOCs) emitted from *A. flavus*-infected peanut and maize kernels, we performed GO term enrichment analysis on the significantly upregulated genes. This allowed us to identify potentially responsive elements for constructing whole-cell bioreporters. The GO enrichment analysis revealed distinct biological processes that were significantly enriched in response to the HVOCs from infected maize (Fig. 13a). The enriched terms predominantly involved metabolic processes related to carbohydrates, transport activities, electron transfer, and specific enzymatic functions. The enrichment of these GO classifications indicates that *E. coli* is adjusting its metabolic pathways and transport systems to respond to the HVOCs from moldy maize. The bacterium may be utilizing these VOCs as a nutrient source (Abis et al., 2020) or coping with potential stress induced by the environmental conditions. This adaptive response reflects *E. coli*'s ability to sense and respond to changes in its environment, optimizing its survival and metabolic efficiency under challenging conditions.

In contrast, the GO enrichment analysis for *E. coli* exposed to HVOCs from moldy peanuts showed a different set of enriched biological processes (Fig. 13b). The enriched processes included regulation of metabolic processes, primary metabolic processes, nitrogen compound metabolic processes, cellular metabolic processes, gene expression, cellular macromolecule biosynthetic processes, and broader biological processes. These findings suggest that the VOCs from moldy peanuts may act as signaling molecules that influence bacterial metabolism, gene expression, and macromolecule synthesis. The low *p*-value adjustment (<0.01) for these GO terms indicated that these processes were significantly regulated in response to the HVOCs, highlighting *E. coli*'s ability to sense and adapt to the environmental cues provided by the volatiles. This adaptive mechanism is crucial for the bacterium's survival and underscores the dynamic interaction between *E. coli* and its environment.





d.

Gene	GO
zyxR	GO:0009100 - phosphorelay signal transduction system
mxhB	GO:0006974 - DNA damage response
y65	GO:0006159 - cytosol
cspA	GO:0009409 - response to cold
yeeN	GO:0006355 - regulation of DNA-templated transcription
ctfA	GO:0043709 - cell adhesion involved in single-species biofilm formation
cyrS	GO:0019847 - response to acidic pH
bciB	GO:0009650 - response to virus
zntT	GO:0019991 - cellular response to hydrogen peroxide
dkgA	GO:0042406 - multi-glycanal catabolic process; GO:0019531 - L-ascorbic acid biosynthetic process
dsu	GO:0015441 - toxic substance binding; GO:0044403 - single-species biofilm formation
suG	GO:0003677 - DNA binding
y4N	GO:0003677 - DNA binding
ydbD	GO:0031594 - response to methylglyoxal
lgiB	GO:0008086 - regulation of growth; GO:0006355 - regulation of DNA-templated transcription
ytIE	GO:0009269 - cytosol
ysiZ	GO:0009608 - response to heat; GO:0005529 - cytosol
nanY	GO:0006974 - DNA damage response
ydsM	GO:0006974 - DNA damage response
yisF	GO:0006159 - cytosol
y4P	GO:0006355 - regulation of DNA-templated transcription
nerB	GO:0006306 - DNA catabolic process
inaP	GO:0009267 - cellular response to starvation; GO:0016054 - cellular response to phosphate starvation
hpsS	GO:0006974 - DNA damage response; GO:0005176 - cellular response to stimulus
yalF	GO:0005886 - plasma membrane
fib	GO:0071844 - cellular response to acidic pH; GO:0033541 - oxalate catabolic process
fspB	GO:0015310 - phosphorolysis; GO:0091702 - organic substance transport
lbuC	GO:0012501 - programmed cell death; GO:0005886 - plasma membrane
relD	GO:0016310 - phosphorylation
mjtB	GO:0005975 - carbohydrate metabolic process
mapG	GO:0015536 - iron-sulfur cluster binding
yeaX	GO:0016491 - oxidoreductase activity
bucC	GO:0009650 - response to virus

Figure 13. GO-term-based enrichment analysis of significantly upregulated genes in *E. coli* exposed to volatile organic compounds (VOCs) from *A. flavus*-infected maize and peanut samples. (a) GO-term enrichment analysis of upregulated genes in *E. coli* in response to head-space VOCs from infected maize samples. (b) GO-term enrichment analysis of upregulated genes in *E. coli* in response to head-space VOCs from infected peanut samples. (c) Heatmap representation of the top 8 upregulated genes in response to VOCs from infected maize samples (left) and the top 25 upregulated genes in response to VOCs from infected peanut samples (right). (d) GO categories associated with the 33 selected upregulated genes. Highlighted in orange are the 8 genes (*evgA*, *yeeN*, *elfA*, *dkgB*, *yiaG*, *hipB*, *yjcF*, and *frc*) whose promoter regions were selected for downstream whole-cell biosensor construction.

To further investigate the transcriptional response of *E. coli* to the VOCs emitted from *A. flavus*-infected peanut and maize kernels, we focused on the significantly upregulated genes. From the list of upregulated genes, we selected key candidates for functional analysis. Specifically, we examined the top 8 upregulated genes in response to maize-derived VOCs and the top 25 upregulated genes in response to peanut-derived VOCs (Fig. 13c). Most of these genes were associated with stress response mechanisms, including *fimB* (DNA damage response), *hicB* (general stress response), and *nanY* (DNA damage response), or signal transduction processes such as *yeeN* (regulation of DNA-templated transcription), *fryB* (phosphorylation), and *ydjF* (regulation of DNA-templated transcription) in response to

environmental stimuli (Fig. 13d). These findings align with previous studies demonstrating the role of VOCs as signaling molecules that influence bacterial behavior (Yung et al., 2016).

In the case of maize-derived volatiles, the most significantly upregulated genes were *frc*, *fryB*, *ibsC*, and *frlD*, with $\log_2$ fold change ($\log_2FC$) values of 4.1, 3.6, and 3.7, respectively (Fig. 13c). The *frc* gene, known for its role in acidic pH stress response and oxalate catabolism, likely aids in mitigating the toxic effects associated with such environmental conditions (Masuda & Church, 2002). The upregulation of *fryB* and *frlD*, both implicated in phosphorylation, suggests their involvement in global signaling systems within *E. coli*, enabling the bacterium to sense environmental cues and activate stress-related pathways (Tchieu et al., 2001). The gene *ibsC*, linked to programmed cell death, further underscores the severe stress experienced by *E. coli* upon exposure to VOCs from infected maize (Mok et al., 2009). These findings align with the notion that VOCs emitted from infected plants can induce oxidative stress, DNA damage, and membrane disruption, leading to broad cellular stress responses (Yung et al., 2016).

In contrast, *E. coli* exposed to VOCs from infected peanut samples exhibited a different pattern of gene upregulation. The most significantly upregulated genes included *yeeN* ($\log_2FC = 9.0$), *elfA* ($\log_2FC = 8.5$), and *evgS* ($\log_2FC = 8.2$) (Fig. 13c). *YeeN* is involved in the regulation of DNA-templated transcription, *elfA* (Serina et al., 2004) is associated with biofilm formation, and *evgS* is known to respond to acidic pH (Masuda & Church, 2002).

Overall, the high expression levels of these 33 genes indicate that *E. coli* mounts a robust transcriptional response to the complex and potentially harmful VOCs produced during the interaction between *A. flavus* and food substrates. The differential gene expression patterns observed in *E. coli* exposed to VOCs from *A. flavus*-infected peanut and maize kernels suggest that specific genes and their associated promoters could be harnessed to develop whole-cell bioreporters for spoilage detection. By integrating these promoters into genetic circuits, it may be possible to develop sensitive and specific bioreporters capable of detecting spoilage VOCs in real-time.

5.2.3. Construction and Functional Validation of a Whole-Cell Biosensor Array for Time-Dependent VOC Response Analysis

Subsequently, from the initial pool of 33 highly upregulated genes identified through RNA-seq analysis, a subset of 16 candidate promoters

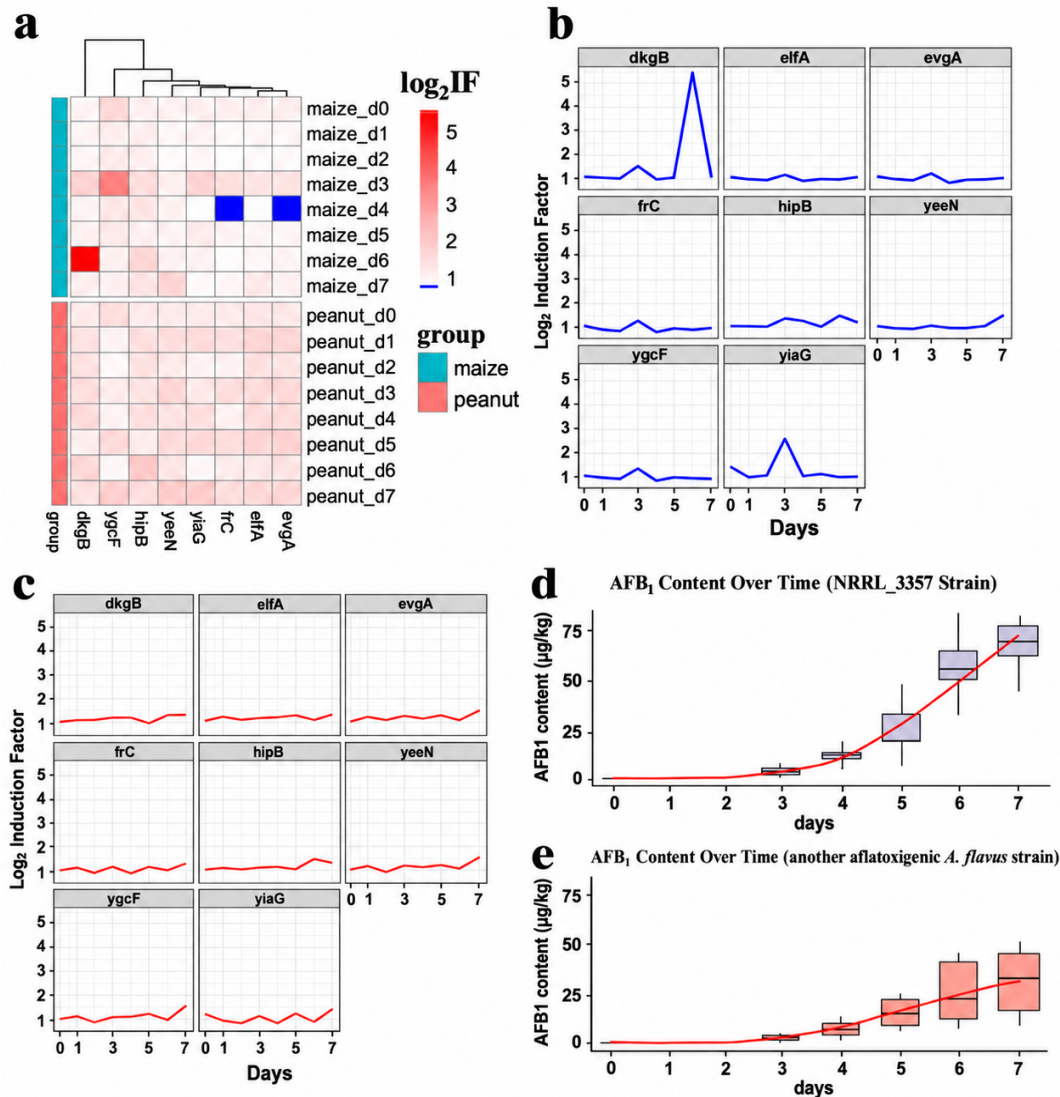
(Figure S6) was selected based on a dual criterion: exhibiting substantial relative gene expression increases and successful cloning into luciferase reporter plasmids suitable for whole-cell biosensor construction. To further prioritize these candidates and streamline downstream efforts towards an effective biosensor array, we conducted a preliminary functional validation using calcium alginate-immobilized whole-cell biosensors harboring these 16 distinct promoter-luciferase fusions as described in section 2.6. Following exposure to headspace VOCs from *A. flavus*-infected kernels, bioluminescence signals were collected and analyzed for relative fold change and Z-score normalization (detailed in Figure S6). This screening revealed that eight specific promoters (namely those associated with the genes *evgA*, *frC*, *elfA*, *yiaG*, *ygcF*, *yeeN*, *dkgB*, and *hipB*) demonstrated particularly prominent and significant bioluminescence responses upon VOC exposure. Consequently, these eight highly responsive promoters were chosen as the optimal candidates for constructing the final multi-channel whole-cell biosensor array, providing a robust and focused set for subsequent machine-learning model development and predictive analysis.

To enable the dynamic and real-time monitoring of VOCs released from *A. flavus*-infected maize and peanut kernels, we engineered a whole-cell biosensor array comprising eight *A. flavus* reporter strains. Each strain incorporated a distinct, highly VOC-responsive promoter, previously identified via a high-throughput functional screening pipeline, driving bioluminescence. The biosensor array was systematically exposed to VOCs emanating from both infected and uninfected control kernels under sealed incubation conditions. Bioluminescence signals were quantitatively recorded in triplicate at eight discrete time points over a seven-day period post-inoculation (0 to 7 dpi), generating a comprehensive dataset for temporal analysis. To accurately quantify promoter induction in response to VOCs, an induction factor (IF) was calculated for each biosensor at every time point, using signals from non-infected samples as the baseline reference (detailed in Section 2.8). All IF values were subsequently $\log_2$ -transformed (Log_2IF) to facilitate comprehensive visualization and comparative analysis, and presented as a heatmap (Fig. 14a) illustrating the temporal dynamics of the biosensor responses. Analysis of the heatmap revealed a predominant pattern of positive Log_2IF values (indicated by red coloration), signifying a robust upregulation of biosensor activity upon exposure to VOCs emitted from *A. flavus*-infected samples. This finding aligns consistently with our prior transcriptional profiling (Fig. 14c), thereby reinforcing the functional responsiveness and specificity of the selected promoters. Notably, VOCs released from infected maize and peanut kernels elicited distinct response profiles, with the biosensors

forming separate temporal clusters based on the host substrate. Specifically, biosensors exposed to maize-derived VOCs consistently exhibited generally higher Log_2IF values throughout the time course compared to those exposed to peanut-derived VOCs, implying a stronger or more sustained transcriptional activation. This observed substrate-dependent difference critically suggests that the nutritional composition and metabolic environment provided by the host material directly influences the metabolic activity and secondary metabolite production of *A. flavus*, consequently altering the specific profile of emitted VOCs that trigger differential biosensor responses.

