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Risk Assessment and Prediction of Aflatoxin in Agro-Products

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Abstract

Aflatoxin (AFT), highly toxic and carcinogenic to humans, seriously threatens consumption safety of agro-products. It is necessary to conduct risk assessment of aflatoxin contamination in agro-food products to find out critical control points (CCPs) and develop prediction, prevention and control theories and technologies. In this chapter, risk assessment and prediction of aflatoxin contamination in peanut were taken as an example. The values under the limit of detection (LOD) were replaced by zero, 1/2 LOD or LOD according to their respective proportion, and the distribution of values higher than the LOD was fitted by @RISK software. AFB₁ dietary exposure was evaluated based on non-parametric probability risk assessment and margin of exposure (MOE). A risk ranking method was adopted for mycotoxins based on food risk expectation ranking. Spatial analysis of AFB₁ contamination was conducted using geographic information system (GIS). Average climatic conditions were calculated by Thiessen polygon method and the relationship between AFB₁ concentration and average pre-harvest climatic conditions was obtained through multiple regression. To fulfill the purposes of reducing cost, increasing efficiency, maximizing the role of risk assessment and prediction, and improving the quality and safety of agricultural products, we will continuously focus on developing advanced and integrated technologies and solutions.

Keywords: peanut, aflatoxin, dietary exposure, risk ranking, risk prediction

1. Introduction

Risk prediction of agro-product, especially oil and grain products, is becoming more and more important. In this chapter, risk assessment and prediction of aflatoxin (AFT) in peanuts were taken as an example. We presented the development and research progress on risk assessment and prediction of aflatoxin in agro-products in the following aspects: (1) data processing and

simulation methods of peanut aflatoxin contamination (determination and simulation of highly skewed data); (2) risk assessment methods (non-parametric probability risk assessment method and margin of exposure (MOE) method); (3) risk ranking method (multi-mycotoxin risk ranking method based on the expert scoring method); (4) risk prediction technologies (large-scale aflatoxin prediction based on ArcGIS) and (5) prospect of future research.

Aflatoxin (AFT) is highly toxic and carcinogenic and has been therefore classified as a Group I carcinogen by the International Agency for Research on Cancer [1]. The most important types of aflatoxins occurring naturally in agro-products are aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁) and aflatoxin G₂ (AFG₂) [2, 3]. The total output and output per acre of peanuts always rank first of all oil crops cultivated in China. Peanuts produced in China account for about 40% of the world's peanut trade. In addition, peanuts contribute large amounts of vegetable oil, protein and vitamin E to developed countries [3–5]. Unfortunately, aflatoxin has been detected in more than 100 kinds of agro-products, especially in peanut and maize. Aflatoxin contamination might occur during the whole process of agro-products from production, storage, processing to trade, which seriously threatens consumption safety. To control aflatoxin contamination and ensure consumption safety, it is necessary to assess the risk of aflatoxin contamination in agro-products to identify critical control points (CCPs) and develop prediction, prevention and control theories and technologies for precise control in practice.

The mechanism of aflatoxin contamination is still not unclear since it is complex and multifactor dependent. Moreover, the aflatoxin contamination processes are significantly different over several consecutive years, and the contamination shows seriously skewed distribution. Among agro-products, peanuts are most seriously contaminated by aflatoxin. Since peanuts are popular food and oilseed worldwide, the prediction and control of aflatoxin contamination in peanuts are hot issues difficult to be resolved.

2. Data processing and simulation methods of peanut aflatoxin contamination

2.1. Data processing of peanut aflatoxin contamination

According to post-harvest peanut aflatoxin data in China from 2009 to 2010, the proportion of “trace data” (below the detection limit) was over 70%, and the proportions of trace data for AFB₁, AFB₂, AFG₁ and AFG₂ were 78.3, 78.3, 98.8 and 97.2%, respectively. The aflatoxin data for the Chinese peanuts were positively skewed, and the Kolmogorov-Smirnov test proved that AFB₁ and the total aflatoxin did not conform to the normal distribution, with about 90% of the aflatoxin data concentrated in the range of 0–2 µg/kg, so that the aflatoxin data for Chinese peanuts were left censored data and in line with a left skewed distribution. The “trace data” were distributed between 0 and the detection limit, which could not be accurately quantified

due to the accuracy limitation of available instruments or equipment or unsatisfactory detection techniques. The presence of these trace data posed some difficulties to subsequent statistical analyses and could not be simply ignored because they had influences on the results of risk assessment.

