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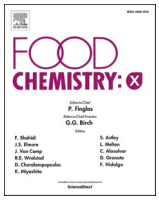

Predictive models for assessing the risk of *Fusarium pseudograminearum* mycotoxin contamination in post-harvest wheat with multi-parameter integrated sensors

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ABSTRACT

Reliable prediction of the risk of mycotoxin contamination in post-harvest wheat will aid in improvement of the quality and safety. To establish the relationship between *Fusarium pseudograminearum* mycotoxins and CO_2 production, changes in their respective concentrations were monitored for the artificial contamination of wheat under different values of water activities (0.84 a_w, 0.92 a_w, and 0.97 a_w) and temperatures (20 °C, 25 °C, and 30 °C). Water activity played a significant role in all these processes. CO_2 concentration together with moisture content and temperature were used as the main parameters to establish DON and ZEN contamination prediction models. The prediction accuracy for DON was 98.15 % ($R^2 = 0.990$) and 90.74 % for ZEN ($R^2 = 0.982$). These models were combined with T/RH/MC/CO_2 multi-parameter integrated sensors to form an early warning system, which offers a great prospect to minimise the risk of DON/ZEN contamination in post-harvest wheat.

Introduction

Grains are easily contaminated by various microorganisms during production, harvesting, transportation and storage, among which fungi are the main microbial species affecting the quality and safety of the grains. *Fusarium*, *Aspergillus*, *Penicillium*, and *Alternaria* are filamentous fungal species found in grains (Balendres et al., 2019; Paterson et al., 2018). These fungi produce toxigenic secondary metabolites, which can cause severe carcinogenicity, mutagenicity, genetic toxicity, growth and reproduction toxicity, immunotoxicity, and neurotoxicity in humans and animals (Luo et al., 2021). *Fusarium species* is the most common pathogenic fungus in wheat, often causing *Fusarium* head blight (FHB) and *Fusarium* crown rot (FCR), resulting in yield and quality loss (Wegulo et al., 2015; Kazan and Gardiner, 2017). *Fusarium pseudograminearum* is the main FCR pathogen in wheat, reported in Australia, the United States, Canada and other countries. In recent years, it has become the dominant causative pathogen of FCR in wheat in Eastern China, and its incidence and severity have increased rapidly (Deng et al., 2020; Jin et al., 2021).

Deoxynivalenol (DON) and zearalenone (ZEN) are the two main hazardous secondary metabolites produced by *F. pseudograminearum*. (Ji et al., 2016; He et al., 2016). DON, also known as the vomiting toxin, is a trichothecene with the highest detection rate in wheat (Binder et al., 2007). ZEN, also known as the F-2 toxin, causes strong reproductive toxicity and teratogenicity (Gao et al., 2017; Rogowska et al., 2019). The risk of DON and ZEN contamination has gained worldwide attention, and a strict maximum limit has been set to control their levels in cereals and cereal by-products intended for human and animal consumption (Cheli et al., 2014). Both mycotoxins can be produced pre- and post-harvest; if grain is not dried immediately post-harvest and stored in poor conditions, *Fusarium* species will thrive and produce mycotoxins (Portell et al., 2020). The main cause for the loss of up to one-third of the total annual global grain production (cereals, oil seeds, and pulses) is because of poor post-harvest management (Singh and Fielke, 2017). Therefore, it is crucial to assess the dynamic situation of mycotoxins contamination in storage to ensure the quality and safety of grain (Buszewska-Forajta, 2020; Haque et al., 2020).

Temperature (T) monitoring of grains in storage is a common method

Abbreviations: DON, deoxynivalenol; ZEN, zearalenone; FCR, Fusarium crown rot; FHB, Fusarium head blight; DMLs, dry matter losses; a_w, water activity; T, temperature; RH, relative humidity; MC, moisture content.