Further temporal analysis, illustrating Log_2IF changes for individual promoters across the 7-day infection period (0-7 dpi), elucidated distinct substrate-specific response patterns (Fig. 14b,c). In maize, the responses of biosensors (Fig. 14b) exhibited a transient upregulation, generally peaking around 3 dpi, followed by a subsequent decline or plateau. For instance, promoter *dkgB* showed the most pronounced activation, reaching a peak Log_2IF value exceeding 4.8 at 5 dpi, while *yiaG* displayed significant but less pronounced induction kinetics ($\text{Log}_2\text{IF} \approx 2.7$). Conversely, promoters like *elfA* and *yeeN* showed only marginal upregulation, indicating limited responsiveness to the volatile profile associated with *A. flavus* infection in maize. In stark contrast, peanut samples induced a markedly different biosensor response profile (Fig. 14c), characterized by a more gradual and sustained increase in Log_2IF values over time, with most VOC-responsive promoters reaching maximal activation at 7 dpi. This extended activation window in peanuts suggests either a delayed or prolonged release of specific VOCs, or sustained microbial activity, which maintains biosensor stimulation. These contrasting kinetics highlight significant substrate-dependent variations in VOC dynamics and biosensor responsiveness, carrying implications for designing time-sensitive detection systems tailored to specific host matrices. To contextualize these biosensor dynamics, we concurrently assessed the temporal production of AFB₁ in maize kernels infected with two different *A. flavus* strains under identical sealed incubation conditions. As shown in Fig. 14d, infection by the NRRL_3357 strain led to a pronounced exponential surge in AFB₁ accumulation starting at 4 dpi, reaching a peak median concentration of approximately 75 $\mu\text{g}/\text{kg}$ by 7 dpi, albeit with notable inter-replicate variability reflecting differential colonization or toxin biosynthesis rates. In comparison, the second aflatoxigenic *A. flavus* strain induced a more gradual AFB₁ accumulation, rising steadily from 3 dpi and plateauing around 35 $\mu\text{g}/\text{kg}$ by 7 dpi (Fig. 14e). Importantly, despite these quantitative differences, the temporal AFB₁ profiles of both strains exhibited a strong positive correlation (Pearson's $r = 0.986$; Fig. 14f), which supports the potential applicability of

predictive models developed from NRRL_3357 data for estimating AFB₁ levels induced by other aflatoxigenic strains. Collectively, these results demonstrate that our constructed multi-channel biosensor array effectively captures both the magnitude and intricate temporal patterns of VOC emissions during *A. flavus* infection with high sensitivity and distinct substrate specificity. The observed divergence in transcriptional response profiles between maize and peanut samples further underscores the profound influence of the host metabolic environment on the precise composition of emitted VOCs, thereby establishing a critical foundation for developing pathogen detection strategies that are specifically tailored to crop-specific biochemical contexts.



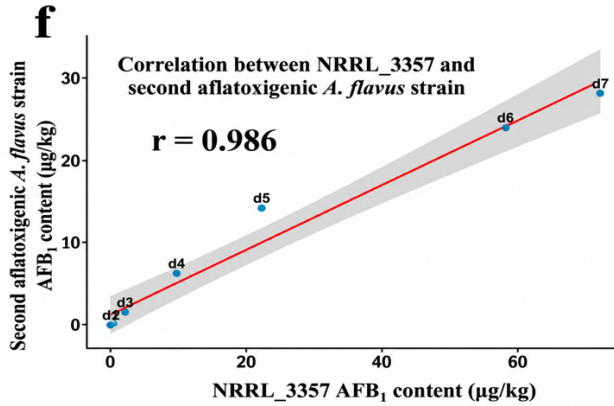


Figure 14. Temporal dynamics and clustering of whole-cell bioreporter responses to *A. flavus*-induced VOCs in maize and peanut. (a) Hierarchical clustering heatmap of Log₂-transformed induction factor (Log₂IF) values across 11 promoter-driven biosensors exposed to VOCs emitted from *A. flavus*-infected kernels, illustrating distinct temporal activation profiles. (b) Time-course plots of Log₂IF values in maize samples (blue), measured at 0, 1, 2, 3, 4, 5, 6, and 7 days post-inoculation (dpi). Each panel corresponds to a specific promoter. (c) Corresponding Log₂IF trajectories in peanut samples over the same infection timeline. (d,e) Aflatoxin B₁ (AFB₁) accumulation dynamics in maize kernels infected with *A. flavus* strain NRRL_3357 (d) and a second aflatoxigenic strain (e), quantified across identical dpi stages. (f) Pearson correlation coefficient of AFB₁ levels between the two strains across all time points, indicating concordance in temporal AFB₁ accumulation patterns.

5.2.4. Regression Model Performance for Predicting *A. flavus* Infection Stages in Maize Kernels

To develop a reliable, non-destructive pipeline for early detection of *A. flavus* colonization in maize kernels, we benchmarked six supervised learning algorithms—Random Forest, XGBoost, CatBoost, SGB, SVM, and SPLSDA—for their ability to regress infection stage (0–7 dpi) from integrated VOC profiles and bioluminescent biosensor readouts. After comprehensive hyperparameter optimization (Table S6, Supplementary Information), CatBoost and SGB achieved the highest apparent accuracies on internal datasets (training $R^2 \approx 0.98$; test $R^2 \approx 0.95$), but their predictive performance declined markedly when applied to an external dataset derived from a second aflatoxigenic *A. flavus* strain ($R^2 \leq 0.5$), reflecting overfitting and limited strain generalizability (Fig. 15a–c). In contrast, XGBoost maintained consistently high performance across training ($R^2 = 0.95$), test ($R^2 = 0.94$), and external validation datasets ($R^2 = 0.92$), underscoring its robustness against pathogen-specific VOC

variability and its capacity to capture stable, non-linear relationships between fungal VOC emissions and biosensor responses. Feature importance analysis further revealed that XGBoost predictions were strongly driven by early infection-associated biosensor outputs, with key contributors including *hipB_3h*, *elfA_4h*, *evgA_0h*, and *dkgB_3h*, which regulate processes such as growth, biofilm formation, phosphorelay signaling, and methylglyoxal detoxification (Fig. 15d). These findings are mechanistically consistent with the oxidative stress responses triggered by early-stage fungal VOCs in host tissues.

To evaluate novelty relative to our previous approach, which employed 14 literature-reported stress-responsive promoters, we constructed regression models under identical preprocessing and tuning protocols. Although the SVM model achieved perfect fit on training data ($R^2 = 0.94$; Fig. S7a), its predictive accuracy decreased substantially on internal test ($R^2 = 0.84$) and external validation datasets ($R^2 = 0.76$; Fig. 15e), confirming overfitting. By contrast, the transcriptomic-guided selection of infection-specific promoters coupled with XGBoost modeling in the present study achieved superior and more balanced accuracy across all datasets (training $R^2 = 0.95$; test $R^2 = 0.94$; external $R^2 = 0.92$; Fig. 15e), demonstrating enhanced specificity, reduced variance, and improved biological interpretability. These results demonstrate that transcriptomics-guided promoter selection not only enhances biosensor specificity and signal strength but also mitigates model overfitting, thereby improving predictive accuracy in heterogeneous biological contexts. Such integration of molecularly informed biosensor design with machine learning establishes a scalable and transferable framework for early, non-destructive detection of fungal contamination, consistent with previous evidence highlighting transcriptomic analysis as a powerful approach for identifying VOC-responsive promoters in diverse biosensing applications (Veltman et al., 2023; Yung et al., 2016; Tan et al., 2015).

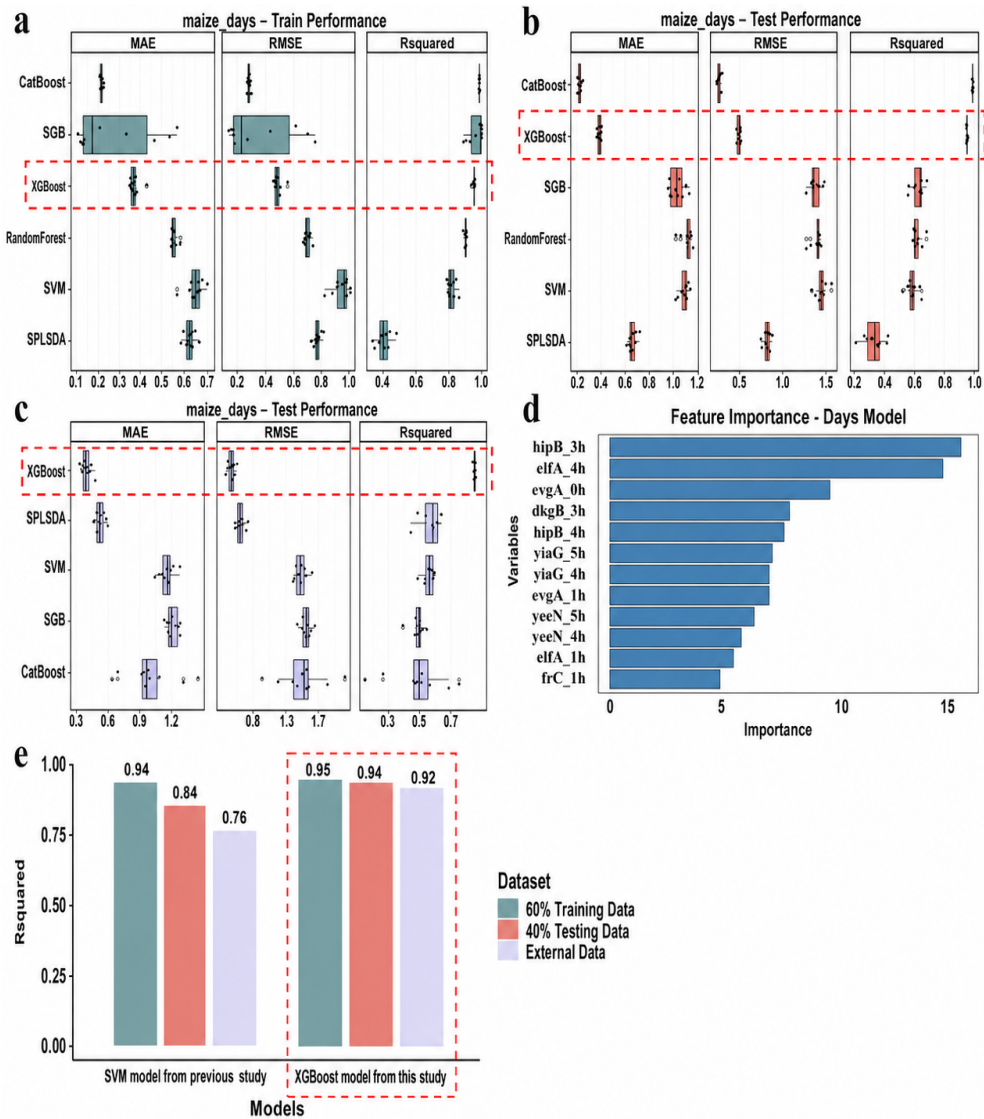


Figure 15. Comparative performance of regression models for predicting *A. flavus* infection stages in maize kernels. (a–c) Boxplots summarizing the predictive accuracy of six regression algorithms—Random Forest (RF), Stochastic Gradient Boosting (SGB), CatBoost, XGBoost, Support Vector Machine (SVM), and Sparse Partial Least Squares Discriminant Analysis (SPLSDA)—evaluated using mean absolute error (MAE), root mean square error (RMSE), and coefficient of determination (R^2). Models were trained and tested on: (a) 60% of maize kernel samples inoculated with *A. flavus* strain NRRL_3357 (training set); (b) 40% of the same dataset (testing set); and (c) an independent external maize kernel dataset inoculated with a distinct aflatoxigenic *A. flavus* strain. (d) Feature importance plot

showing the top 20 variables contributing to infection stage prediction, as identified by the best-performing XGBoost model, with importance scaled from 0 to 100%. (e) Parallel comparison of R^2 values between an SVM model which utilized previously-published 14 promoters for whole-cell biosensor array construction, and the current XGBoost model informed by transcriptomic-guided selection of 8 promoters in this study, revealing enhanced predictive accuracy in the latter across testing (40%), and external datasets. The red dashed rectangles in panels (a–c) highlight the most accurate and robust models across metrics and datasets.

5.2.5. Modeling Aflatoxin B₁ Accumulation in Maize Kernels

To assess the practical applicability of the proposed biosensing platform in food safety, we applied predictive modeling to quantify AFB₁ levels in maize kernels, a critical indicator of *A. flavus* colonization. Among the algorithms evaluated, CatBoost showed the highest accuracy on the training (60 %) and internal testing (40 %) datasets ($R^2 \approx 0.98$). However, its predictive performance significantly declined on an external dataset derived from a second aflatoxigenic strain ($R^2 \approx 0.5$), highlighting its susceptibility to strain-specific variations (Fig. 16a–c). In contrast, XGBoost, optimized with the hyperparameters provided in Table S2 (Supplementary Information), demonstrated robust predictive power, achieving R^2 values of 0.98 on both the training and internal testing datasets, and maintained high accuracy ($R^2 = 0.91$) on independent external validation (Fig. 16a–c). Feature importance analysis revealed that model predictions were primarily driven by transcripts associated with stress adaptation and fungal developmental regulation, including *yiaG_5h* (DNA binding), *evgA_0h* (phosphorelay signaling), *elfA_1h* (biofilm formation), *yeeN_3h* (transcriptional regulation), and *hipB_3h* (growth control), which are all involved in early stages of fungal infection (Fig. 16d). These molecular markers corroborate the known role of oxidative stress and transcriptional reprogramming as key triggers for the aflatoxin biosynthetic pathway, providing mechanistic support for the predictive accuracy of our model. In comparison, a previously reported whole-cell biosensor array incorporating 14 promoters and six regression models exhibited lower performance, with CatBoost achieving the best results on the 60 % training dataset but underperforming on the 40% testing ($R^2 = 0.84$) and external datasets ($R^2 = 0.75$) (Fig. 16e). Notably, our novel method, based on the transcriptomic selection of 8 promoters, demonstrated superior stability and robustness, achieving R^2 values of 0.98, 0.98, and 0.91 on the 60 % training, 40 % testing, and external datasets, respectively (Fig. 16e). The capacity of XGBoost to integrate biologically relevant signals from biosensor data further highlights its potential for non-invasive, real-time monitoring of mycotoxin contamination across diverse environmental conditions. Taken together, these results establish the XGBoost-based biosensing platform as a scalable, field-deployable early warning system for postharvest management, advancing food safety monitoring by combining mechanistic insight with high predictive accuracy.