During the process of building a risk assessment model for the peanuts' aflatoxin exposure, it is important to consider how to deal with the considerable values below the detection limit.

In accordance with the previous studies, there were mainly two solutions, which were point substitution and theoretical distribution substitution.

The point substitution method has been widely used in risk assessment of chemicals, such as heavy metals or pesticides. International aflatoxin risk assessment, conducted by JECFA or EFSA, also adopted this approach. Global Environment Monitoring System-Food Contamination Monitoring and Assessment Program [6] suggested that when the proportion of non-quantified or non-detected results was greater than 60%, the value under the limit of detection (LOD) was replaced by zero or LOD to produce upper and lower boundaries; when the proportion was less than 60%, LOD/2 was chosen as the substitute to produce statistical estimates. The point substitution method was a convenient operation, but its results were relatively rough and could not be used to evaluate uncertainty and variability.

Taking the process of total aflatoxin in peanuts as an example, the values below the LOD were all replaced by 0, 1/2LOD and LOD at first to generate three data sets, respectively. Then, the percentiles were calculated and it showed the difference occurred at <65th percentile, the three alternative results of total aflatoxin approached gradually from 65th to 85th percentile, and the maximum absolute difference was reduced from 0.16 to 0.14 $\mu\text{g/kg}$ and then to 0.11 $\mu\text{g/kg}$ at 95th percentile. The difference was mainly from detection limit of test method for aflatoxins. So, low detection limit was the main approach for reducing the difference among three alternative methods and improving the evaluation accuracy. The optimal detection method for aflatoxin was liquid chromatography coupled with immunoaffinity chromatography, which had relatively higher sensitivity and accuracy.

The theoretical distribution substitution method was based on the characteristics of contamination data. Taking AFB₁ in post-harvest peanuts as research object, the method was performed in two steps as follows. First, we sorted all data and eliminated the trace values that were lower than LOD from the entire dataset. Second, we fitted the distribution function with the values that were higher than the LOD by @RISK software, and then used the Kolmogorov-Smirnov (K-S) or Anderson-Darling (A-D) method to perform statistical tests on the fitting results. Through screening and optimization, Pearson V, Inverse Gauss and log-normal distributions were suitable to aflatoxin distribution in peanuts, and the comparison of frequency distribution and probability density indicated that Pearson V for goodness of fit was the best.

2.2. Risk assessment methods

2.2.1. Risk assessment based on non-parametric probability

AFB₁ dietary exposure was evaluated based on a probability distribution of aflatoxin contamination and consumption in agro-products, and the results were standardized by human bodyweight. The Monte Carlo method was chosen to perform the entire simulation process by @RISK program and the uncertainty was described by 90% confidence interval or quartile. The risk posed by dietary exposure to AFB₁ was modeled by the following formula: Population risk = exposure × average potency; exposure (daily intake of AFB₁ expressed as ng kg⁻¹ bw day) = (contamination level × consumption amount)/bw; average potency = $0.3 \times P + 0.01 \times (1 - P)$, where P represents the hepatitis-B-virus surface antigen (HBsAg) prevalence rate for different age groups.

For example, AFB₁ risk assessment in peanuts was conducted on the basis of dietary exposure to AFB₁ and its potential to cause hepatic cancer. Based on the results of peanut aflatoxin survey in China conducted in 2009–2010, as well as peanut consumption data and average bodyweight in each age-gender group from the 2002 Chinese Residents Nutrition and Health Survey Report [7–9], dietary exposure to AFB₁ was calculated and simulated by Monte Carlo. In line with the guidelines of Global Environment Monitoring System-Food Contamination Monitoring and Assessment Program [6], the values which were less than the LOD, were estimated, assuming that the proportion of non-quantified or non-detected results was more than 60% but less than 80%, the values under the LOD were substituted by zero or the LOD, which could provide a lower or higher boundary [10].

Excess risks for liver cancer incidence per year, resulting from AFB₁ dietary intake through peanut consumption, were calculated from dietary exposure to AFB₁ multiplied by the average AFB₁ cancer potency. According to the AFB₁ risk assessment report from JECFA [11], the average cancer potency was produced by setting the individual potencies of HBsAg⁺ and HBsAg⁻ to 0.3 and 0.01 cancers/year/100,000/ng kg⁻¹ bw day⁻¹, respectively. In this assessment, the age-adjusted HBsAg⁺ prevalence rate was obtained from the 2006 National Sero-epidemiological Survey report.