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for detecting spoilage in grains. However, owing to the low thermal conductivity of the grains, T must be measured within about 0.5 m or less of an active spoilage spot to detect the self-heating. This means that deterioration occurring in the stored grain more than 0.5 m away from the temperature sensor; may progress to an advanced stage before any significant rise in temperature is recorded. Therefore, T measurement is not a sensitive parameter (Ileleji et al., 2006). Grain T is also affected by the environment, and few fungi emit less heat and does not raise the T of grain piles (Cai et al., 2013). Therefore, this approach requires a high-density arrangement of monitoring points to exert its advantages (Plumier and Maier, 2021). All living organisms in stored grain piles produce carbon dioxide (CO_2) as a metabolite (Neethirajan et al., 2010). Due to its sensitivity and reliability, CO_2 concentration is an effective indicator for monitoring grain quality (Abalone et al., 2011; Fleurat-Lessard, 2017; Aby and Maier, 2020). The early warning sign for grain mycotoxin contamination has been initially achieved by monitoring CO_2 concentration; however, a major challenge remains for large-scale grain storage. Many studies have showed that fungal growth, which causes spoilage, is associated with the utilisation of lipids in the grain, resulting in quality decline and dry matter losses (DMLs). Therefore, CO_2 production and respiration rate can be used to establish a relationship between DMLs and mycotoxin contamination and would aid in prediction of quality and mycotoxin contamination in stored cereals (Garcia-Cela et al., 2018; Garcia-Cela et al., 2019; Castaño et al., 2017a,b; Mylona et al., 2012a,b; Magan et al., 2010). The technology obtains DMLs values using relevant parameters such as grain respiration rate and mass and storage volumes. Subsequently, it determines the early warning value according to the maximum limit set, to achieve prevention and control of mycotoxin contamination. However, relying only on gas monitoring values to predict the amount of mycotoxin contamination is challenging, because T affects CO_2 release rate, and grain moisture content (MC) affects respiration rate (Kaleta and Gornicki, 2013). Monitoring food quality using parameters such as T, MC, and CO_2 has received considerable attention from researchers, farmers, and businesses (Ramachandran, 2022). Portable air extraction CO_2 detection devices have been used to monitor CO_2 concentration in warehouses. However, it is time-consuming, labour-intensive, and the accuracy of detection results is affected by various factors such as trachea size, air flow rate and gas exchange (Zhang et al., 2014); therefore, it is not suitable for safety monitoring of large grain depots. With the advancement of sensor technology, real-time monitoring sensors for CO_2 in grain piles have been developed. By integrating T, relative humidity (RH) and other related sensor elements, these sensors can be used as effective tools for grain storage management.

This study aims to establish a mycotoxin contamination early warning model in wheat, by evaluating the direct relationship between related storage parameters such as CO_2, T, RH, MC, and mycotoxins. This study uses a new type of multi-parameter: T/RH/MC/CO_2 integrated sensors in real-time. The accuracy and effectiveness of the monitoring system have been verified in a typical wheat warehouse in China, with real-time sensor multi-parameter acquisition of grain piles and the establishment of an early warning platform for storage risks. This monitoring system would provide early warning guidance for wheat storage management, for early detection of problems and proffering relevant solutions to ensure the safety of grain storage.

Materials and methods

2.1. Wheat samples

Samples of wheat produced in Henan Province, China in 2020, were tested and it was confirmed that the contents of 16 common mycotoxins were all <LOD (limit of detection) (Ye et al., 2018). The disinfected wheat kernels were prepared as follows. Firstly, the wheat kernels were soaked in 75 % alcohol for 1 min, followed by 3 % sodium hypochlorite solution for 12 min. Secondly, wheat kernels were washed three times with distilled water. In a biological safety cabinet, the disinfected wheat kernels were spread on sterile gauze, ventilated, dried, and water activity (a_w) was measured using an Aqualab Dew Point Water Activity Meter 4 TEV DUO (Decagon, WA, USA) at 25 ℃, until it equilibrated to 0.75 a_w.

To confirm the fungal infection rate of the disinfected wheat, 10 grains/plate from each group were inoculated into potato dextrose agar (PDA) (AOBOX, Beijing, China) medium and incubated at 28 ℃ for 7 d, and 10 technical repetitions were made for each group. To confirm the germination rate, 50 grains/plate from each group were placed into the petri dish covered with moist gauze, incubated at 20 ℃ for 7 d, and 4 technical repetitions were made for each group.

2.2. Development of the wheat moisture adsorption curve

The disinfected wheat kernels were distributed into zip-lock bags (100 g/bag), distilled water of known volume (2 mL to 40 mL) was added and inverted several times until the water was completely absorbed by the wheat kernels. Later, they were sealed and placed at 4 ℃ to equilibrate for 72 h. Each group of wheat kernels was then incubated at 25 ℃ and a_w was measured using an Aqualab Dew Point Water Activity Meter 4 TEV DUO (Decagon, WA, USA), the MC was determined by drying at 105 ℃ in an oven after reaching constant weight.