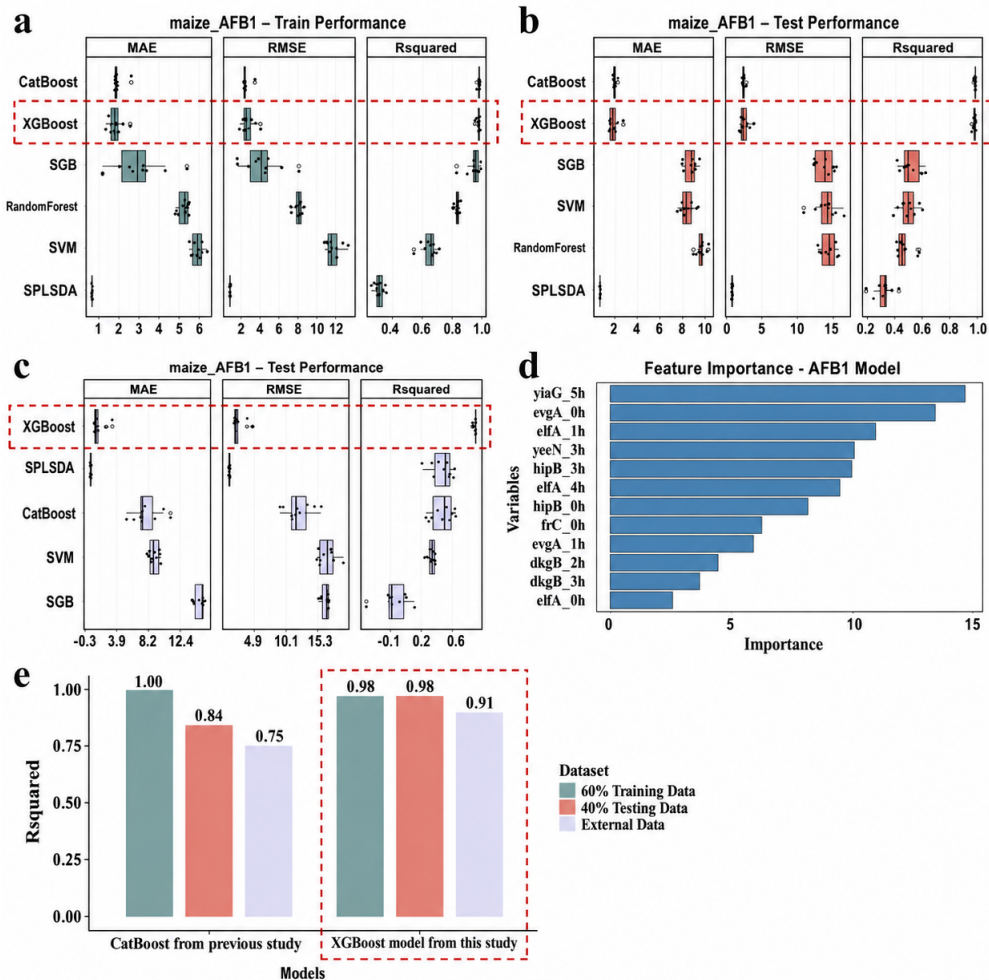


Figure 16. Predictive performance benchmarking of regression models for estimating AFB₁ production by *A. flavus* in maize kernels. (a–c) Boxplots illustrating the predictive accuracy of six machine learning regression algorithms—Random Forest (RandomForest), Stochastic Gradient Boosting (SGB), CatBoost, XGBoost, Support Vector Machine (SVM), and Sparse Partial Least Squares Discriminant Analysis (SPLSDA)—in forecasting aflatoxin B₁ (AFB₁) accumulation. Model performance was assessed using mean absolute error (MAE), root mean square error (RMSE), and coefficient of determination (R²) on: (a) 60% of maize kernel samples inoculated with *A. flavus* strain NRRL_3357 (training set), (b) the remaining 40% of the same dataset (testing set), and (c) an independent external maize kernel dataset inoculated with a genetically distinct aflatoxigenic *A. flavus* strain. (d) Relative importance (scaled 0–100%) of the top 20 predictive variables for AFB₁ estimation, as determined by the best-performing XGBoost model. (e) Parallel comparison of R²

values between a CatBoost model which utilized previously-published 14 promoters for whole-cell biosensor array construction, and the current XGBoost model informed by transcriptomic-guided selection of 8 promoters in this study, revealing enhanced predictive accuracy in the latter across testing (40%), and external datasets. The red dashed rectangles in panels (a–c) highlight the most accurate and robust models across metrics and datasets.

5.2.6. Regression Model Performance for Predicting *A. flavus* Infection Stages in Peanut Kernels

To assess the robustness and generalizability of the proposed modeling framework, we applied it to a distinct agricultural system by predicting infection stages in peanut kernels inoculated with *A. flavus* using six regression models trained on eight transcriptomics-guided promoters. Among them, the SGB model achieved the lowest MAE and RMSE with an R^2 of 0.97 on the 60 % training set, yet its performance declined markedly on the 40 % test ($R^2 = 0.60$) and external datasets ($R^2 < 0.70$), indicating overfitting (Fig. 17a–c). The CatBoost model displayed better balance, yielding R^2 values of 0.96 on both training and test sets, but its external validation ($R^2 = 0.68$) similarly revealed limited generalization. In contrast, XGBoost consistently outperformed the other models, maintaining high predictive accuracy across training ($R^2 = 0.94$), test ($R^2 = 0.94$), and external datasets ($R^2 = 0.92$), thereby underscoring its superior capacity to capture infection dynamics in heterogeneous biological contexts (Fig. 17a–c). Feature importance analysis further revealed that promoters such as *evgA_5h*, *yiaG_0h*, *ygcf_0h* and *ygcf_1h*, *yeeN_0h*, and *frc_0h* were critical determinants, suggesting that early-stage fungal VOCs rapidly activate cellular signaling pathways in whole-cell biosensors. For comparison, models constructed using 14 literature-reported stress-responsive promoters exhibited strong training fits (CatBoost, $R^2 = 1.0$) but showed poor generalization on internal ($R^2 = 0.86$) and external ($R^2 = 0.77$) datasets (Fig. 17e), highlighting the inadequacy of non-specific promoters. Collectively, these results demonstrate that transcriptomics-guided promoter selection combined with XGBoost modeling achieves stable, biologically interpretable predictions with reduced overfitting, thereby providing a more reliable strategy for biosensor-based infection monitoring across diverse host–pathogen systems.

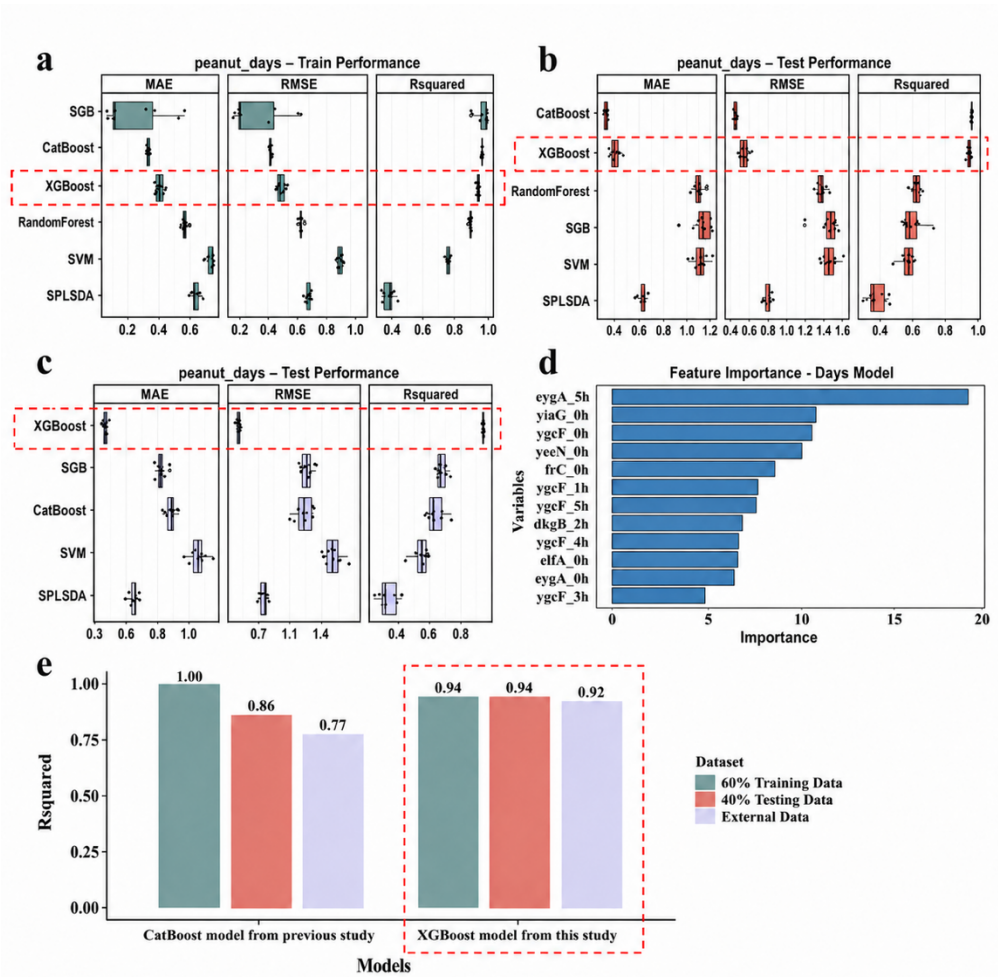


Figure 17. Comparative performance of six regression models for predicting *A. flavus* infection stages in peanut kernels. (a–c) Boxplots illustrating the predictive performance of Random Forest (RandomForest), Stochastic Gradient Boosting (SGB), CatBoost, XGBoost, Support Vector Machine (SVM), and Sparse Partial Least Squares Discriminant Analysis (SPLSDA) across three evaluation metrics: mean absolute error (MAE), root mean square error (RMSE), and coefficient of determination (R^2). Models were trained and validated on: (a) 60% of peanut kernel samples inoculated with *A. flavus* strain NRRL_3357 (training set), (b) 40% of the same dataset (testing set), and (c) an independent test set comprising kernels infected with a distinct aflatoxigenic *A. flavus* strain. (d) Ranked feature importance plot from the top-performing CatBoost model, highlighting the 20 most influential variables contributing to model predictions, with normalized importance scores (0–100%). (e) Parallel comparison of R^2 values between a CatBoost model which utilized previously-published 14 promoters for whole-cell biosensor array construction, and

the current XGBoost model informed by transcriptomic-guided selection of 8 promoters in this study, revealing enhanced predictive accuracy in the latter across testing (40%), and external datasets. The red dashed rectangles in panels (a–c) highlight the most accurate and robust models across metrics and datasets.

5.2.7. Prediction of AFB₁ Production in Peanut Kernels

Our predictive framework was further evaluated for its capacity to quantify AFB₁ accumulation in peanut kernels, a critical parameter for food safety assessment. The XGBoost regression model exhibited strong predictive performance, achieving coefficients of determination (R^2) of 0.97 on both training and internal testing datasets (Fig. 18 a, b). Importantly, its robustness was maintained in an independent external validation set containing peanuts infected by a genetically distinct toxigenic *A. flavus* strain, where an R^2 of 0.86 was obtained (Fig. 18c). This consistency across fungal genotypes underscores the model's high generalization capability, a prerequisite for deployment in heterogeneous agricultural environments. Feature importance analysis further revealed that the most influential predictors corresponded to transcriptional markers of stress responses and metabolic regulation (Fig. 18d), including *elfA_0h* and *elfA_1h*, *yeeN_0h*, *evgA_5h*, and *dkgB_2h/3h*. These pathways are tightly coupled with fungal stress adaptation and aflatoxin biosynthesis, supporting the mechanistic relevance of the predictors and highlighting how VOC-based profiles capture the pathogen's physiological state during infection. For comparative benchmarking, we constructed regression models using 14 literature-reported stress-responsive promoters under identical preprocessing and optimization protocols. Although CatBoost achieved a perfect fit on the training dataset ($R^2 = 1.0$; Fig. S7d), its performance dropped significantly on internal test ($R^2 = 0.84$) and external validation datasets ($R^2 = 0.75$; Fig. 18e), indicative of overfitting and limited generalizability. In contrast, transcriptomic-guided promoter selection coupled with XGBoost modeling produced consistently higher and more balanced performance across all datasets (training $R^2 = 0.97$; test $R^2 = 0.97$; external $R^2 = 0.86$; Fig. 18e), confirming enhanced specificity, reduced variance, and improved biological interpretability. Collectively, these results demonstrate that integrating transcriptomics-driven feature selection with robust machine learning architectures not only improves prediction accuracy but also establishes VOCs fingerprints as reliable, non-invasive indicators of *in situ* toxigenesis. The ability to estimate both fungal infection and toxin accumulation with high fidelity, consistent with previous findings in maize systems, suggests broad applicability across crops and provides a valuable framework for pre-harvest and early postharvest risk assessment in real-world agricultural settings.

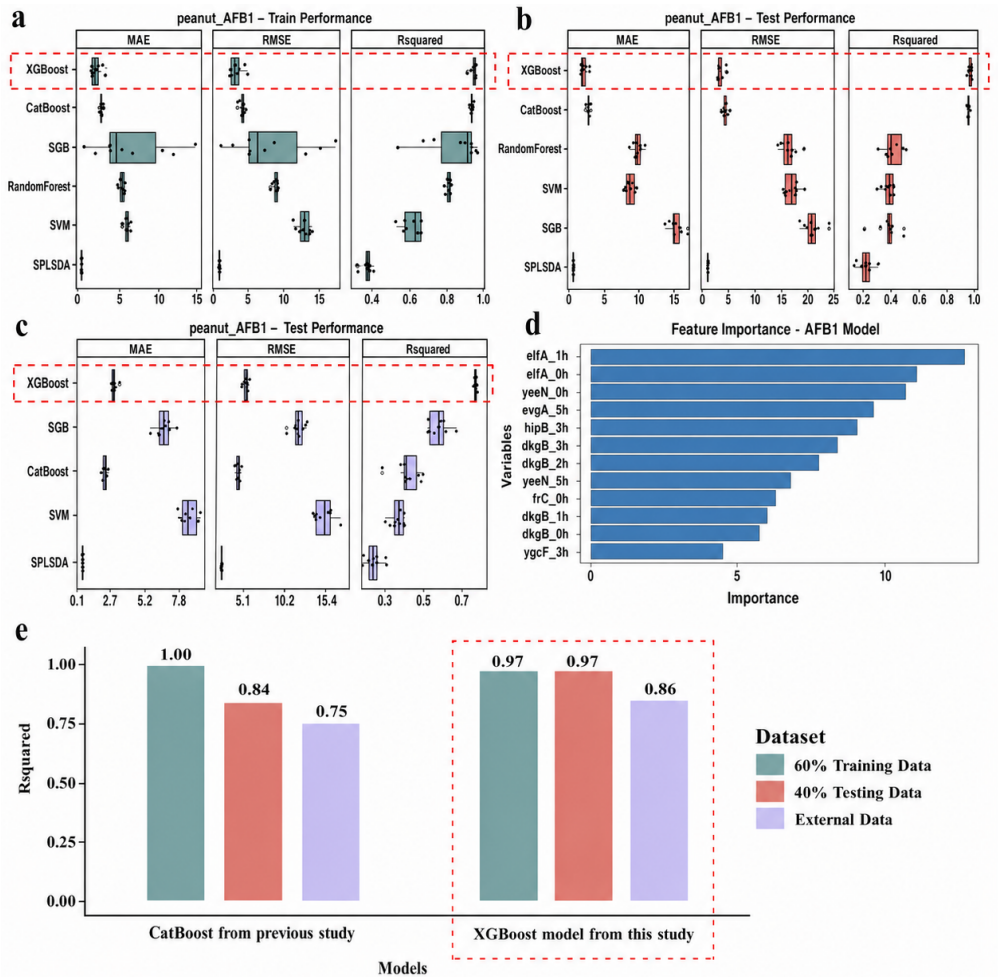


Figure 18. Predictive performance benchmarking of regression models for estimating AFB₁ production by *A. flavus* in peanut kernels. (a–c) Boxplots illustrating the predictive performance of Random Forest (RandomForest), Stochastic Gradient Boosting (SGB), CatBoost, XGBoost, Support Vector Machine (SVM), and Sparse Partial Least Squares Discriminant Analysis (SPLSDA) across three evaluation metrics: mean absolute error (MAE), root mean square error (RMSE), and coefficient of determination (R²). Models were trained and validated on: (a) 60% of peanut kernel samples inoculated with *A. flavus* strain NRRL_3357 (training set), (b) 40% of the same dataset (testing set), and (c) an independent test set comprising kernels infected with a distinct aflatoxigenic *A. flavus* strain. (d) Ranked feature importance plot from the top-performing CatBoost model, highlighting the 20 most influential variables contributing to model predictions, with normalized importance scores (0–100%). (e) Parallel comparison of R² values between a CatBoost model which utilized previously-published 14 promoters for whole-cell biosensor array

construction, and the current XGBoost model informed by transcriptomic-guided selection of 8 promoters in this study, revealing enhanced predictive accuracy in the latter across testing (40%), and external datasets. The red dashed rectangles in panels (a–c) highlight the most accurate and robust models across metrics and datasets.

