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The remote monitoring of aflatoxin levels in grain corn relative to temperature and humidity

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Abstract

Aflatoxin contamination of grain-based animal feeds in silos presents a food security concern, since animals feeding on aflatoxin-contaminated feed may have reduced productivity. Aflatoxins are produced by several members of the genus *Aspergillus*. Malaysia's tropical climate provides suitable conditions for *Aspergillus* spp. proliferation and subsequent aflatoxin production. Therefore, throughout a two-week storage period, a remote monitoring system was used to record the temperature and humidity data inside downscaled corn silos under two conditions: indoor (control) and outdoor. The temperature and humidity recorded for outdoor storage were 25.3°C to 33.6°C and 65.4°C to 69.6%, respectively, while those for indoor storage were 25.1°C to 28.7°C and 64.9% to 65.7%, respectively. The average moisture content of grain corn before storage was significantly lower (2.86 ± 0.03%) than in outdoor (3.523 ± 0.012%) and indoor (3.908 ± 0.002%) silos. Aflatoxin levels before and after storage were below the detection level even under favourable conditions. However, mycological analyses showed higher fungal loads for *Fusarium* spp. and *Penicillium* spp. following the two-week storage. The present work demonstrated that different storage conditions could affect fungal loads in grain corn in downscale silos.

Keywords: Aflatoxins, *Aspergillus* spp., Grain corn, Remote monitoring, Downscale silo

1. Introduction

Global corn production is around a hundred million tonnes annually, which serves as a staple food and provides more than one-third of the calories and protein for populations in certain countries [1]. Corn (*Zea mays* L.) is typically divided into sweet corn for human food, and grain corn for animal feed. In Malaysia, grain corn is imported from Argentina, Brazil, China, and Indonesia [2]. As a practice, grain corn is usually stored in silos under warm and humid conditions upon arrival and prior to further processing at feed mills. Fungal spoilage and mycotoxin contamination of grain corn in silos are major concerns in the agricultural industry, especially in tropical regions. Mycotoxins are often associated with health problems in livestock, which could reduce the economic efficiency of the livestock industry [3]. Among the various mycotoxins, aflatoxins are the most economically relevant and have been classified as carcinogenic (Group 1) by the International Agency for Research on Cancer [4]. Aflatoxins are produced by several members of the genus *Aspergillus*, notably the *Aspergillus* section *Flavi* (*A. flavus*, *A. parasiticus*) growing on various economically relevant crop commodities, including corn [5]. Aflatoxins are also mutagenic, teratogenic, and immunosuppressive, and they are produced at a wide range of conditions, such as at humidity levels above 17.5% and temperature levels above 24°C [6].

Although fungal spoilage and the subsequent mycotoxin contamination of animal feed materials have been widely reported [7,8], fewer studies have been focused on silo contamination. Corn grains stored in silos are highly susceptible to aflatoxin contamination, generally brought about by inadequate drying and storage under damp conditions [9]. However, it is very costly for a tropical country like Malaysia to maintain low temperatures and humidity during storage, with temperatures ranging from 28°C to 31°C and humidity of 70-80% during the wet season, and 50-60% during the dry season [10]. In Malaysia thus far, limited focus on silo contamination is rather perplexing considering that (i) the Malaysian tropical climate (also that of the neighbouring regions) is highly favourable for fungal contamination and the subsequent mycotoxin contamination; (ii) agriculture, including the livestock industry, is the third engine that drives and sustains Malaysian economic growth, after services and manufacturing [11]; and (iii) Malaysian regulatory limits for mycotoxins in foods do exist. To date, however, no mycotoxin regulation has been prescribed for animal feeds in Malaysia [12].

Muga et al. [13] stored grain corn inoculated with *A. flavus* spores in a climatic test chamber for ten days in which the temperature and relative humidity were regulated. They reported that aflatoxin contamination was higher at temperatures of 20°C and 30°C and a relative humidity of 90% (3.9-11,179.7 µg/kg. Most researchers collected grain corn during storage in facilities and determined the aflatoxin contamination [7,14,15]. For example, a study assessing 464 corn silage samples collected from 58 silos in Brazil revealed aflatoxin contamination of up to 100 µg/kg [16], while the EU's prescribed limit for aflatoxin in animal feed is only 20 µg/kg [17]. However, they did not monitor changes in temperature and humidity of the silo throughout storage of grain corn, or their impacts on aflatoxin contamination. Currently, as far as we are aware, there is a lack of published reports on the risk or the presence of aflatoxins in animal feed materials stored in silos in Malaysia, despite the health hazards aflatoxins pose to humans' and animals' health. Therefore, the present work was aimed at quantifying the levels of aflatoxin contamination in grain corn stored in downscale silos and establishing the relationship between temperature and humidity profiles using a remote monitoring system inside the silos to the aflatoxin levels.

2. Materials and methods

2.1 Development of a downscale silo equipped with a temperature and humidity monitoring system

Due to limited access to grain corn stored in industrial silos, experiments were instead conducted in downscale silos. A tin-coated steel container with a 20-liter capacity (Figure 1) was used as the downscale silo. Steel was used in the construction of many industrial silos due to its durability in storing large amounts of grain. Therefore, it was assumed that the tin-coated steel containers were suitable for simulating conditions in an industrial silo.