2.3. Fungal strain and spore suspension

The strain of *F. pseudograminearum* (JN862234.1) used in this study was isolated from wheat and stored in our laboratory. *F. pseudograminearum* was inoculated into the PDA medium, incubated at 28 ℃ for 5 d and the mycelium was transferred into the carboxymethylcellulose (CMC) medium (1.5 % CMC-Na, 0.165 % $(NH_4)_2SO_4$, 0.5 % yeast extract, 0.05 % $MgSO_4{\cdot}7H_2O$, and 0.1 % KH_2PO_4). After 7 d shaker cultivation (120 rpm/min) at 25 ℃, the culture medium was filtered using four layers of gauze to collect spores. They were then washed once with spore washing solution (0.85 % NaCl solution with 0.005 % Tween 80) and the number of spores in suspension was adjusted to 1.5×10^6 spores/mL.

2.4. Wheat inoculation culture under different water activity and temperature conditions

The disinfected wheat kernels were adjusted to a target a_w of 0.85, 0.90, and 0.95 according to the moisture adsorption curve, later 5 g of wheat were distributed into 20 mL headspace injection vial. The *F. pseudograminearum* spore suspension was added to each bottle of wheat samples to a final concentration of 4.5×10^4/g mixed well, sealed using culture sealing film, and labelled. Non-inoculated disinfected wheat samples used as control. After re-testing, the actual a_w and the MC values of the samples were 0.84 (18.04 %), 0.92 (21.16 %) and 0.97 (26.87 %), respectively. The sample vials were divided into plastic fresh-keeping storage boxes, and an appropriate amount of sterile water was added to the bottom of the box for moisturizing, and then cultured at 20 ℃, 25 ℃, and 30 ℃ for 12 d.

2.5. Monitoring the concentration of CO_2 and calculating the respiration rate

The CO_2 release rate was analysed every 24 h. The specific operation followed was as follows: The vial cap was replaced with a screw-sealed cap with a silicone gasket inside, and the samples were then placed back into the original storage boxes and incubated for 1 h. The CO_2 concentration percentages were detected using gas chromatography (GC). The GC equipment used was an Agilent 7890A Gas Chromatograph (Agilent Technologies, UK) with a Thermal Conductivity Detector (TCD) that used helium as a carrier gas. The column used for the analysis was PoraBOND Q (Agilent Technologies, UK) and the data were analysed