5.2.8. Comparative Assessment: Our Whole-Cell Biosensor Array versus Established Techniques for Mold Monitoring in Postharvest Agricultural Products

Benchmarking our transcriptome-guided whole-cell biosensor array against existing non-invasive detection platforms revealed notable differences in predictive performance and operational feasibility. Near-infrared (NIR) spectroscopy, which has been extensively applied for rapid AFB₁ quantification in maize and barley, typically reports coefficients of determination (R^2) ranging from 0.82 to 0.88 under controlled conditions (Fernández-Ibañez et al., 2009). Similarly, colorimetric sensor arrays utilizing chemically responsive dyes for detecting VOCs from fungal contamination in wheat have demonstrated classification accuracies exceeding 97 % (Jiang et al., 2021; Jiang et al., 2022). Although these optical and chemical platforms offer quick, minimally invasive measurements, they often require high-precision instrumentation and specialized chemistries, which contribute to substantial capital and maintenance costs, limiting their scalability in decentralized agricultural settings.

In contrast, our biosensor platform integrates genetically encoded luminescent reporters within Ca-alginate-immobilized *E. coli* and employs ensemble learning regression models, specifically XGBoost, trained on time-resolved bioluminescence profiles. This design achieved consistently high internal cross-validation performance, with R^2 values ≥ 0.94 for both infection staging and AFB₁ quantification in *A. flavus*-infected maize and peanut kernels (Fig. 15–18). More critically, the framework maintained robust generalization to independent external validation datasets, achieving R^2 values of 0.92, 0.91, 0.92, and 0.86 for infection staging and toxin prediction across both maize and peanut substrates (Fig. 15e–18e). These metrics not only surpass the upper range of NIR spectroscopy but also do so with a much lower material and instrumentation overhead. Bioluminescence readouts can be captured using inexpensive CMOS sensors or even smartphone cameras (Ma et al., 2020a; Ma et al., 2020b), making it a cost-effective solution suitable for field deployment in agricultural environments.

Beyond cost-effectiveness, our biosensor demonstrates superior specificity compared to our earlier 14-promoter RandomForest classifier, which achieved only 80 % substrate distinction accuracy (Ma et al., 2023). By using transcriptomic data to select eight promoters specifically induced during *A. flavus* infection and aflatoxin production—rather than relying on broadly stress-responsive promoters—we established a more biologically relevant predictor set. This

refinement, combined with XGBoost regression, resulted in balanced and reproducible performance across internal ($R^2 > 0.94$) and external ($R^2 > 0.86$) datasets. In contrast, models trained on the previous 14-promoter set, such as CatBoost (the best-performing model among six trained regressors; [Figure S7](#), supplementary information), suffered from overfitting and considerably lower external accuracy. Thus, transcriptome-informed promoter selection not only improves predictive fidelity but also enhances the biological interpretability of the biosensor by linking output signals directly to pathogen-specific processes.

From a mechanistic perspective, early predictive features linked to oxidative stress, signal regulation, and biofilm formation emerged as key markers, highlighting transcriptional programs associated with *A. flavus* infection and aflatoxin production. Such mechanistic resolution, which provides insight into the biological basis of toxigenesis, is not readily achievable using NIR or colorimetric assays, which infer contamination indirectly. Nevertheless, established optical platforms retain certain advantages: NIR spectroscopy offers analyte-agnostic detection, making it less susceptible to biological variability, while colorimetric arrays are adaptable for multiplex VOCs profiling. Nonetheless, our model's performance ceiling is currently constrained by the diversity of training datasets, emphasizing the need for expansion to include a broader range of geographic isolates, environmental conditions, and substrate types. Future developments in synthetic biology, such as promoter engineering ([Liu et al., 2023](#)) and directed evolution ([Chen et al., 2023](#)), could further enhance biosensor robustness and sensitivity, particularly in varying environmental contexts.

In conclusion, while NIR and colorimetric assays remain valuable for centralized, high-throughput screening, our transcriptome-guided whole-cell biosensor array offers a highly competitive alternative, combining strong internal validation, robust external generalization, low instrumentation demands, and mechanistic interpretability. The integration of promoter-level transcriptomic signals with ensemble learning creates a versatile, non-invasive platform that bridges laboratory precision with real-world agricultural applicability. Future efforts to expand datasets and optimize biosensor performance will further position this approach as a leading tool for pre- and postharvest aflatoxin risk management in diverse agricultural systems.

5.3. Conclusion

This study establishes a transcriptome-guided whole-cell biosensor array as a robust platform for early detection of *A. flavus* contamination and AFB₁ production in maize and peanuts. By selecting infection-induced promoters identified through transcriptomic analysis and embedding them in calcium alginate-immobilized *E. coli*, the biosensor achieved substantial improvements over previous designs that relied on general stress-responsive promoters. When integrated with machine learning

regression models, particularly XGBoost, the system consistently demonstrated high predictive accuracy ($R^2 > 0.94$ in internal validation) while maintaining strong generalizability in external validation datasets ($R^2 = 0.86$), surpassing the performance of conventional non-invasive detection methods such as NIR spectroscopy. Importantly, feature importance analysis revealed that transcriptional programs associated with DNA-templated signal transduction, cytosolic regulation, growth regulation, methylglyoxal catabolism, and biofilm formation served as critical early predictors of fungal infection and toxin accumulation, thereby providing mechanistic interpretability alongside predictive performance.

The validation of this approach across two distinct crop substrates underscores its versatility and scalability for broader agricultural contexts. Coupling promoter-level biological specificity with low-cost luminescence readouts, which can be captured using CMOS sensors or smartphone cameras, further highlights the feasibility of this platform for decentralized, field-based monitoring. Nonetheless, practical deployment will require systematic evaluation of biosensor stability under variable storage and environmental conditions, as well as adaptation to diverse crop types and fungal isolates. Future optimization through synthetic biology strategies, such as promoter engineering or directed evolution, combined with expansion of the training dataset to include geographically and genetically diverse strains, is expected to enhance sensitivity, specificity, and robustness. Collectively, these results position the transcriptome-guided biosensor as a promising tool for scalable, real-time aflatoxin risk assessment, offering significant potential to strengthen food safety monitoring systems and reduce postharvest losses in global supply chains.

Summary

This chapter extended the early-warning framework from the detection of fungal contamination to the quantitative prediction of mold progression and AFB₁ production using whole-cell biosensors and regression models. Together with the VOC-based investigations presented in the preceding chapters, these results establish a complementary strategy that links early biochemical signals, fungal development, and mycotoxin risk prediction. Building on these findings, Chapter 6 integrates the main outcomes of the thesis, discusses their broader scientific and practical significance, addresses the principal limitations of the current work, and considers future directions for the development of more robust, transferable, and application-oriented early-warning systems for fungal contamination and mycotoxin risk.

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Chapter 6

Integrated Discussion and Perspective

6.1. General discussion

The work presented in this thesis was motivated by a practical challenge in food safety: fungal contamination often becomes apparent only after grain quality has already deteriorated and mycotoxins may already have accumulated to hazardous levels. As discussed in Chapter 1, mycotoxin contamination remains a persistent problem in food and feed systems in Asia, particularly under storage and postharvest conditions that favor fungal growth (Sun et al., 2023). That review also highlighted an important point: once contamination has progressed, intervention becomes more difficult, less effective, and more costly. From this perspective, recognizing fungal activity before visible spoilage or substantial toxin accumulation would allow monitoring to intervene earlier, when grain lots may still be managed, separated, dried, or prioritized for confirmatory testing.

Against this background, the thesis was designed around two related questions. The first was whether fungal contamination could be recognized during the asymptomatic stage, when no obvious visual symptoms are yet present. The second was whether this early disturbance could help estimate later AFB₁ risk. In this sense, the thesis moves beyond endpoint contamination assessment and develops a framework that connects early recognition with risk-oriented evaluation.

Chapter 2 considered this issue from the broader perspective of volatile organic compounds (Sun et al., 2025b). VOCs are particularly relevant in this context because they can be monitored non-destructively and because they reflect ongoing metabolic interactions between host tissues and microorganisms (Dudareva et al., 2013). Importantly, the review showed that VOC-based monitoring can combine sensor-based detection with machine-learning techniques, rather than relying on a single analytical method. This perspective guided the experimental work that followed, especially the integration of rapid sensing, volatile profiling, and predictive modelling.

The two experimental chapters then addressed these questions more directly using *Aspergillus flavus* and two important commodity systems, maize and peanut. Chapter 3 demonstrated that *A. flavus* infection in maize kernels can be recognized during the asymptomatic stage through integrated analysis by E-nose, GC-IMS, and GC-MS. Exploratory GC×GC-MS analysis further supported a progressive expansion of differential VOCs during the first three days after inoculation. Even before visible symptoms developed, infection produced reproducible changes in the headspace volatile profile. Although some cultivar-related variation in signal intensity remained, the effects of infection status and time after inoculation were more pronounced than those of cultivar background. These findings suggest that early fungal colonization is already accompanied by detectable metabolic disturbance, and that VOC changes during the asymptomatic stage may therefore serve as early warning signals before mold growth becomes visible.

Chapter 4 extended this logic from recognition to prediction (Sun et al., 2025a). It shifted the focus from simple detection of fungal activity to the quantitative

interpretation of infection-related VOC responses in relation to toxin-associated risk. By combining transcriptome-guided whole-cell biosensors with regression models, this chapter established a biologically informed strategy for estimating infection progression and AFB₁-related risk in moldy maize and peanut kernels. Instead of relying only on empirical sensor selection, this study selected promoter elements from the transcriptomic responses of immobilized *E. coli* exposed to mold-associated headspace VOCs. This linked the reporter output more directly to biological responses triggered by the infected-kernel environment. This strategy improved predictive performance by linking volatile exposure to bio-reporter luminescence response. This approach should be understood not as a replacement for direct toxin assays, but as a complementary early-warning and dynamic monitoring strategy. Its value lies in the possibility of identifying risk before contamination becomes fully established and of supporting decisions on whether further confirmatory analysis is needed.

In conclusion, the thesis supports a staged monitoring framework built around two connected but distinct levels of interpretation. The first is the recognition of early biological disturbance before overt spoilage becomes visible. The second is the evaluation of whether that disturbance is likely to develop into significant toxin-related risk. The thesis therefore connects two analytical layers: VOC-based recognition of early fungal disturbance and biosensor-regression modelling for subsequent AFB₁-related risk estimation. It provides both a conceptual and methodological basis for improving the timeliness of fungal contamination monitoring in stored postharvest agricultural commodities.

6.2. From postharvest contamination to early warning

Aflatoxin control is still largely organized around endpoint assessment. In practice, contaminated materials are often identified only after visible spoilage, quality decline, or direct toxin measurement. While such approaches remain necessary for confirmation, they provide limited opportunity for timely intervention. This is a basic limitation of endpoint-oriented monitoring: contamination is recognized only after the process has already progressed to a more advanced stage.

Postharvest contamination should be understood as a progressive biological process rather than a single final state. This view highlights the importance of identifying early signals before contamination becomes visually evident or toxicologically severe. Fungal colonization, host response, volatile release, and toxin accumulation do not occur simultaneously, but unfold over time. This means that kernels may release detectable metabolic signals before spoilage becomes visible. On the contrary, it is likely to contain early biochemical and metabolic signals that reflect contamination-related disturbance before visible symptoms appear and before toxin levels become the only obvious warning. In the context of *A. flavus* infection, these early changes may be particularly relevant because shifts in the volatile profile can precede substantial aflatoxin accumulation and therefore provide an earlier view of the contamination process.

Within this framework, early warning is useful because it creates a decision window before contamination reaches an advanced stage. At that point, storage lots may still be separated, monitored more closely, dried again if moisture conditions are unfavorable, or selected for confirmatory toxin testing. This would help operators target laboratory-based AFB₁ analysis to lots showing early biological disturbance, thereby reducing unnecessary testing and avoiding delays until visible deterioration becomes apparent. In large storage systems, where frequent testing of every batch is costly and difficult, early warning could serve as a triage step to guide segregation, intensified monitoring, and targeted confirmatory analysis. For operators, this could mean fewer blind tests and more targeted use of confirmatory AFB₁ analysis. Therefore, the work presented in this thesis was built around this premise: early signals do not replace confirmatory toxin analysis, but they can make postharvest monitoring more timely, more informative, and more responsive to developing risk. Such signals may also provide the basis for subsequent evaluation of whether early biological disturbance is likely to progress into significant toxin-related hazard.

6.3. Volatile changes during the asymptomatic stage of *A. flavus* infection

One of the main findings of this thesis is that *A. flavus* infection in maize kernels is already accompanied by measurable volatile changes during the asymptomatic stage. This stage is particularly important because kernels may still appear visually healthy, even though fungal colonization and contamination-related metabolic disturbance has already started.

The results in Chapter 3 suggest that the early headspace response is not incidental, but follows a recognizable pattern. E-nose analysis showed a progressive separation between infected and control samples over time, while GC-IMS and GC-MS provided additional support at both the fingerprint and compound levels. Across cultivars, infection altered the relative abundance of multiple VOC classes, although the extent of these changes was not identical in every genotype. This indicates that host background can modulate the intensity of the response, but does not eliminate the underlying infection-related signal. At the same time, these volatile shifts should not be interpreted too simplistically. The observed profile is unlikely to reflect a single biochemical pathway or emission source. It more plausibly represents the combined effects of fungal metabolism, early host tissue alteration, and dynamic host–pathogen interactions during colonization. Changes in ketones, esters, sulfur-containing compounds, and other stress-associated volatiles are broadly consistent with this interpretation and support the view that the asymptomatic stage is already metabolically active and biologically disorder.