To evaluate potential health risk to Chinese under AFB₁ exposure in food, the excess risk for liver cancer in adults was estimated based on the mean and 97.5th percentile of the contamination and consumption data. The estimated AFB₁ intake from raw peanuts was between 0.11 and 5.66 ng kg⁻¹ bw day⁻¹ and the population risk was 0.003–0.17 cancer cases/year/100,000 from raw peanut consumption. The population risk was 0.03–2.06 cancer cases/year/100,000 from peanut oil intake of 0.84–68.8 ng kg⁻¹ bw day⁻¹. These data indicated that the risk from peanut oil was 10 times or more that from raw peanuts.

2.2.2. Margin of exposure (MOE) method

A “margin of exposure” was calculated from a chosen point of departure (POD) on a dose-response curve divided by the human dietary exposure estimate, which was obtained based on the benchmark dose (BMD) developed by EFSA [12, 13]. The PODs, employed to quantify an increased cancer risk, were summarized in **Table 1**. When the POD was determined, a smaller

Sources	BMDL10 ^a	BMDL10 ^b	BMDL1 ^b	T25
BfR	150	—	—	—
Brien et al. [14]	160	—	—	500
EFSA [10]	170–340	870–1100	78–121	—
Dybing et al. [15]	160–300	—	—	500
Benford et al. [16]	250	—	—	390

^aDerived from animal carcinogenicity data.
^bDerived from Chinese epidemiological data.

Table 1. Reference points/PODs derived from animal carcinogenicity and Chinese epidemiological data (ng kg⁻¹ bw day⁻¹).

MOE value represented a greater risk. Compared with a traditional low-dose extrapolation approach, the MOE value easily indicated what the risk level was, provided that the POD value had been defined. The MOE value would be smaller when exposure became greater. In general, a smaller MOE represented a greater risk.

Taking risk assessment of peanut aflatoxin exposure expressed by the MOE in China as an example, the MOEs were calculated on the basis of Chinese peanut aflatoxin exposure and PODs from the reported literature, which were developed based on Chinese epidemiological data by EFSA [10] or rodent experimental data by Benford et al. The relative PODs were summarized in **Table 1**. Here, BMDL10 (140 ng kg⁻¹ bw day⁻¹ for rodent [16] and 870 ng kg⁻¹ bw day⁻¹ for human) and BMDL1 (78 ng kg⁻¹ bw day⁻¹ for human), were introduced into the MOE calculation, which represented the 95% lower confidence limit (CL) of the BMD for a 10 or 1% increased cancer risk.

The estimated MOE values ranging from 24.1 to 1272 were higher than the results estimated by EFSA (88–483) [10] for Africa (0.2–121.4) [17]. Far lower than 10,000, would be regarded as low concern [12], and a higher MOE value implied a lower risk. The MOE values of peanuts based on the rodent data were 24.7–1272 and 2.0–167, respectively. In other words, the cancer risk, which originated from direct consumption of post-harvest peanuts or raw peanuts, was much lower than that from peanut oil. The above results were consistent with the conclusions, which were calculated on the basis of the cancer potencies of aflatoxin employed by JECFA. However changing index among two different methods was not found by now.

3. Risk ranking methods

The Codex Alimentarius Commission (CAC) recommended a food risk expectation ranking method. This method is usually based on literature review, authoritative data and database records of the countries that have evaluated the food hazard/consumption frequency and detected frequency information. Then the main food contamination factors are accurately identified, and the risks from different sources are compared. According to the calculated scores of the indexes, ranking of the risk will be obtained. This approach has the advantage of

clear scoring criteria and that direct use of the defined scoring criteria. In 1999, Houghton et al. [18] studied the ranking of risk factors for anxiety disorders in the UK using the risk expectation approach. In 2011, the method of risk expectation was used to systematically study the ranking and change in the relative risk index of liquefied petroleum gas transportation in Mexico metropolitan area [19]. In 2014, Speybroeck et al. [20] studied the ranking of risk factors in food chain by means of sampling survey and risk expectation. Nevertheless, there are few studies on risk factor of mycotoxins in peanuts and no study on risk ranking of mycotoxins in China until now.