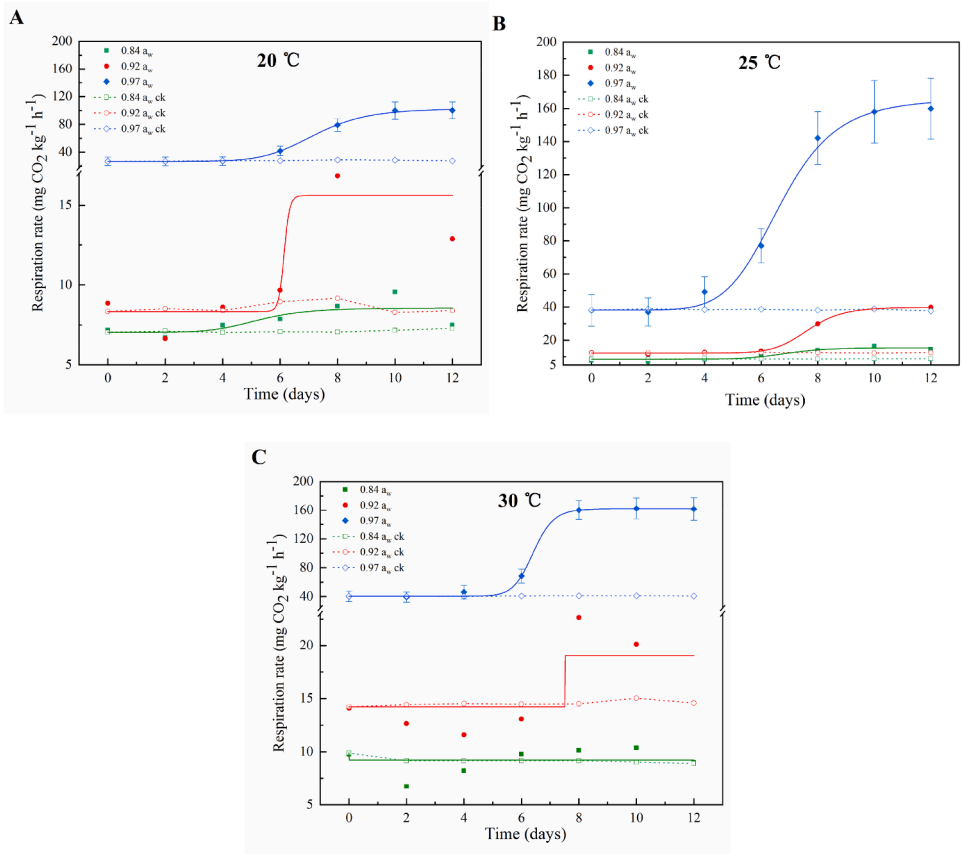

Fig. 1. Variation curves of respiration rates of *Fusarium pseudograminearum* inoculated wheat (Solid line, data fitting), and blank wheat (dotted line) with different water activities (0.84 a_w, 0.92 a_w, and 0.97 a_w) stored at 20 ℃ (A), 25 ℃ (B), and 30 ℃ (C). Error bars indicate the standard errors of the means of three repeated experiments.

using Agilent Chemstation Software (Agilent Technologies, UK). The respiration rate of each sample was calculated following the relationship described by Castao, S.M., Medina, A., Magan, N. (Castano et al., 2017b).

2.6. Monitoring mycotoxin production

Samples were taken every 48 h to detect the toxin content. Liquid chromatography tandem mass spectrometric method was used for the determination of multiple mycotoxins in cereals using stable isotope dilution established by reference laboratories for sample processing (Ye et al., 2018).

2.7. Real-time monitoring of temperature, relative humidity, moisture content, and CO_2 in wheat horizontal warehouses

Wheat horizontal warehouse (60 m × 21 m × 6 m) was monitored for one year using a set of customized real-time monitoring of T/RH/MC/CO_2 multi-parameter integrated sensors (GESCASER SA, Spain), including three probes of 1.80 m with 3 sensors (Fig. S1 A. B.). Regular sampling was done to measure MC, detect mycotoxins and other related indicators. The sampling depth was 4 layers, and each layer has 4 sampling points located in four directions 20 cm away from the centre of the sensor (Fig. S1 C).

2.8. Statistical analysis

SPSS 20 (IBM, Armonk, NY, USA) was used for *t*-test and analysis of variance (ANOVA). The Grain T, RH, MC, and CO_2 monitoring data fast processing software V1.1 (developed by our group) was used to process sensor real-time monitoring data and obtain the summary results of each parameter of each sensor in different periods. OriginPro 2018 (OriginLab Corporation, Northampton, MA, USA) was used for graph drawing and data fitting.

The respiration rates of wheat inoculated with *F. pseudograminearum* under simulated storage conditions was fitted by logistic regression model. The expression is as follows:

$$R = \frac{R_0 - R_1}{1 + (t/t_0)^r} + R_1$$

where R represents the respiration rates of wheat, R_0 represents the initial respiration rates of blank wheat, R_1 represents the maximum respiration rates of wheat, t represents the simulated storage time, t_0 represents the time it takes for the respiration rate to reach half the maximum value, r represents the maximum growth rate of respiration rates.

Results and discussion

3.1. Effect of water activity and temperature on the respiration rates of wheat inoculated with F. pseudograminearum

The respiration rates of wheat inoculated with *F. pseudograminearum* and blank wheat under simulated storage conditions were investigated (Fig. 1). A significant increase in respiration rate during the 12-d storage period was observed for wheat inoculated with *F. pseudograminearum* except for 0.84 a_w at 30 ℃, whereas the respiration rates for blank wheat showed initial elevation of a_w and T, and later remained stable throughout the storage period. This demonstrates that the disinfection of wheat is satisfactory and still maintains biological activity and is