Overall, these findings indicate that the first few dpi represent a critical transition period, during which contamination-related changes can already be detected before visible deterioration becomes evident. This is important not only because it supports the feasibility of asymptomatic-stage recognition, but also because it suggests that early volatile disturbance may provide a foundation for subsequent risk-oriented

monitoring before toxin accumulation becomes fully established.

6.4. Linking early fungal warning with AFB₁-related risk estimation

The integration of whole-cell biosensors with regression modelling extends this thesis from contamination recognition to risk-oriented estimation (Ma et al., 2023; Veltman et al., 2023). This shift is important because it separates two questions that are often conflated in contamination monitoring: whether fungal activity has started, and whether this activity is likely to develop into a toxin-related hazard. This distinction is important because fungal presence alone is not a sufficient proxy for toxin burden. Some contaminated samples may remain at relatively low risk, whereas others may progress toward substantial AFB₁ accumulation. A monitoring strategy that cannot distinguish between these situations has limited practical value for postharvest decision-making. This distinction was reflected in the experimental results: the volatile profiles in Chapter 3 captured early infection-related disturbance, whereas the biosensor-regression models in Chapter 4 provided a quantitative link to AFB₁ accumulation.

Previous work had already demonstrated that this whole-cell biosensor could detect mold contamination through its response to VOC signals (Ma et al., 2023). Building on that foundation, the present study further shows that the same biologically responsive sensing system can be extended beyond simple contamination recognition toward AFB₁ prediction when coupled with regression analysis. The significance of this approach therefore lies not in directly tracking fungal aflatoxin biosynthetic pathways, but in providing a biologically mediated sensing layer for complex mold-associated volatile environments. In the system developed here, an engineered *E.coli* reporter was exposed to the volatile environment generated by *A. flavus*-infected kernels, and its output signal served as a measurable biological response to mold-related headspace changes.

In this context, the differential volatile analysis in Chapter 3 demonstrates that early infection alters the biochemical environment of maize kernels during the asymptomatic stage, whereas the biosensor-regression platform in Chapter 4 shows that this altered environment can be translated into quantifiable biological signals and further linked to AFB₁ estimation. Taken together, these two analytical branches support a staged view of contamination, in which early headspace perturbation and later toxin burden are related through a changing chemical environment, but are not interchangeable measures of the same process. This perspective avoids the oversimplified assumption that early VOC shifts alone can directly predict toxin concentration, while still providing a conceptual basis for integrating volatile early-warning signals with downstream toxin-risk estimation.

The inclusion of both maize and peanut, together with validation using an additional independent aflatoxigenic *A. flavus* strain, further strengthens the broader relevance of this work. Although these commodities differ in composition, storage

ecology, and contamination context, both are highly relevant targets for aflatoxigenic contamination. The results therefore suggest that the analytical concept developed here may have value beyond a single crop system. At the same time, broader application should be interpreted cautiously, since further validation under more diverse commodity backgrounds, fungal populations, and realistic storage conditions remains necessary.

6.5. Practical application value

A practical question raised by this work is how the biosensor platform should be positioned relative to the many existing rapid aflatoxin detection methods already available. This question is important because low-cost toxin assays, including immunochromatographic strips and other rapid screening tools, are already widely used for endpoint detection (Haque et al., 2020; Liu et al., 2022). The present biosensor platform addresses a different stage of monitoring and a different operational need from established rapid toxin assays.

Most conventional rapid toxin tests are designed to determine whether aflatoxin is already present in a sample and, in some cases, to estimate its concentration. They are therefore highly useful as confirmatory or endpoint-oriented tools. However, they still require direct sample collection, extraction, and contact-based handling, and they are usually applied only after contamination is already suspected or after quality deterioration becomes evident. In contrast, the biosensor-based strategy developed in this thesis is better understood as a front-line screening and risk-assessment tool. Its main advantages are its low cost, rapid response, and minimal-contact operation, which make it more suitable for repeated testing, high-frequency surveillance, and early risk triage in storage systems (Ali et al., 2023).

From an application perspective, this distinction is important. In large postharvest systems, the key challenge is often not the lack of a final toxin assay, but the difficulty of deciding when, where, and how often such assays should be used. A low-cost biosensor platform may provide value by serving as an intermediate decision layer between routine observation and definitive toxin analysis. For example, it could be used to screen storage lots repeatedly, flag materials with increasing biological risk, and thereby reduce the number of samples that need to undergo more labor-intensive confirmatory toxin testing. Used in this way, the platform could help concentrate confirmatory toxin testing on lots that show increasing biological risk, rather than applying more labor-intensive assays blindly or too late.

Another practical advantage is that the platform is more compatible with non-destructive or low-disturbance monitoring logic than conventional extraction-based methods. This feature is particularly relevant when repeated surveillance is required over time, such as during storage, transport, or batch management. Under these conditions, the operational value of a method depends not only on its analytical performance, but also on whether it can be deployed frequently, cheaply, and with limited handling burden. The biosensor platform developed here is therefore better

viewed as part of a hierarchical monitoring strategy: early low-cost screening and risk warning first, followed by targeted confirmatory toxin analysis where necessary.

The practical contribution of the present platform lies in its complementary role alongside existing rapid toxin tests, particularly for repeated, low-cost, and minimally invasive early risk evaluation. Its practical deployment, however, depends on how well this screening logic can be maintained under the biological and environmental complexity of real storage systems.

6.6. Translational challenges in real storage environments

The experiments were intentionally conducted under controlled conditions to resolve infection-related signal structures more clearly. Real storage environments are far more heterogeneous. Moisture gradients, temperature fluctuations, kernel damage, microbial competition, and background volatiles all complicate signal interpretation (Magan & Aldred, 2007; Haque et al., 2020; Liu et al., 2017). Under those conditions, analytical separation becomes harder, and the meaning of any early warning signal becomes less straightforward. This means that the transition from experimental proof of concept to practical deployment will require more than scale-up; it will also depend on understanding how biological and environmental variability reshapes signal reliability.

A particularly important challenge is interference from non-aflatoxigenic strains and background fungi. In storage ecosystems, kernels are rarely colonized by a single target organism (Fan et al., 2023; Snyder et al., 2024). Non-toxigenic *A. flavus* strains, *Aspergillus niger*, *Penicillium* spp., and other molds may coexist and contribute overlapping metabolic outputs. This is especially relevant to the VOC branch of the thesis. Several of the volatile classes altered during infection, including alcohols, ketones, sulfur-containing compounds, and some terpene-related signals, are chemically plausible indicators of fungal deterioration, but they are not guaranteed to be exclusive to aflatoxigenic *A. flavus* (Börjesson et al., 1999; Josselin et al., 2021; Fan et al., 2023). Under mixed-fungi conditions, an early VOC warning may therefore indicate real biological disturbance without necessarily identifying its source with full specificity. In practice, this creates a realistic risk of false alarms or non-specific alerts in environments with a complex fungal background. This means that a VOC alert should be interpreted first as a warning of biological disturbance, not as definitive evidence of aflatoxigenic *A. flavus* activity. VOC-based monitoring is well suited to broad early warning during the asymptomatic stage, but it should not be expected to resolve every case of fungal identity or toxin relevance on its own. That is precisely why the biosensor-regression branch is important. Because it is grounded in promoter selection and biologically informative responses linked to aflatoxin-related activity, it has the potential to provide a more specific second layer of interpretation. In other words, the value of the biosensor platform lies not in eliminating the ambiguity of volatile signals altogether, but in helping translate biologically complex headspace changes into a more risk-relevant form of interpretation. Even so, it would be

premature to claim that this issue has been fully solved. The biosensor platform should also be challenged against broader panels of non-aflatoxigenic strains, mixed fungal communities, and more variable substrate conditions.

A second limitation concern scale and generalizability. The maize VOC study showed a cross-cultivar signal structure, but this does not yet establish universal transferability across all storage histories and natural contamination scenarios. Similarly, the biosensor-based prediction system showed promising performance in laboratory, yet wider validation remains necessary before it can be considered robust across the diversity likely to be encountered in practice. This is particularly important because both volatile responses and biosensor outputs may shift with substrate composition, fungal ecology, and storage environment. Nevertheless, the consistency of infection-related VOC patterns and the performance of the biosensor-regression models support the feasibility of this approach as an early screening tool.

A third limitation lies in how early fungal disturbance should be interpreted in relation to toxin risk. The two are closely connected in this thesis, but they should not be treated as the same signal or used as interchangeable measures. An early VOC shift can tell us that something has started to change inside the kernel, the fungal activity may be developing, the headspace environment is being altered, and the risk may be moving upward. But this does not mean that the VOC profile can be read as a direct value of final AFB₁ concentration. That distinction matters. If early volatile changes are overinterpreted, the method risks being used beyond what the data can support. A more appropriate interpretation is that Chapter 3 provides an early warning layer, identifying asymptomatic-stage disturbance, while Chapter 4 adds a second layer by estimating whether that disturbance is associated with AFB₁-related risk. The two chapters therefore are closely connected, but they do not answer exactly the same question.

6.7. Future directions

The findings of this thesis suggest that aflatoxigenic contamination could be monitored earlier in the postharvest process, but several steps are still required before practical use. In particular, future research should improve analytical performance while strengthening specificity, robustness, and practical deployability.

A key challenge that remains is the differentiation of aflatoxigenic contamination from non-aflatoxigenic fungal backgrounds. Under practical storage conditions, kernels may be colonized by toxigenic *A. flavus*, non-toxigenic *A. flavus* strains, and other common storage fungi. Therefore, future studies should examine the system under biologically complex background conditions to determine whether it can reliably distinguish general fungal disturbance from specifically aflatoxigenic contamination. This issue is particularly important for the interpretation of early-warning signals in mixed-fungi environments. To further improve diagnostic specificity under such complex storage conditions, future studies may move beyond single-input biosensors toward multi-input genetic circuits (Chen et al., 2023). In

principle, whole-cell biosensors could be engineered to respond only when multiple contamination-relevant signals are simultaneously present, for example through logic-gated designs that integrate asymptomatic-stage VOC markers with aflatoxin pathway-specific transcripts in an “AND-gate” configuration (Guo et al., 2024). Such a strategy could help reduce interference from non-toxigenic fungal strains and other background microorganisms, which remains a major limitation for early warning in heterogeneous storage environments.

Validation under conditions that more closely resemble practical storage systems remains essential, as the volatile changes identified during the asymptomatic stage were characterized under controlled experimental conditions, whereas real storage environments are generally complex. Future studies should therefore assess whether the early-warning patterns observed in this work remain robust under natural or semi-natural contamination scenarios and throughout extended storage periods. At the same time, the biosensor platform itself could be further refined through protein- and circuit-level engineering. For example, directed evolution of key regulatory components, combined with screening under simulated environmental stress, may improve sensing performance under fluctuating temperature and humidity conditions and thereby enhance thermal stability, signal reliability, and shelf life for practical deployment beyond laboratory settings.

In parallel, more advanced machine-learning strategies may help strengthen the prediction performance developed in this thesis. While XGBoost provided strong performance in the present study, future iterations could explore models that incorporate biological or transport-related constraints from the postharvest system itself. In this respect, physics-informed or mechanism-aware machine-learning approaches may offer a useful direction for integrating fungal growth behaviour, environmental variation, and volatile transport into more robust prediction models. Such developments could move the model from purely empirical correlation toward a more process-informed representation of contamination dynamics.

In the longer term, these advances may support the development of miniaturized and field-deployable monitoring devices integrating sample handling, signal transduction, and computational interpretation within a single platform. Although such systems remain beyond the scope of the present thesis, they would be the practical form in which asymptomatic-stage warning and toxin-risk evaluation could be used for continuous surveillance during storage and transport.

6.8. Conclusion

This thesis started from a practical problem in postharvest food safety: by the time aflatoxigenic contamination is visually recognized, grain quality may already have declined and toxin accumulation may already be underway. Against this background, the work examined whether *A. flavus* contamination could be recognized during the asymptomatic stage, whether such early biological disturbance could be

linked to toxin-related risk evaluation, and whether these signals could further be used to predict mold development stages and mycotoxin contamination levels.

The results showed that *A. flavus* infection in maize kernels is already associated with measurable and structured volatile changes before visible symptoms appear. By integrating E-nose, GC-IMS, and GC-MS, the volatile profiling study identified a set of candidate VOCs that differentiated infected from non-infected maize across cultivars and infection time points, providing a basis for asymptomatic-stage warning. The findings demonstrate that early fungal colonization is accompanied by detectable headspace disturbance before substantial aflatoxin accumulation becomes evident.

Building on this, the transcriptome-guided whole-cell biosensor study showed that biologically responsive signals can be translated into estimates of infection progression and AFB₁-related risk. By selecting infection-induced promoters and integrating them into calcium alginate-immobilized *E. coli* reporters, the biosensor platform substantially improved predictive performance over earlier designs based on general stress-responsive promoters. When coupled with regression models, particularly XGBoost, the system showed strong predictive accuracy and external generalizability across maize and peanut kernels, while also providing a degree of mechanistic interpretability through the identification of early transcriptional programs associated with infection and toxin accumulation.

Although the transcriptome-guided biosensor provides a promising tool for aflatoxin risk monitoring, further work is still needed before this approach can be translated into routine storage surveillance. Both VOC interpretation and biosensor performance should be evaluated under more heterogeneous environmental conditions, across broader fungal backgrounds, and in a wider range of commodity systems.

In conclusion, this thesis shows that *A. flavus* contamination can be detected before visible mold development and that early biological changes can be further linked to AFB₁-related risk. Volatile profiling provided the first warning of fungal activity in apparently healthy kernels, while transcriptome-guided whole-cell biosensors and regression models helped estimate infection progression and toxin accumulation. These findings provide a promising basis for developing scalable and potentially real-time aflatoxin risk-monitoring strategies, with value for improving food safety surveillance and supporting earlier intervention in postharvest systems.