In order to give a reference for risk monitoring and assessment of peanut quality and safety, the risk ranking method of mycotoxins in peanuts was proposed on the basis of the food risk expectation ranking method. A total of 604 peanut samples from 8 provinces were collected. Based on the mycotoxins concentration in peanuts and maximum residue levels, hazard degrees were identified. The effective evaluation indicators were chosen, and a normalized method was established for searching, identifying and ranking peanut mycotoxin risk factors.

3.1. Hazard degree identification

This study referred to the risk ranking sample tool recommended by the CAC and considered human health threats caused by the risk factors. The hazard severity and probability of occurrence were considered from the qualitative and quantitative points of view. The hazard degrees of the risk factors and risk ranking score evaluation criteria (Table 2) were identified with their toxicity, degree of difficulty in risk control, severity, social reputation, maximum amount of detection residue and detection rate considered.

According to the basic requirements of risk identification, the modified risk identification method was developed for peanut mycotoxins after several cycles of discussion, screening and expert opinion collection (Table 3).

3.2. Risk factor analysis and ranking

According to the risk ranking score evaluation criteria (Table 2) and identification of peanut mycotoxin risk degrees (Table 3), in addition to the toxicities, degrees of difficulty in risk

Index	Index value (score = 5)	Index value (score = 4)	Index value (score = 3)	Index value (score = 2)
Toxicity	High	Relatively high	Medium	Low
Degree of difficulty in risk control	Difficult	Poor	Potentially poor	Capable
Severity	Serious	Relatively serious	Medium	Noteworthy
Social reputation	Serious	Relatively serious	Medium	Noteworthy
Maximum amount of detection residue/(μg/kg)	>5000	1000–5000	500–1000	0–500
Detection rate/%	>10	8–10	6–8	4–6

Table 2. Identification of food hazards and risk ranking score evaluation criteria.

Risk factors	Toxicity	Degree of difficulty in risk control	Severity	Social reputation
AFB ₁	High toxicity, class 1 carcinogens, damage to liver	Difficult	Serious	Serious
AFB ₂		Poor	Relatively serious	Relatively serious
AFG ₁		Poor	Serious	Relatively serious
AFG ₂		Poor	Relatively serious	Relatively serious
OTA	Medium toxicity, class 2 possible carcinogens	Potentially poor	Medium	Medium
DON	Medium toxicity, class 2 possible carcinogens	Potentially poor	Medium	Medium

Table 3. Identification of peanut mycotoxin risk degrees.

control, severities, social reputations, maximum amounts of detection residue and detection rates of peanut AFB₁, AFB₂, AFG₁, AFG₂, Ochratoxin A (OTA) and Deoxynivalenol (DON) in peanuts in China, the mycotoxin risk factor scores for peanuts were calculated by Formula (1).

$$s = \frac{\sum_{i=1}^n X_{Ai}}{n} \times \frac{\sum_{i=1}^n (X_{Bi} + X_{Ci} + X_{Di} + X_{Ei} + X_{Fi})}{n} = U_A \times (U_B + U_C + U_D + U_E + U_F) \quad (1)$$

S: mycotoxin risk factor scores of peanuts; X_{Ai}: mycotoxin toxicity score of sample i; X_{Bi}: mycotoxin score of the degree of difficulty in risk control of sample i; X_{Ci}: mycotoxin severity score of sample i; X_{Di}: mycotoxin social reputation score of sample i; X_{Ei}: mycotoxin score of the maximum amount of detection residue of sample i; X_{Fi}: mycotoxin detection rate score of sample i; n: number of samples; U_A: average score of mycotoxin toxicity; U_B: average score of the degree of difficulty in risk control; U_C: average score of severity; U_D: average score of social reputation; U_E: average score of the maximum amount of detection residue; U_F: average score of the detection rate.

Mycotoxin risk factor scores and ranking for peanuts in China were listed in **Table 4**. It indicated that high attention needed to be paid to AFB₁, relatively high attention needed to be paid to AFG₁, moderate attention needed to be paid to AFB₂ and AFG₂, and low attention needed to be paid to OTA and DON.

Mycotoxin	UA	UB	UC	UD	UE	UF	S	Risk degree
AFB ₁	5	5	5	5	5	4	120	High
AFB ₂	4	4	4	4	4	3	76	Moderate
AFG ₁	4	4	4	4	5	5	88	Relatively high
AFG ₂	4	4	4	4	4	3	76	Moderate
OTA	3	3	3	3	2	3	42	Low
DON	3	3	3	3	2	3	42	Low

Table 4. Mycotoxin risk ranking for peanuts in China.