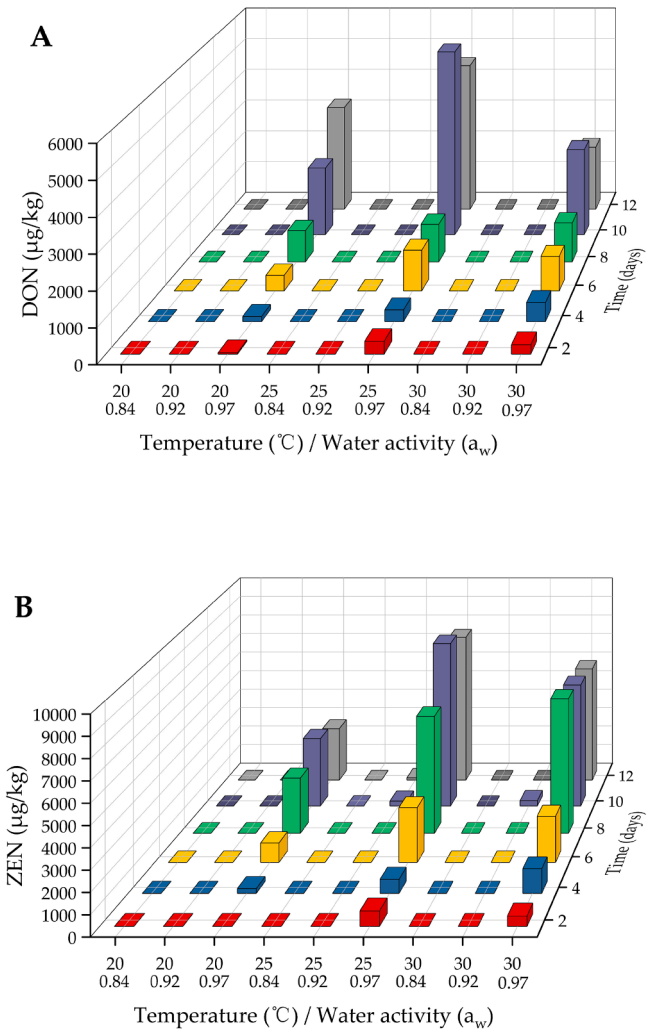

Fig. 2. The production and accumulation of DON (A) and ZEN (B), in inoculated wheat with different water activities (0.84 a_w, 0.92 a_w, and 0.97 a_w) stored at 20 ℃, 25 ℃, and 30 ℃.

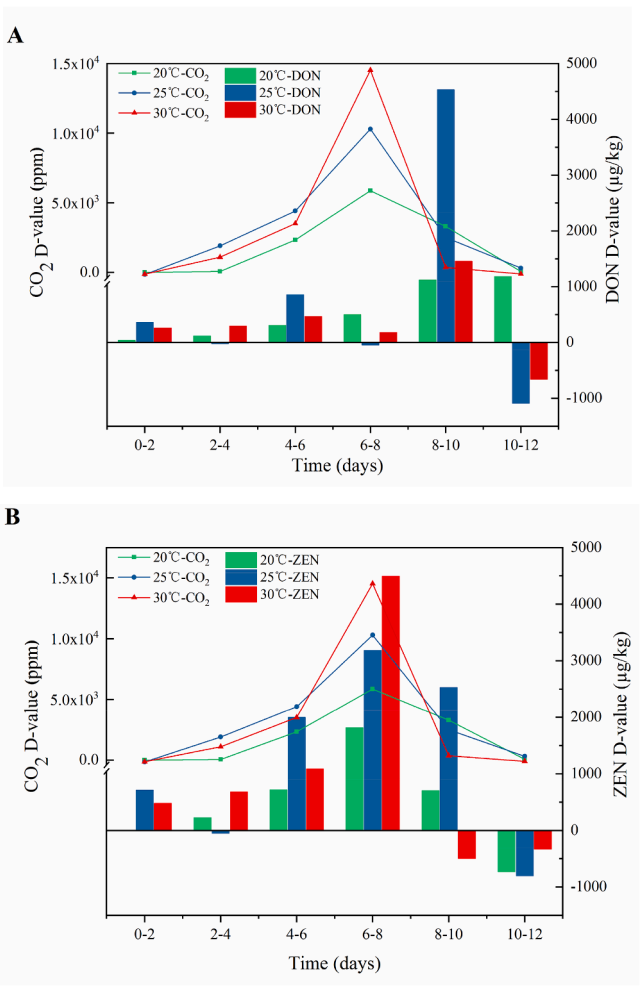

Fig. 3. Comparison of CO_2 with DON (A) and ZEN (B) production of 0.97 a_w inoculated wheat at different temperatures. The D-value was calculated by subtracting the previous day's data from the current day's data.

consistent with the 93.3 % disinfection and 86.7 % germination rate of our disinfection method. This indicated that the increase in respiration rates of inoculated wheat was due to the growth of *F. pseudograminearum*.