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Supplementary Material

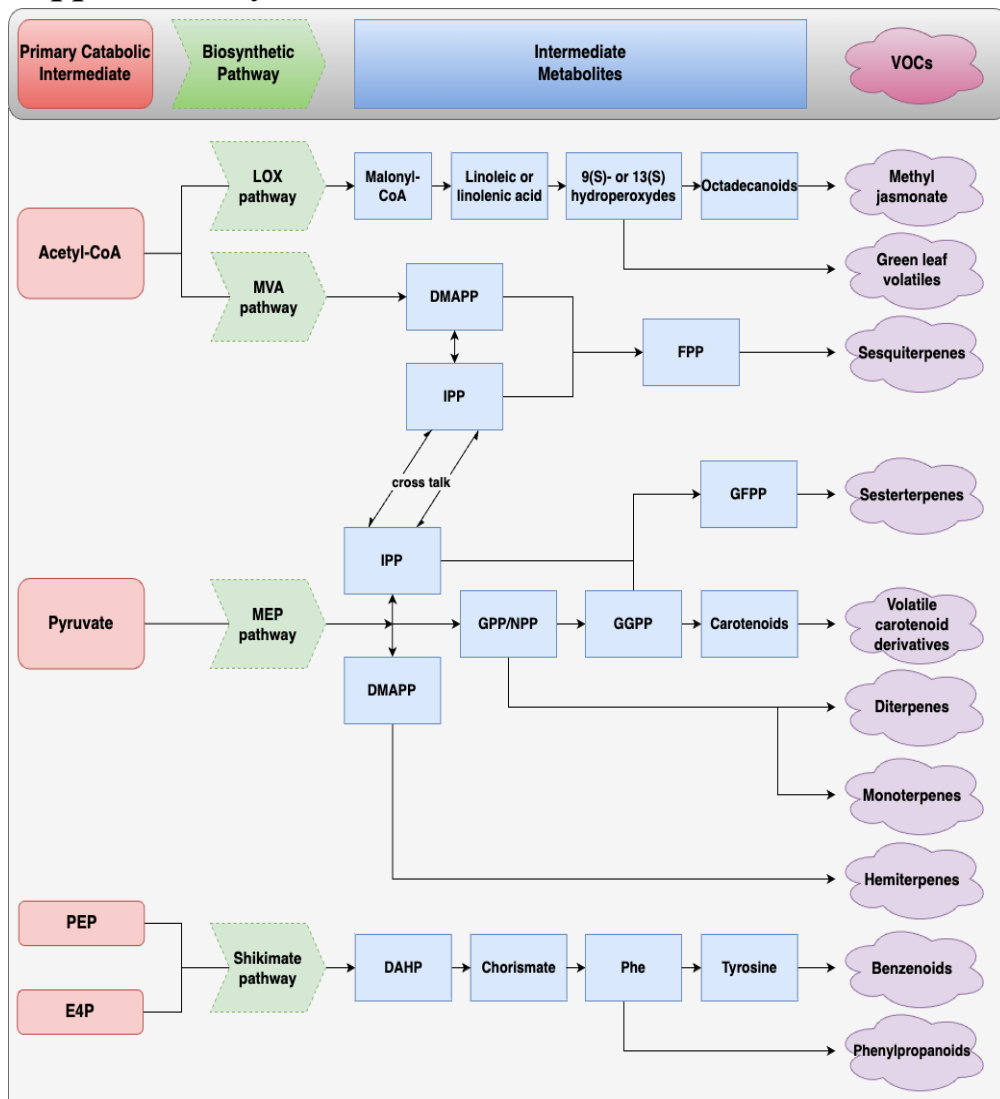


Figure S1. Overview of biosynthetic pathways leading to the emission of plant VOCs (Dudareva et al., 2013).

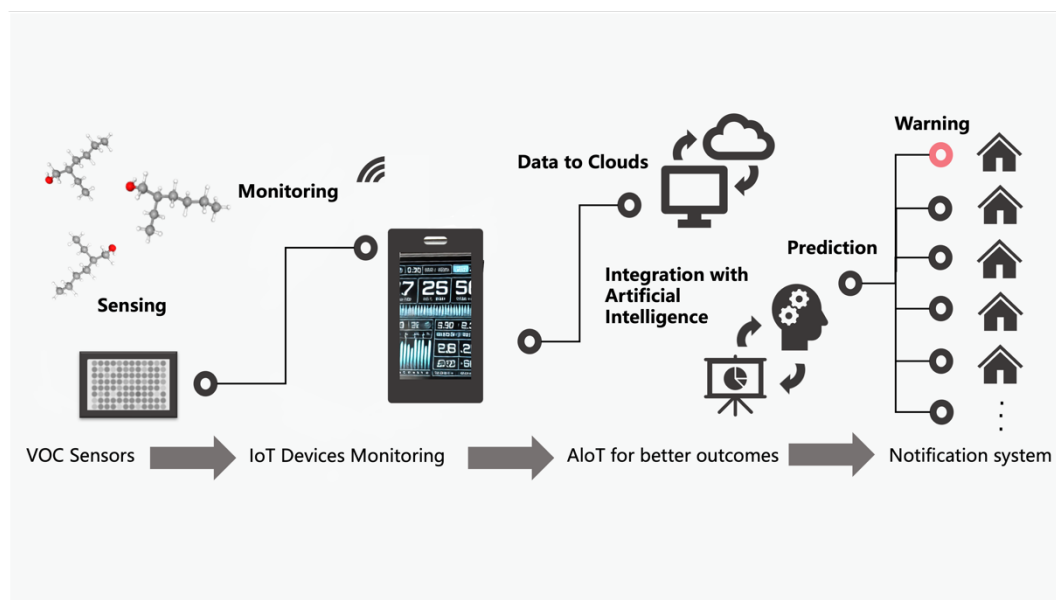


Figure S2. From smart "Things" in IoT systems to adopting Artificial Intelligence techniques in deterioration prediction

Supplementary Material

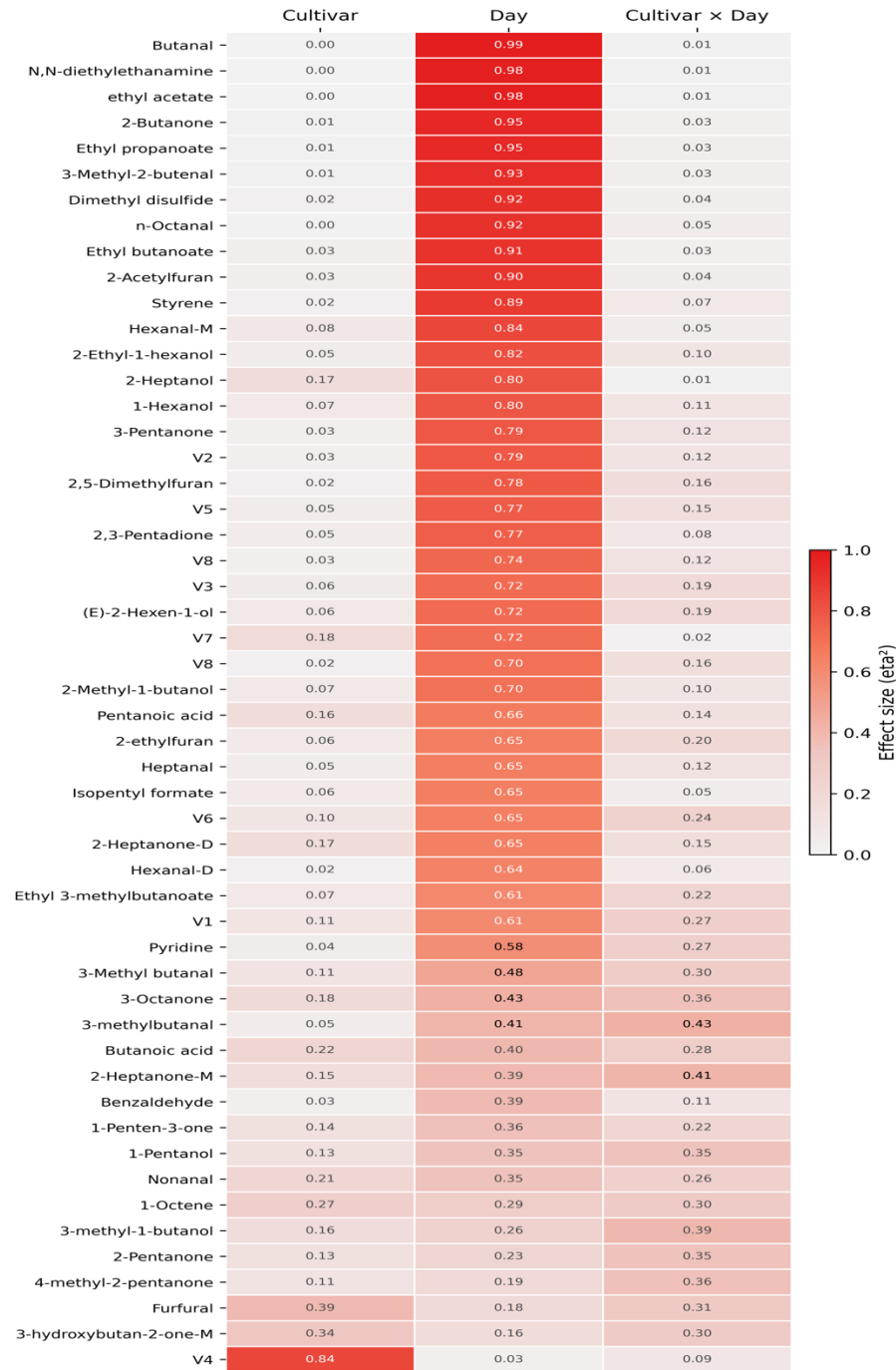


Figure S3. Two-way ANOVA effect-size analysis.

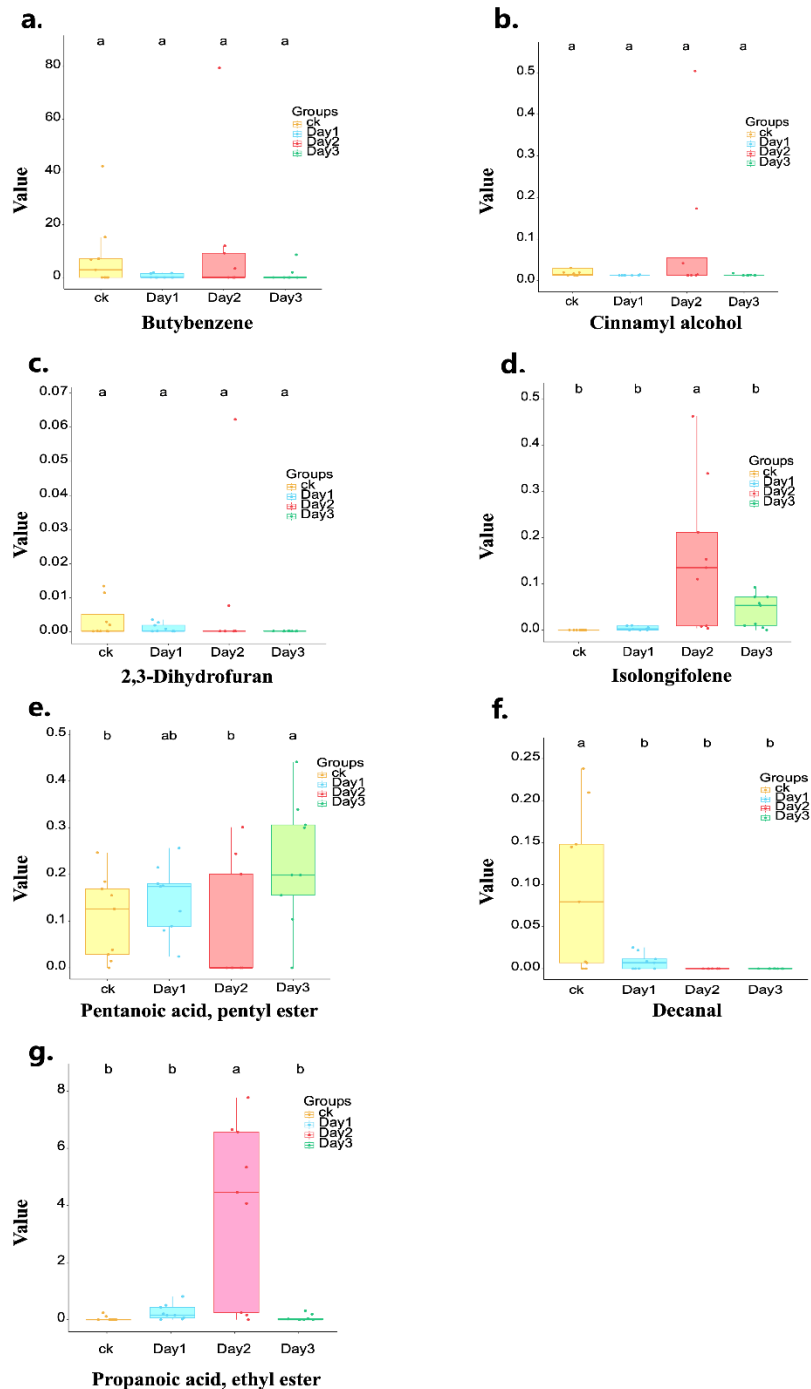


Figure S4. Boxplots of seven overlapping candidate VOCs in GC-MS analysis.

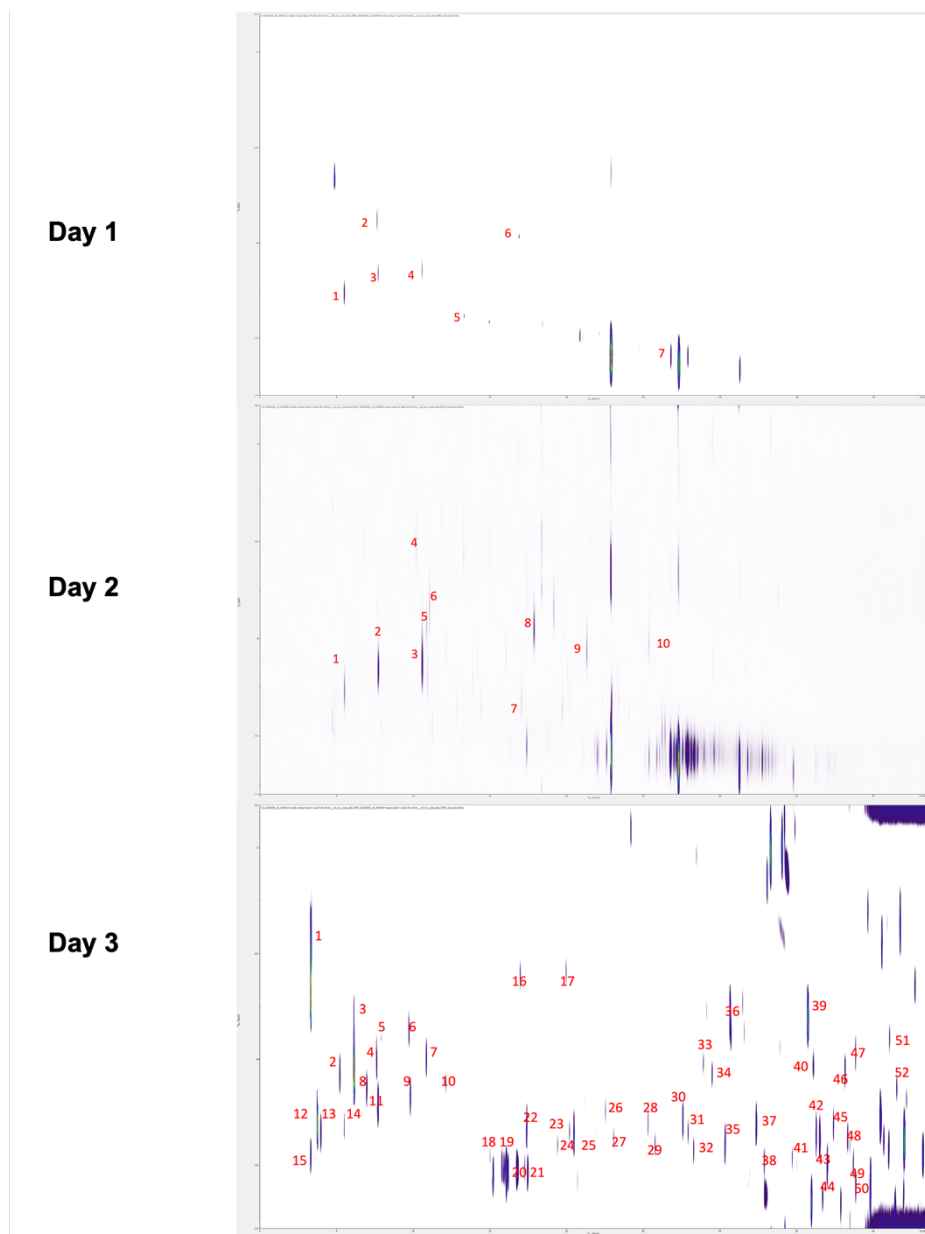


Figure S5. Time-dependent GC×GC-MS fingerprints of differential volatile compounds between *A. flavus*-inoculated maize and corresponding CK samples at 1, 2, and 3 dpi.