4. Risk prediction technologies

Geographic information systems (GIS) and geostatistics can be used to describe, analyze and display spatial patterns of a wide variety of variables at any scale and, by improving resource management and revealing causal relationships among geographically variable factors, assist in real world problems [21]. Kriging, a regression technique for interpolation of spatially correlated data, is the most common geostatistical procedure for surface interpolation, can be used to locally average the weights of data from sampled locations surrounding an unsampled location based on statistical similarity to unsampled locations; it gives unbiased estimates with the estimated variance minimized. The weights are determined using semivariance analysis between sampled locations [22]. Areas in China with the highest risk of AFB₁ contamination were identified by geostatistical analyses and Kriging maps. According to different locations, terrain features, climatic conditions, variety distributions and cultivation systems, the peanut planting areas in China were divided into four sections: Northeast, North, Yangtze River and South [23]. Agricultural practices including crop rotation, tillage, irrigation and fertilization, as well as the planting date, genetic resistance, soil type and climatic conditions all impact AFT contamination of peanuts before harvest [24]. Nevertheless, climatic conditions significantly influence the AFT contamination level. In serious drought and/or high temperature conditions before harvest, fungus invasion and AFT accumulation become accelerated [25, 26].

4.1. Spatial analysis of AFB₁ contamination of peanuts in China

A total of 9741 peanut samples were collected from main produce area in China from 2009 to 2014 and on the AFB₁ content of these peanut samples were analyzed. Geostatistical analyses were performed on the annual average AFB₁ content to obtain the patterns of AFB₁ contamination throughout China. Kriging of AFB₁ showed that aflatoxin contamination of peanuts in China was a perennial problem presenting both temporal and spatial (regional) variations. Kriging interpolation of AFB₁ contamination indicated a patchy distribution, which varied with the seasons. Results showed that aflatoxin contamination was almost not found in the Northeast region during the study period. And it presented significantly temporal and spatial (regional) variations in the Yangtze River Basin region and Southeast Coast region.

4.2. Relationship between AFB₁ contamination levels in peanuts and climatic conditions before harvest

Cole *et al.* found that ripe and integral peanuts exposed to simultaneously drought and heat (25.7–31.3°C) stress became be prone to *Aspergillus flavus* invasion and AFT production in the last 4–6 weeks of the growing season [27]. A total of 2983 peanut samples were collected from 122 counties in 6 provinces of China's Yangtze River ecological region from 2009 to 2014. Based on Thiessen polygon interpolation, average precipitation and mean temperature data in 2009–2014 in the Yangtze River ecological region were calculated by the climatic conditions of 118 weather stations. In **Figure 1**, we found that there was less precipitation and higher daily mean temperature (around 25°C) during peanut growing season (June–August) in 2013, which aggravated the AFB₁ contamination. Taking Hunan province as an example, the