There was a consistent link between respiration rates and the storage moisture and temperature conditions (Garcia-Cela et al., 2019). The logistic regression fitting results showed that the maximum respiration rates (R_1) elevated with the increase of a_w and reached the maximum at 25 ℃ (Table S1). The maximum respiration rates would decline, particularly at lower a_w conditions, when the T increased to 30 ℃. The logistic regression fitted best for of 0.97 a_w, and under this condition the t_0 values tended to decrease with the increase of T. Correspondingly, the r values tended to increase, particularly for T at 30 ℃, which was quite higher than those at 20 ℃ and 25 ℃. These results showed that a_w had a greater effect on the respiration rate than T, which was probably attributed to the fact that field fungi usually required a higher water activity (>0.9) to survive (Magan and Lacey, 1984, 1985; Fleurat-Lessard, 2017).

3.2. Effect of water activity and temperature on the accumulation of mycotoxins

The most effective way to reduce mycotoxins is to prevent the fungus from growing in the first place (Liu et al., 2020). Interestingly, factors influencing fungal growth are varied (water, air, temperature, etc.), and storage conditions conducive to fungal growth do not necessarily lead to mycotoxins production (Fleurat-Lessard, 2017; Mannaa and Kim, 2017). The yields of DON and ZEN from *F. pseudograminearum* inoculated wheat with different water activities were variable when stored at different T (Fig. 2). Highest values of both mycotoxins in different storage periods appeared in the inoculated wheat at 0.97 a_w and a storage T of 25 ℃. This is consistent with the pattern of the respiration rate. The inoculated wheat at 0.92 a_w showed a small amount of ZEN production during the late storage period at 30 ℃, whereas the inoculated wheat at 0.84 a_w did not produce any toxins within the 12-d storage at various T.

The ANOVA showed that the effect of a_w on DON and ZEN production was significant ($P < 0.05$), whereas T had a significant effect on DON and ZEN production only during suitable a_w for toxin production. This indicates that a_w is the main limiting factor for mycotoxin production, which is consistent with the reported view that free water content has a higher role in the production of mycotoxins (Magan et al., 2010).

3.3. Relationship between mycotoxins and CO_2

The changes in CO_2 and mycotoxins levels in the 12-d simulated storage process of inoculated wheat at 0.97 a_w were compared (Fig. 3). The overall changes in the trend of CO_2 with the DON or ZEN levels were consistent, and the changes in case of the ZEN level was particularly significant. The changes in CO_2 and toxins levels were not significant in

Table 1
The coefficients b_0 to b_6 of the logDON and zearalenone (ZEN) prediction models and the statistical significance of the relevant factors in the model equations on the toxin production of logDON and ZEN were determined by the forward stepwise regression.

	logDON			ZEN		
	Unstandardized Coefficients B	*P*-value	Standardized Coefficients beta	Unstandardized Coefficients B	*P*-value	Standardized Coefficients beta
b_0	20.518	< 0.001	/	−14.343	< 0.001	/
b_1	0.044	< 0.001	5.128	8.429E-007	< 0.001	2.021
b_2	−1.928	< 0.001	−4.976	−4.141E-009	< 0.001	−1.050
b_3	8.72E-06	< 0.001	1.271	/	/	/
b_4	0.008	< 0.001	−0.979	/	/	/
b_5	−6.77E-06	< 0.001	0.703	/	/	/
b_6	−0.137	0.001	−0.396	/	/	/
R^2	0.990			0.982		