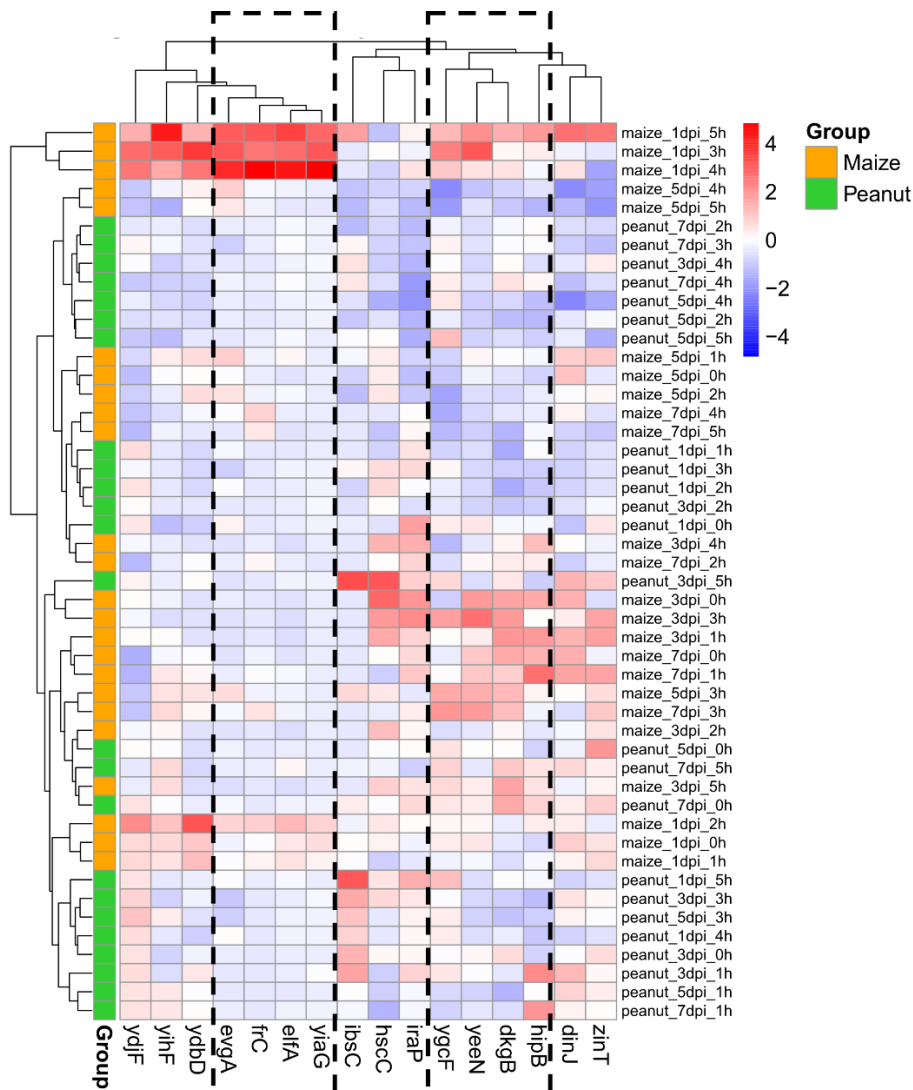


Figure S6. Functional screening of candidate promoters for whole-cell biosensor construction in response to *A. flavus*-derived volatile organic compounds (VOCs). Sixteen candidate promoters were selected from RNA-seq-identified upregulated genes based on both transcriptional induction levels and compatibility with luciferase reporter plasmid assembly. Each promoter was fused to a luciferase reporter and functionally tested using calcium alginate-immobilized whole-cell biosensors. Bioluminescence responses were measured following exposure to VOCs emitted by *A. flavus*-infected maize kernels. Among these, eight promoters—*evgA*, *frc*, *elfA*, *yiaG*, *ygcF*, *yeeN*, *dkgB*, and *hipB*—highlighted with black dashed rectangles, exhibited significantly elevated and consistent luminescence signals, demonstrating

strong responsiveness to VOCs and suitability for incorporation into the final biosensor array.

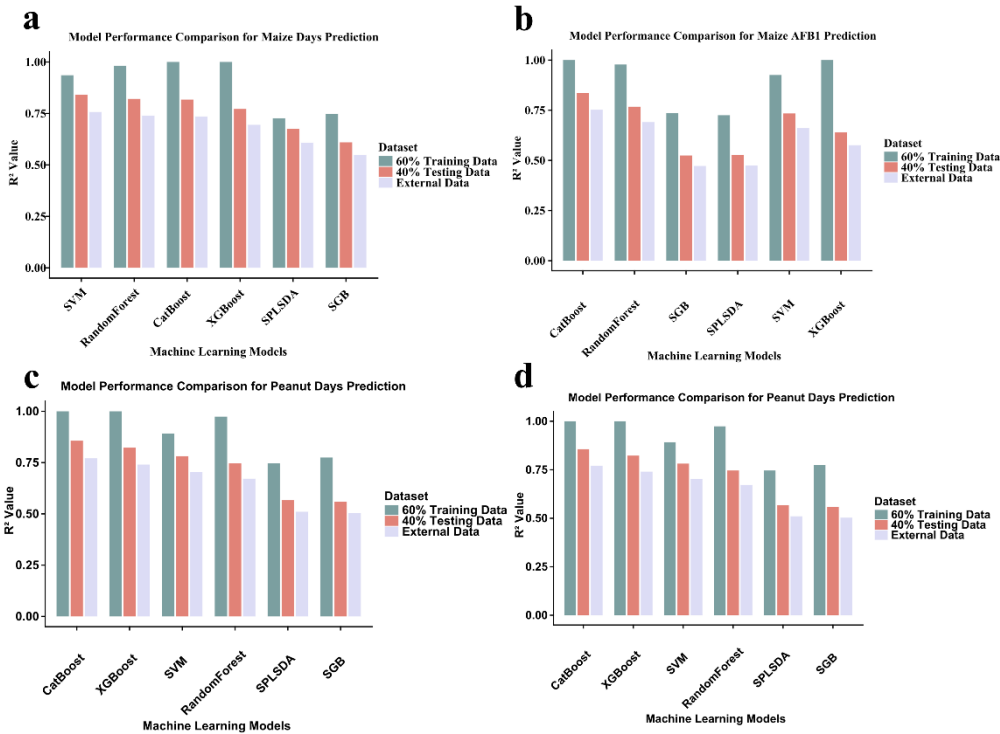


Figure S7. Comparison of six regression models combined with fourteen previously published promoters (pspA, soxS, grpE, dnaK, fabA, glgS, katG, leuA, ompF, oxyR, recA, spy, uvrA, yibT) for the construction of whole-cell biosensor arrays designed to stage *A. flavus* infection and predict aflatoxin B₁ production in infected maize and peanuts. (a) R² metrics for the six regression models in predicting *A. flavus* infection staging in infected maize. (b) R² metrics for the six regression models in predicting aflatoxin B₁ production in infected maize. (c) R² metrics for the six regression models in predicting *A. flavus* infection staging in infected peanuts. (d) R² metrics for the six regression models in predicting aflatoxin B₁ production in infected peanuts.

Table S1. The 10 MOS sensors of PEN3 E-nose system

Sensor	Main sensitive compounds
W1C	Aromatic compounds
W5S	Nitrogen oxides

Sensor	Main sensitive compounds
W3C	Ammonia and aromatic compounds
W6S	Hydrogen
W5C	Alkanes, aromatic and aliphatic compounds
W1S	Broad-range methane compounds
W1W	Terpenes and organosulfur compounds
W2S	Broad-range alcohols
W2W	Aromatic, sulfur-containing, and chlorine-containing compounds
W3S	Methane and aliphatic compounds

Table S2. Information on the identified compounds in moldy maize kernels based on GC-IMS.

Count	Compound	CAS#	Formula	MW	RI	Rt [sec]	Dt [a.u.]	Comment
1	Nonanal	C124196	C9H18O	142,2	1108,5	563,561	147,941	null
2	2-Ethyl-1-hexanol	C104767	C8H18O	130,2	1034,6	434,127	141,315	null
3	Benzaldehyde	C100527	C7H6O	106,1	985,3	367,117	11,576	null
4	3-Octanone	C106683	C8H16O	128,2	991,1	373,462	130,767	null
5	Heptanal	C111717	C7H14O	114,2	900,2	285,766	134,024	null
6	Isopentyl formate	C110452	C6H12O2	116,2	790,1	217,879	126,762	null
7	Hexanal-D	C66251	C6H12O	100,2	789,9	217,773	155,859	Dimer
8	3-Methyl-2-butenal	C107868	C5H8O	84,1	790,1	217,879	136,731	null
9	Pyridine	C110861	C5H5N	79,1	759,1	201,884	125,602	null
10	V1	unidentified	*	0	758,5	201,566	129,776	null
11	Pentanal	C110623	C5H10O	86,1	730,3	188,008	139,397	null
12	3-methyl-1-butanol	C123513	C5H12O	88,1	728,5	187,164	124,559	null
13	2-ethylfuran	C3208160	C6H8O	96,1	729,4	187,584	128,964	null
14	4-Methyl-2-pentanone	C108101	C6H12O	100,2	729,4	187,584	149,715	null
15	3-Methyl butanal	C590863	C5H10O	86,1	657,4	160,125	118,659	null
16	2,3-Pentadione	C600146	C5H8O2	100,1	659,7	160,794	128,092	null
17	2-Pentanone	C107879	C5H10O	86,1	658,3	160,374	139,966	null
18	3-Methylbutanal	C590863	C5H10O	86,1	626,6	151,309	117,739	null

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19	Butanal	C123728	C4H8O	72,1	619,7	149,37 9	128,713	null
20	V2	unidentified	*	0	694,6	172,112	157,275	null
21	Hexanal-M	C66251	C6H12O	100, 2	790,0	217,85 7	12,426	Monomer
22	Dimethyl disulfide	C624920	C2H6S2	94,2	770,8	207,80 2	117,445	null
23	3-hydroxybutan-2-one-M	C513860	C4H8O2	88,1	710,5	178,98 9	107,703	Monomer
24	V8	unidentified	*	0	693,8	171,75 6	14,322	null
25	1-Penten-3-one	C1629589	C5H8O	84,1	694,2	171,95 1	129,351	null
26	Ethyl propanoate	C105373	C5H10O	102, 2	695,5	172,47 1	119,024	null
27	Ethyl butanoate	C105544	C6H12O	116,2	788,6	217,08 1	119,437	null
28	2-Heptanol	C543497	C7H16O	116,2	909,2	293,39 8	13,488	null
29	n-Octanal	C124130	C8H16O	128, 2	993,4	376,04 3	139,649	null
30	2-Acetylfuran	C1192627	C6H6O2	110,1	910,2	294,31 6	14,286	null
31	Styrene	C100425	C8H8	104, 2	900,1	285,72	143,801	null
32	Ethyl 3-methylbutanoate	C108645	C7H14O	130, 2	867,6	262,91 9	164,695	null
33	1-Pentanol	C71410	C5H12O	88,1	759,0	201,80 6	15,218	null
34	N,N-diethylethanamine	C121448	C6H15N	101, 2	714,2	180,64 3	121,248	null
35	1-Octene	C111660	C8H16	112,2	788,5	217,06 6	142,917	null
36	V3	unidentified	*	0	759,6	202,14 4	13,324	null
37	2-Butanone	C78933	C4H8O	72,1	588,9	141,16 4	124,549	null
38	V4	unidentified	*	0	829,0	239,42 6	126,831	null
39	pentanoic acid	C109524	C5H10O	102, 2	883,4	273,22 5	123,851	null
40	2,5-Dimethylfuran	C625865	C6H8O	96,1	695,2	172,35 5	135,523	null
41	3-Pentanone	C96220	C5H10O	86,1	688,9	169,67 8	111,432	null
42	(E)-2-Hexen-1-ol	C928950	C6H12O	100, 2	867,3	262,73 5	136,566	null
43	ethyl acetate	C141786	C4H8O2	88,1	609,0	146,46 7	13,352	null
44	2-Heptanone-M	C110430	C7H14O	114,2	891,0	278,29 5	126,044	Monomer
45	Butanoic acid	C107926	C4H8O2	88,1	829,5	239,75 7	136,961	null
46	2-Heptanone-D	C110430	C7H14O	114,2	889,8	277,51	162,169	Dimer
47	V5	unidentified	*	0	890,4	277,86 9	136,148	null
48	V6	unidentified	*	0	867,5	262,87 9	140,452	null
49	1-Hexanol	C111273	C6H14O	102, 2	868,5	263,54 5	13,275	null
50	V7	unidentified	*	0	810,4	228,90 9	147,521	null

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51	2-Methyl-1-butanol	C137326	C5H12O	88,1	751,8	198,25 7	147,535	null
52	Furfural	C98011	C5H4O2	96,1	780,1	212,60 9	134,703	null

Table S3. Information on the identified compounds in moldy maize kernels based on GC-MS.