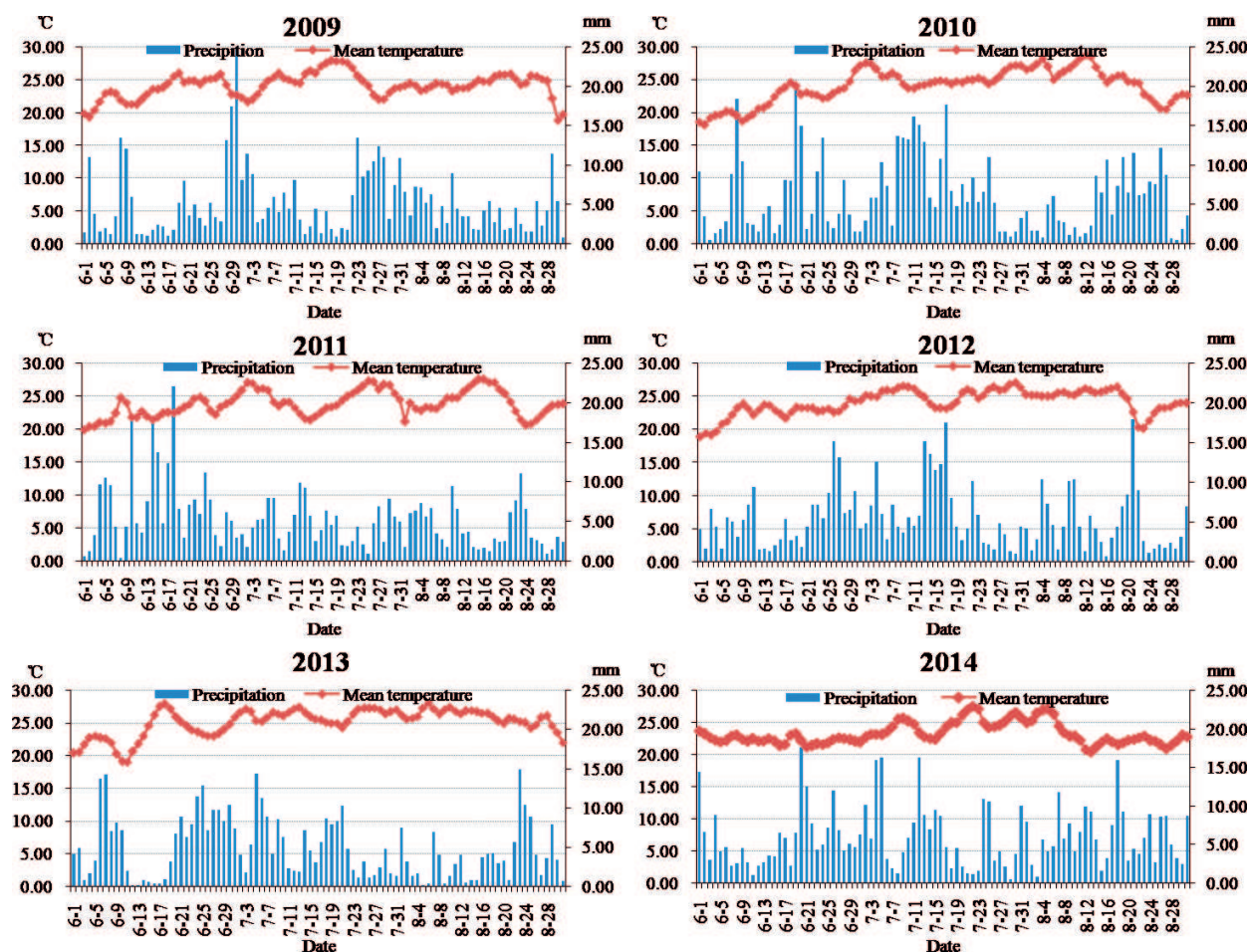


Figure 1. Precipitation and mean temperature of the Yangtze River ecological region during the peanuts' growing season (2009–2014).

determination coefficient (R^2) fitted by the AFB₁ content with the average climatic conditions in different pre-harvest periods was obtained by multiple regression (**Figure 2**). Results indicated that the average precipitation and mean temperature of 1 month before harvest had a significant influence on AFB₁ contamination. Moreover, Hunan and Jiangxi were greatly affected. Due to the annual and climatic variation of AFB₁ contamination level, it is necessary to build a prediction model by developing a continuous and effective AFB₁ monitoring program for pre-harvest peanuts during its growing season. Up to now, there had been some progress on model building in Australia and USA [28, 29].

5. Prospect of future research

The occurrence and control of aflatoxin contamination in agro-products are world wide hot issues difficult to resolve. Studies on risk monitoring, risk assessment and early risk prediction of aflatoxin in peanuts, maize and other agro-products have long been considered as an important premise for effective aflatoxin contamination control. Hence, our further efforts