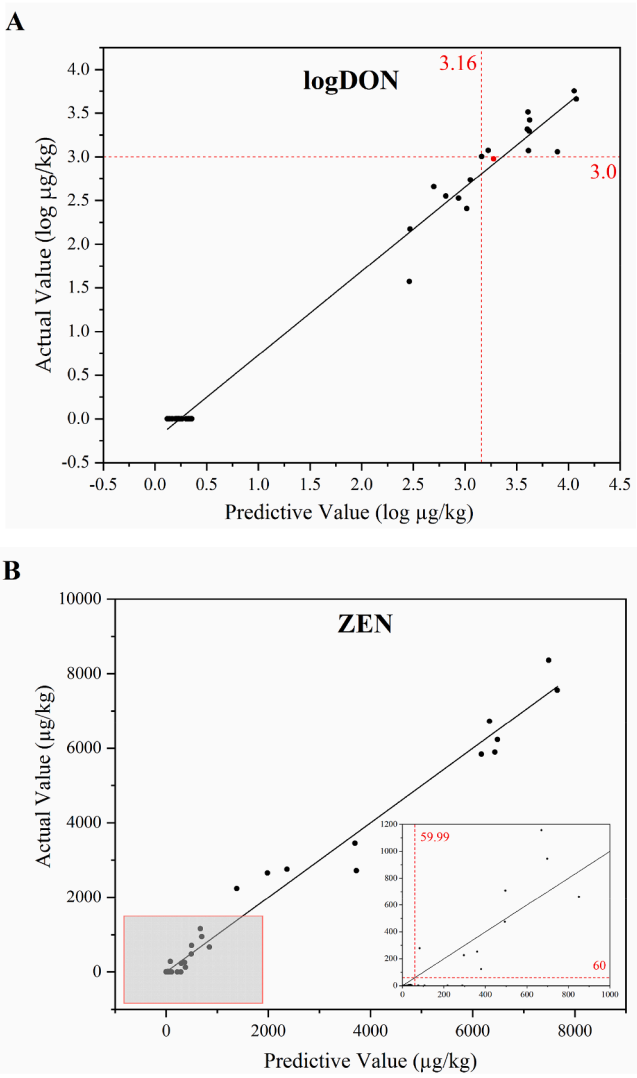

Fig. 4. Scatter-plot of predictive and actual values of logDON (A) and ZEN (B) produced by *Fusarium pseudograminearum* colonising stored wheat for under 12 days. The red dotted line (inset) on the x-axis indicates the China legislative limits for grains. The red dotted line (inset) on the y-axis indicates the early warning value. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the early stage of simulated storage (0 d to 4 d). This may be because the *F. pseudograminearum* inoculated in wheat during this period was in a mixed state of spore germination and mycelial growth. The growth rate in different samples was not uniform, and *F. pseudograminearum* and wheat had a mutual inhibitory effect. From day 4–6 of the simulated storage, the rising trend of CO_2 was evident, and the increase was as follows: 25 ℃ > 30 ℃ > 20 ℃; the corresponding increase in ZEN and CO_2 was consistent, while DON relatively lagged behind. From day 6–8 of simulated storage, the rising trend of CO_2 changed to 30 ℃ > 25 ℃ > 20 ℃, which was similar for ZEN, whereas DON had a maximum growth at 25 ℃.

Although the production changes of DON and ZEN are volatile, the correlation analysis results showed that CO_2 had a significant correlation with DON and ZEN, and the correlation coefficients were 0.862 ($P < 0.001$) and 0.938 ($P < 0.001$), respectively.

3.4. Establishment and validation of toxin prediction model

A comprehensive analysis of the results of all the experimental samples were performed to build toxin prediction models for different parameter combinations. (Table S2). The accuracy of the prediction results was verified and the best prediction models for each toxin were identified. At the end, a multiple linear regression (MLR) was performed to predict the base 10 logarithm of DON (logDON) and ZEN production. To match the actual monitor parameters of the T/RH/MC/CO_2 multi-parameter integrated sensor, we used MC instead of a_w to build the toxin prediction model. As RH made no significant contribution ($P > 0.05$), it was excluded from the final prediction model.

The step-by-step forward regression analysis confirmed that the factors influencing DON production were MC^2, MC, $MC \times CO_2$, $MC \times T$, and $T \times CO_2$. Although temperature alone was not a significant factor, $MC \times T$ and $T \times CO_2$ interactions were. Table 1 shows the value of each coefficient from b_0 to b_6 and its statistical significance.

$$LogDON = b_0 + b_1 \bullet MC^2 + b_2 \bullet MC + b_3 \bullet MC \bullet CO_2 + b_4 \bullet MC \bullet T + b_5 \bullet T \bullet CO_2 + b_6 \bullet T \quad (1)$$

It was confirmed that the influencing factors were $MC \times CO_2^2$ and $T \times lnCO_2^2$ for ZEN production. The values of the coefficients from b_0 to b_2 and their statistical significance are shown in Table 1.

$$ZEN = b_0 + b_1 \bullet MC \bullet CO_2^2 + b_2 \bullet T \bullet \ln CO_2^2 \quad (2)$$

The strongest correlations were observed between the predicted and actual values of logDON ($R^2 = 0.990$) and ZEN ($R^2 = 0.983$) (Fig. 4) and the delivery line was delineated according to the maximum limit value of mycotoxins in China (DON is 1000 μg/kg and ZEN is 60 μg/kg). To reduce the false negative rate and improve the prediction accuracy, the early warning value of logDON was set to 3.16, with a validation accuracy rate of 98.15 % and a false positive rate of 1.85 %; the early warning value of ZEN was set to 59.99 and the validation accuracy was 90.74 % with a false positive rate of 9.26 %.