Super Class	Compound Name	CA S	Formula	Molecular Weight(g/mol)	Main difference day	Average relative abundance on main difference day	Rt	Reference m/z
Esters	Oxalic acid, isobutyl pentyl ester	N/A	C11H20O4	216.27	Day 2	1.01	24.142	57.12
	Pentanoic acid, pentyl ester	2173-56-0	C10H20O2	172.26	Day 3	0.23	30.112	84.951
	Oxalic acid, isohexyl neopentyl ester	N/A	C15H28O4	272.38	Day 2	5.29	35.873	57.098
	Ethyl propanoate	105-37-3	C5H10O2	102.13	Day 2	3.91	8.619	57.12
	Butanoic acid, butyl ester	109-21-7	C8H16O2	144.21	Day 2	0.21	24.745	71.42
	Acetic acid, pentyl ester	628-63-7	C7H14O2	130.18	Day 1	0.33	20.664	43.089
	Pentanoic acid, butyl ester	591-68-4	C9H18O2	158.24	Day 2	0.56	28.165	85.33
	Oxalic acid, butyl cyclobutyl ester	N/A	C10H16O4	200.23	Day 2	0.83	30.467	57.098
	Oxalic acid, isobutyl octyl ester	N/A	C14H26O4	258.35	Day 2	0.13	33.15	57.139
	Oxalic acid, butyl propyl ester	N/A	C9H16O4	188.22	Day 2	0.97	24.598	57.12
Aromatic Hydrocarbons	Methylbenzene	108-88-3	C7H8	92.14	Day 2	0.15	12.109	91.072
	p-Xylene	106-42-3	C8H10	106.17	Day 2	0.14	18.639	91.076
	Styrene	100-42-5	C8H8	104.15	Day 2	12.63	19.655	104.106
	Butylbenzene	104-51-8	C10H14	134.22	Day 1	0.56	27.654	134.01
	1,2,3,5-Tetramethylbenzene(tentatively identified)	527-53-7	C10H14	134.22	Day 2	1.15	29.082	118.989
	Ethylbenzene	100-41-4	C8H10	106.17	Day 3	0.03	18.203	91.087
	Propylbenzene	103-65-1	C9H12	120.19	Day 2	0.19	21.284	91.065
	1,2,4,5-Tetramethylbenzene(tentatively identified)	95-93-2	C10H14	134.22	Day 2	0.27	28.012	118.989
	Octanal	124-13-0	C8H16O	128.21	Day 2	0.01	23.534	43.276
	Decanal	112-31-2	C10H20O	156.27	Day 1	0.04	30.598	57.121
Aldehydes	Tetradecanal	124-25-4	C14H28O	212.37	Day 2	10.80	38.21	191.149
	Heptanal	111-71-7	C7H14O	114.19	Day 1	0.02	20.735	44.01
	Ethanol	64-17-5	C2H6O	46.07	Day 2	21.08	4.497	31.009
Alcohols	3-Methyl-1-butanol	123-51-3	C5H12O	88.15	Day 2	0.48	11.798	55.166
	3-Phenyl-2-propen-1-ol	104-54-1	C9H10O	134.18	Day 2	0.07	36.232	92.365

Supplementary Material

Ketones	3-Pentanone	96-22-0	C5H10O	86.13	Day 2	0.37	6.25 ₁	57.115
	4-Methylacetophenone(tentatively identified)	122-00-9	C9H10O	134.18	Day 2	0.05	33.0 ₀₃	119.989
	Limonene	5989-27-5	C10H16	136.24	Day 2	0.61	25.2 ₀₂	67.014
Terpenes	Isolongifolene	1135-66-6	C15H24	204.35	Day 2	0.16	35.8 ₁₆	161.155
	Benzene, methyl(1-methylethyl)-	2515-5-15-1	C10H14	134.22	Day 2	0.24	25.0 ₁₁	118.968
Nitrogenous Compound	Nonanal oxime	821-67-8	C9H19NO	157.25	Day 2	0.01	32.3 ₃₉	57.876
others	2,5-Dihydrofuran(tentatively identified)	1708-29-8	C4H6O	70.09	Day 2	0.02	6.25 ₁	69.74
	2,3-Dihydrofuran(tentatively identified)	1191-99-7	C4H6O	70.09	Day 2	0.01	6.38 ₄	69.74
	Cyclopentene	142-29-0	C5H8	68.12	Day 2	1.64	5.25 ₅	67.014
	2,4,6-Trimethyl-1-nonene	N/A	C12H24	168.32	Day 2	0.61	26.7 ₁₁	69.125

Table S4. Total differential volatile compounds detected by GC×GC between mold-inoculated maize and the corresponding CK samples at 1, 2, and 3 dpi

	1tr	2tr	RT	compounds	cas	library RI	RI	MF	RMF
1	3.7499	1.6965	3.7615	Acetaldehyde	992-94-9	363	272	717	718
2	3.958	1.6532	3.9945	Methanethiol	74-93-1	414	286	713	818
3	5.4978	1.7336	5.4989	Dimethyl sulfide	75-18-3	508	397	768	768
4	6.1145	1.9641	6.1329	2-propanol, 2-methyl	75-65-0	512	443	707	718
5	7.5626	1.8672	7.899	2-Butanone	78-93-3	575	568	710	744
6	7.6299	2.0784	7.6679	2,3-Butanedione	431-03-8	595	546	805	817
7	8.1837	1.8153	8.1831	Furan, 2-methyl-	534-22-5	594	588	695	800
8	9.8083	1.7607	9.8315	Butanal, 3-methyl-	590-86-3	652	703	709	712
9	10.57	1.8489	10.5974	2-Pentanone	107-87-9	685	727	765	768
10	11.0415	2.1488	11.0694	2-Pentanol	6032-29-7	703	747	656	718
11	12.1079	1.8925	12.1314	Disulfide, dimethyl	624-92-0	730	776	827	747
12	12.2208	1.8174	12.2852	2-Hexanone	591-78-6	768	780	629	813
13	12.3608	2.2602	12.4044	1-Butanol, 3-methyl-	123-51-3	736	783	762	794
14	12.3953	2.3782	12.4399	Pyridine	110-86-1	746	785	868	871
15	14.5692	2.1196	14.7161	Pyridine, 3-methyl-	108-99-6	845	857	562	901
16	15.6964	1.7737	15.7384	Ethylbenzene	100-41-4	850	888	861	898
17	17.3821	1.7377	17.0283	Heptanal	111-71-7	880	942	749	798
18	17.8565	2.0487	17.9008	Cyclohexanone	108-94-1	894	781	811	854

Supplementary Material

19	19.456	1.6596	19.4268	Furan, 2-pentyl-	3777-69-3	979	1007	608	782
20	19.6955	1.6366	19.7273	Butanoic acid, butyl ester	109-21-7	995	1018	625	737
21	20.6875	1.5855	20.6081	Limonene	138-86-3	1023	1049	843	906
22	21.2873	1.9337	21.3323	1-Hexanol, 2-ethyl-	104-76-7	1016	1074	813	856
23	23.0668	1.6243	23.0937	2-Nonanone	821-55-6	1071	1137	658	820
24	25.2904	1.7008	25.3282	2-Nonenal	2463-53-8	1134	1221	656	819
25	25.3966	1.9721	25.3997	Butanedioic acid, diethyl ester	123-25-1	1182	1225	752	811
26	25.7518	1.5893	25.7932	2-Decanone	693-54-9	1193	1240	599	762
27	27.9185	1.6512	27.9261	2-Decenal, (E)-	3913-81-3	1263	1327	697	845
28	28.2772	1.6206	28.2927	2-Undecanone	112-12-9	1273	1341	699	845
29	28.7416	1.6292	27.9592	Undecanal	112-44-7	1287	1355	603	794
30	30.3126	1.594	30.3589	Dodecenal	4826-62-4	1465	1459	688	761
31	32.349	1.6864	32.3605	1-Dodecanol	112-53-8	1473	1535	683	759
32	32.915	1.5228	32.9256	2-Tridecanone	593-08-8	1477	1546	686	811
33	35.1069	1.5434	35.99	longifolene	475-20-7	1408	1416	887	918

Table S5. Information of Primers used in this study. (Note: bolded nucleotides in the primer sequence column denote restriction site for PmeI in F primer or SnaBI in R primer)

Genes	Primer sequences (flanking PmeI or SnaBI restriction site) for promoter cloning (5'-3')	Length of cloned promoters (bp)	Purpose
elfA	F: GTTAGCGTTTAAACATTCGGTCAGTCTGTCGGAG R: AGCAGTTACGTAGTGTCCGCCATTTAAATCAAGCA	236	Promoter cloning
evgA	F: GTTAGCGTTTAAACTGCACATTATCCCAGGAACG R: AGCAGTTACGTAAAGCATCTCCCTTCTCCCTG	312	Promoter cloning
iraP	F: GTTAGCGTTTAAACGCTTAAATGGCCATGCGG	344	Promoter cloning

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	R: AGCAGTTACGTACGCACACAGTTCTTTCGACT		
yeeN	F: GTTAGCGTTTAAACCGTGGGCTGTTTGCTTCCT TGG R: AGCAGTTACGTACGTTGCACCGTCTTTAGCCGTTT	325	Promoter cloning
yaG	F: GTTAGCGTTTAAACGATAGTGCCGTTGCCTTT R: AGCAGTTACGTAGGGTAATTTTCTGCGTTTCATC	349	Promoter cloning
yjF	F: GTTAGCGTTTAAACCTGGTTCCAGCGTGAAAA CA R: AGCAGTTACGTAGCCACGATTACACCCCTGTA	324	Promoter cloning
dkgB	F: GTTAGCGTTTAAACAAGAATTCGGTGGAGCGG TA R: AGCAGTTACGTAGGGCTTCAGACTGTTGAGGA	323	Promoter cloning
hscC	F: GTTAGCGTTTAAACTTGCCGAAGTCTGGATTTC C R: AGCAGTTACGTACCAATGGCGAGTTCTGCATT	416	Promoter cloning
yjF	F: GTTAGCGTTTAAACTTCCTCGGCATCTGGTTCT T R: AGCAGTTACGTATGGACTTGTGACAGAGTGGT	356	Promoter cloning
frC	F: GTTAGCGTTTAAACCTCGCGGTGCTATAAACT CG	502	Promoter cloning

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	R: AGCAGTTACGTATATCGTCGTAGCTGCACTCG		
ibsC	F: GTTAGCGTTTAAACCAGATGGGTTATGTGGTT ATTG R: AGCAGTTACGTATGAGGGCTTACCGTTAC	310	Promote r cloning
dinJ	F: GTTAGCGTTTAAACATCGACGGCAAGGACATA CT R: AGCAGTTACGTAGAGCAACAGCGACAATCCAT	367	Promote r cloning
ydbD	F: GTTAGCGTTTAAACCTTTACACCGCTGCAATC GT R: AGCAGTTACGTAACATCGAAGTTCCGCCTGTAC	368	Promote r cloning
zinT	F: GTTAGCGTTTAAACCGGCATACACAGATCGCATC R: AGCAGTTACGTACGCCAAAACAGTTTCCTCCA	203	Promote r cloning
yihF	F: GTTAGCGTTTAAACTGGTGAAATCTCTGGTCG CT R: AGCAGTTACGTACCACCCAGATTACGGCTAA	392	Promote r cloning
hipB	F: GTTAGCGTTTAAAAAGTACGTACCGGGGTTGAG R: AGCAGTTACGTATTGATGCGCCGTTTGAAGTC	391	Promote r cloning

Validate primer	F: cttaccagtcattacataatcg R: ccctaccgtatagagaaa	-	Validate promoter
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Table S6. Optimized hyperparameters for the XGBoost regression model applied to predict the *A. flavus* strain (NRRL_3357) infection stages and AFB₁ levels in maize and peanut

Maize-days-SVM Parameter	Value
Cost	1
Gamma	0.01
Epsilon	0.1
Cross-validation folds	10

Maize-AFB ₁ -CatBoost Parameter	Value
Learning rate	0.1
Maximum depth	6
Minimum child weight	1
Subsample ratio	0.8
Feature sampling ratio	0.8
L1 regularization	1
L2 regularization	3
Minimum split loss	0
Optimal iterations	1000
Cross-validation folds	10
Early stopping rounds	50

Peanut-days-CatBoost Parameter	Value
Learning rate	0.1
Maximum depth	6
Minimum child weight	1
Subsample ratio	0.8
Feature sampling ratio	0.8
L1 regularization	1
L2 regularization	3
Minimum split loss	0
Optimal iterations	1000

Cross-validation folds	10
Early stopping rounds	50

Peanut-AFB1-CatBoost Parameter	Value
Learning rate	0.1
Maximum depth	6
Minimum child weight	1
Subsample ratio	0.8
Feature sampling ratio	0.8
L1 regularization	1
L2 regularization	3
Minimum split loss	0
Optimal iterations	1000
Cross-validation folds	10
Early stopping rounds	50

List of Publications

1. Sun, L., Ma, J., Jiang, Y., Purcaro, G., Tian, Y., Wang, G., Li, W., Tai, B., & Xing, F. (2025). A novel approach for predicting aflatoxin B₁ production using regression models and whole-cell biosensors in moldy maize and peanut kernels. *Journal of hazardous materials*, 498, 139883. (IF=11.3)
2. Sun, L., Ma, J., Purcaro, G., Wang, G., Jin, J., & Xing, F. (2025). A comprehensive review of post-harvest agricultural product deterioration signature volatile organic compounds. *Food chemistry: X*, 29, 102866. (IF=8.74)
3. Sun, L., Li, R., Tai, B., Hussain, S., Wang, G., Liu, X., & Xing, F. (2023). Current status of major mycotoxins contamination in food and feed in Asia-a review. *Journal of Food Science and Technology*, 3 (2), 2682-2690. (IF=3.12)
4. Gao, Y., Sun, L., Tian, X., Yang, S., Du, H., Zhao, H., Yang, B., & Xing, F. (2025). Solid-state fermentation of acid-resistant *Aspergillus niger* A515, an approach to degrade AFB₁ and ZEN and enhance nutritional components in maize products. *Journal of Food Science and Technology*, 5 (7), 231–244. (IF=3.12)
5. Sun, L., Purcaro, G., Wang, G., Xing, F. (2025). Comparative Volatile Profiling of Maize Varieties During Early *Aspergillus flavus* Infection by GC-MS, GC-IMS, and E-nose. (submitted)
6. Tian, Y., Zhuo, Q., Sun, L., Li, X., Wang, G., & Xing, F. (2025). FphA Positively Regulates Fungal Development and AFB₁ Biosynthesis and Phosphorylates Ypd1 in *Aspergillus flavus*. *Journal of agricultural and food chemistry*, 73(41), 26377-26387. (IF=6.0)
7. Li M, Yang X, Sun L, et al. (2025). Meat products consumption and risk for Alzheimer's dementia and type 2 diabetes: a systematic review and meta-analysis of cohort studies. *Food Science and Human Wellness*, 2025, 14(8): 9250307. (IF=7.8)

Patents

1. A Whole-Cell Biosensor Array and Early-Warning Model for Early Monitoring of Grain and Oilseed Mold Spoilage and Aflatoxin Contamination
2. A Control System for Leucine Enrichment Processing and a Control Method Thereof