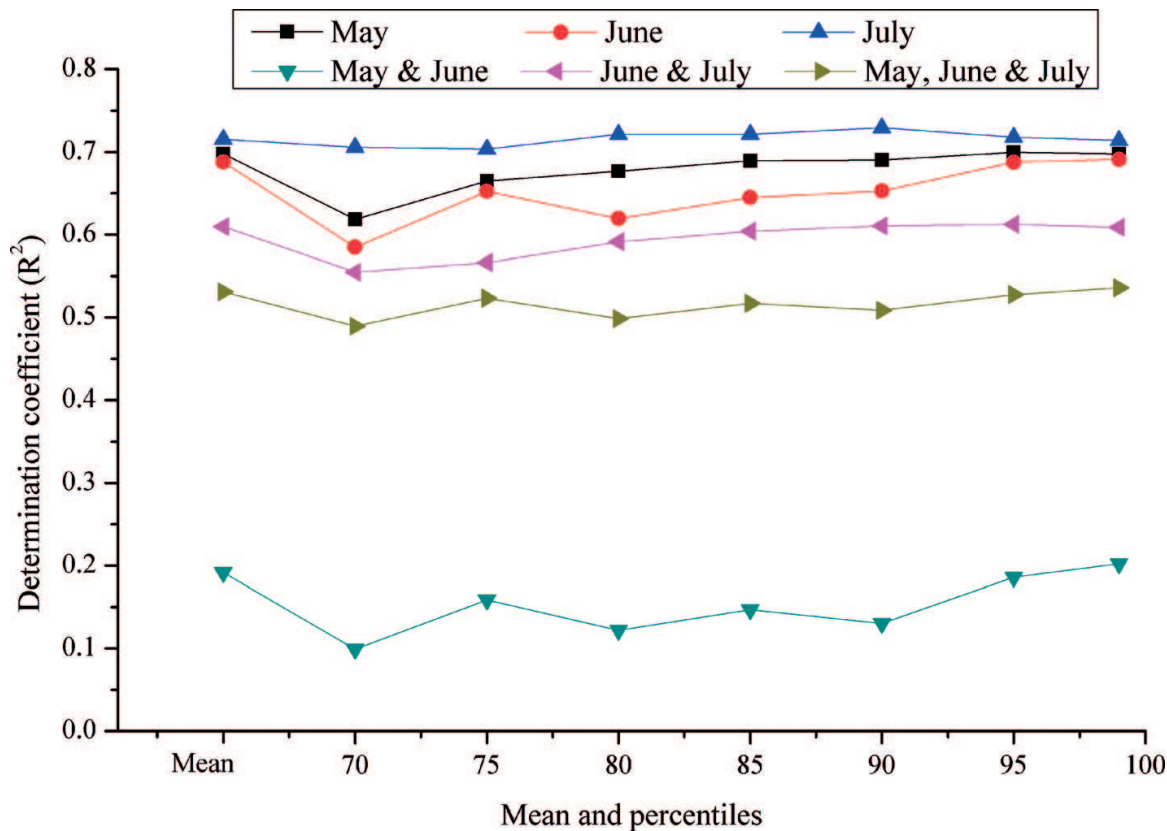


Figure 2. Multiple regression determination coefficient (R^2) fitted by the AFB₁ content with average precipitation and mean temperature in different periods of time (Hunan province).

should be focused on enhancing and perfecting the basic database of aflatoxin contamination, drawing geographic risk maps, developing reliable and accurate risk forecasting techniques and a forecasting system, as well as establishing a smart and low-cost platform for sustainable aflatoxin management and communication in the field and during storage, processing and transportation. Moreover, risk monitoring, simultaneous detection technologies of multi-mycotoxin contamination and their interaction mechanisms should also be taken into account.

5.1. Characteristics and geographic risk maps for aflatoxin contamination and main toxigenic fungal population

To boost the progress of mycotoxin risk assessment, what is crucial is to enhance the basic database construction for mycotoxin contamination, especially aflatoxin, which has the highest acute and chronic toxicity among all mycotoxins. Hence, it is necessary to conduct a continuous and effective AFT monitoring program for obtaining quantitative data from different latitudes, altitudes and ecological regions in a global level via international cooperation by sampling and detecting representative fields. Precise risk maps for aflatoxin contamination will be drawn to highlight the distribution, concentration and trend of annual occurrence. Certainly, advanced and accurate analytical techniques will be an essential part of guaranteeing the quality of monitoring and data. Meanwhile, the main toxigenic fungal population

database, including *A. flavus* and *Aspergillus parasiticus*, should also be determined by collecting and identifying isolates from peanuts and soil in the corresponding areas. Geographic maps of these fungi populations will be defined. The aflatoxin production, biodiversity and phylogenetic clades of these toxigenic fungi will be revealed. Because the changes in climatic conditions will lead to a shift in the fungal population and mycotoxin patterns, more attention should be paid in a climate change scenario. What is more, multi-mycotoxin co-occurrence and interactions with different fungi, such as *A. flavus* and *Fusarium verticillioides*, have been recognized as emerging problems and cannot be ignored.

From a risk assessment perspective, to determine risk maps of aflatoxin contamination and toxigenic fungal population to a global level, which was based on a continuous and effective AFT monitoring program, is a key step in risk prediction. Additionally, aflatoxin and toxigenic fungi risk maps could be used as a communication tool for stakeholders and farmers. Moreover, the maps could be provided as a tool for scientific supervision, decision support and governments' policy-making, as well as prioritization of a more targeted approach and intervention strategies, especially in high-risk zones.