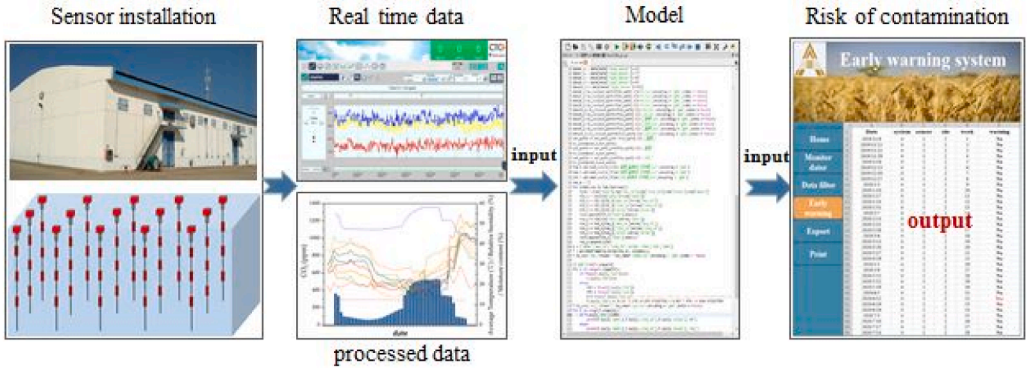

Fig. 5. Real-time monitoring and early warning system workflow.

3.5. Real-time monitoring probe verification

Multi-parameter integrated sensors are the key to obtaining accuracy of monitoring results in real life applications (Singh, 2017). Before the T/RH/MC/CO_2 multi-parameter integrated sensors were used in the horizontal warehouses for verification, the three indicators of stability, accuracy and response time of the probe were confirmed through comparative analysis with laboratory test data. The sensor operated continuously and stably during the one-year working period. Except for RH ($R^2 = 0.933$), the monitoring values and laboratory detection values were monitored for each sensor, for CO_2 ($R^2 = 0.968$), T ($R^2 = 0.956$), and MC ($R^2 = 0.965$); the determination coefficients were all greater than 0.95 (Fig. S2). After changing the wheat conditions near the sensor through manual intervention, the response time of the sensor to the changes of each parameter was < 60 min or within 2 monitoring cycles (monitoring data was recorded every 30 min).

3.6. Real-time monitoring system detection and verification in horizontal warehouses

The established toxin prediction models were embedded in the data processing system developed in the supporting system and combined with the T/RH/MC/CO_2 multi-parameter integrated sensors; a real-time monitoring and early warning system was formed (Fig. 5). The verification and application were initially conducted in a horizontal warehouse. After the one-year monitoring data was corrected according to the determination coefficient obtained from the sensor verification, it was substituted into the DON prediction model (Formula 1); the obtained logDON values were all less than 3.16, and the DON content of the predicted samples did not exceed the limit (1000 μg/kg). After substituting the wheat warehouse monitoring data into the ZEN prediction model, the ZEN values obtained were all less than 59.99, and the ZEN content of the predicted samples did not exceed the limit (60 μg/kg). During the one-year verification period, four sample collections were carried out, and 192 samples were obtained. The toxin test results confirmed that the wheat warehouse did not have DON and ZEN exceeding the standard throughout the monitoring period.

Conclusion

This study demonstrated that accurate prediction of the risk of contamination with DON and ZEN could be achieved by monitoring the T and MC of post-harvest wheat during storage, based on the CO_2 concentration that is produced. The real-time monitoring and early warning system developed using the integrated real-time monitoring sensor of T/RH/MC/CO_2 combined with the developed toxin prediction model can be used for safe storage of wheat. This minimises the risk of mycotoxin contamination and contributes to the interests of related food/feed supply chains and stakeholder decisions. Our findings provide a fundamental tool for establishing an early warning platform for DON and ZEN contamination in storage silos, improving the global food and feed safety while avoiding economic loss to the entire supply chain.

CRediT authorship contribution statement

Hua Cui: Conceptualization, Investigation, Validation, Formal analysis, Writing – original draft. **Songshan Wang:** Formal analysis, Writing – review & editing. **Xu Yang:** Data curation, Validation. **Wei Zhang:** Methodology, Formal analysis, Writing – review & editing. **Mengze Chen:** Methodology. **Yu Wu:** Validation. **Sen Li:** Data analysis. **Li Li:** Data analysis. **Di Cai:** Software, Visualization. **Baoyuan Guo:** Supervision, Writing – review & editing. **Jin Ye:** Conceptualization, Methodology, Writing – review & editing. **Songxue Wang:** Conceptualization, Methodology, Formal analysis, Resources, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100472.

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