5.2. Building an early predictive model of aflatoxin by combining macroscopic and molecular warning technologies

In further studies, there is an urgent need to establish a precise and reliable forecasting system with advanced prediction methods to reflect actual occurrence of aflatoxin contamination so that we can make appropriate management and agronomic strategies especially in high-risk areas, minimize the risk of pre-harvest contamination and therefore protect public and animal health. Moreover, applying the early warning model can significantly reduce the detoxification cost. Until now, a lot of researches indicated that key environmental factors including temperature, humidity and precipitation significantly influenced fungus growth, infection as well as aflatoxin production [30–32]. And some efforts have been devoted to developing models to predict aflatoxin contamination in peanuts and maize with climatic data used as the main or only input, such as the Agricultural Production Systems Simulator (APSIM) and the CSM-CROPGRO-Peanut model [29, 33]. However, few models were actually applied to the field to predict the future aflatoxin risk or just a small region for validation and demonstration. Besides climatic data, the factors such as ecological zones, peanut varieties and microbial structures should not be ignored, which were also believed to be aflatoxin-related factors. In short, there is further work to develop a large-scale risk prediction model based on multi-factors and apply it to different fields.

Firstly, advanced technologies in digital and smart agriculture are essential for effectively monitoring the fields. GIS, environmental sensors or satellite systems will be used to monitor crop conditions and abiotic factors such as humidity, temperature, precipitation, wind and sunshine in real time, in order to acquire spatial and temporal distribution information of the crops and climatic data rapidly and accurately during crop-growing seasons. These local and accurate real-time data will be directly translated for researchers and other stakeholders. And then, correlation analysis will be, respectively, carried out between the aflatoxin contamination data and these real-time data, agronomic information and toxigenic fungus population to

evaluate the role and contribution of these relevant factors in the field of aflatoxin contamination risks. In particular, the role of CO₂ should be taken into account, which is increasingly important in a climate change scenario. At last, a macro-scale predictive model will be built and operated with these “real-time” data as the input to obtain specific early predictions regarding the risk of aflatoxin. Appropriate management decisions and recommendations for farmers and other stakeholders will be formulated when the contamination risk is high, which is based on the output data.

In recent years, with rapid development of molecular biology, molecular prediction technologies have gradually become frontier for early warning of mycotoxins. The interactive conditions of $a_w \times$ temperature \times elevated CO₂ have a significant impact on aflatoxin biosynthetic gene expression, such as the structural genes *aflD* and *aflM* and regulatory genes *aflS* and *aflR*, and the production of AFB₁ [32, 34]. A physical model was built and used to relate gene expression to a_w and temperature conditions to predict AFB₁ production. And its relationship with the observed AFB₁ production provided a good linear regression fit to the predicted production based on the model [34]. The expression data ratio of *aflS/aflR* has a relationship with the amount of AFB₁ or AFG₁. High ratios in the range between 17 and 30°C corresponded to the production profile of AFG₁ biosynthesis. A low ratio was observed at >30°C, which was related to AFB₁ biosynthesis [35]. We therefore believed that it is possible to predict the aflatoxin risk via the expression model of key genes or secondary metabolites. In our future work, we will devote to screening and identifying more effective molecular markers to make the prediction more reliable and build a molecular forecasting model.

Therefore, it is believed that effective integration of macro-scale, molecular, ecophysiological and secondary metabolite data sets could be critical in predicting the risk of aflatoxin contamination under different biotic and abiotic stress scenarios and agronomic strategies. Such combinative technologies will be beneficial to more accurate predictions of the aflatoxin risk in different regions and also the potential for new emerging toxin threats.

5.3. Developing a smart platform for aflatoxin risk communication and management

A convenient and user-friendly platform, such as a mobile app, will be developed. The platform will provide key information about the crops, contamination risks or levels, recommendations, practical solutions, problem consultation and answers to farmers and other stakeholders who require suggestions for rapid and low-cost intervention. The interpretation of the output of the predictive models and recommendations will be transformed into the platform. Thus, farmers can not only obtain the growth status of the crops in the field, but also timely and cost-effective strategies for prevention or remediation of the risks during their harvest, storage, processing and transportation.

In conclusion, it is necessary to further focus on the development of advanced and integrated technologies and solutions to achieve the purposes of reducing costs, increasing efficiency, maximizing the role of risk assessment and risk prediction, and definitely improving the quality and security of agricultural products.

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