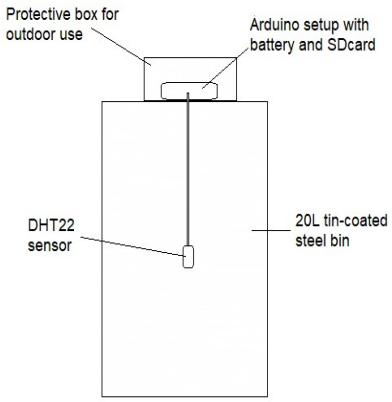

Figure 1 Schematic diagram of a downscale silo outfitted with a temperature and humidity monitoring system.

A system for humidity and temperature monitoring was developed and installed to allow for frequent measurements with minimal disturbance to the environment inside the downscale silos during experiments. The Arduino Uno Rev 3 (Arduino, Italy) was used as the microcontroller board for sensor connectivity. The system can be powered by connecting it to a computer, either via a universal serial bus (USB) cable, an adapter, or a battery. The sensor used was the DHT22 (Aosong Electronics Co. Ltd., Guangzhou, China) sensor module breakout, which simultaneously senses temperature and humidity by using a thermistor and a capacitive humidity sensor, respectively. This sensor generates data in digital signals, with an operating range of -40 to 80 ± 0.5°C and 0 to 100 ± 2%, respectively.

An SD card shield V4.0 was used to provide the system with storage capability to store the data collected by the DHT22 sensor module breakout since the Arduino Uno has limited storage space. This SD card shield can be stacked directly above the Arduino Uno as the pins of the SD card shield have the same form-factor as the Arduino Uno pins. Hence, this allowed the sensor to be connected to the SD card shield pins.

The DHT22 sensor was programmed to collect temperature and humidity data every 10 min, and the data was stored on the SD card. The cost for the entire monitoring system was approximately USD 36.

2.2 Experimental design

A total of four bags (20 kg each) of grain corn (imported from Argentina) were purchased from an agricultural equipment and supplies company. Prior to the silo experiment, 250 g of grain corn was sampled from each of the four bags, thoroughly mixed, placed in a zip-lock plastic bag, and stored in a cold room at 4°C. Subsequently, these samples were analysed for moisture content, fungal load, and aflatoxin contamination.

Four downscale silos were filled with 14.4 kg of grain corn. The bulk density of grain corn was 720 kg/m. The silos were placed under two conditions: outdoors on a rooftop (Silo O1 and Silo O2) and exposed to the ambient local temperature to replicate the conditions of a typical industrial silo; and indoors (Silo I3 and Silo I4), where the room temperature was maintained at 26°C throughout the experiment and served as controls. The humidity and temperature inside all silos were measured and recorded at an interval of 10 min using the remote monitoring system.

2.3 Experimental data collection

The downscale silo storage period lasted for two weeks (March 2nd-16th, 2018), following a storage duration at a Malaysian feed mill. This duration would have been longer if demand for grain corn animal feed was low. At the mill, the grain corn is stored in silos with no environmental (relative humidity or temperature) control. Due to battery capacity limitations, the data collection was done for a full 48 h in three separate sets: days 2-3, 6-7, and 10-11.

Grain corn was sampled (1 kg) before and after storage according to Regulation 401/2006 (18). The incremental samples collected from the three depths (top, middle, and bottom) of the silos were composited to form the aggregate samples of 1 kg. Next, 1 kg of aggregate samples were packed in zip-lock plastic bags, labelled, and stored in a cold room at 4°C prior to analysis.

In total, 24 samples were collected and analysed [4 treatments (2 outdoor silos + 2 indoor silos) × 2 timepoints (before storage + after storage) × 3 replicates].

2.4 Moisture content analysis

The moisture content of the grain corn was determined using the hot air oven method [18] at 105°C to a constant weight.

2.5 Mycological analysis for fungal detection

2.5.1 Preparation of culture media

The Potato Dextrose Agar (PDA) and Dichloran Rose Bengal Chloramphenicol Agar (DRBC) were purchased from Oxoid (UK), while *A. flavus* and *A. parasiticus* Agar (AFPA) were purchased from Sigma-Aldrich (USA). The fungal growth media were prepared following the manufacturer's instructions. Briefly, PDA (39 g), DRBC (31.5 g) and AFPA (45.5 g) powder were separately mixed into 1 L of distilled water and autoclaved at 121°C for 15 min. Chloramphenicol was added (0.001 g/L^{-1}) into all three media prior to autoclaving since it is heat stable. For the screening of mycotoxigenic fungi, Coconut Cream Agar (CCA) was prepared according to Dyer et al. [19], using 50% coconut cream extract (Kara brand purchased from a local supermarket in Selangor) and 1.5% agar, and autoclaved at 121°C for 15 min. After cooling for 30 min, sterilised growth media was poured into Petri plates (90 mm Ø) and allowed to solidify. Subsequently, they were kept in airtight polyethylene bags and refrigerated at 4°C prior to use.

2.5.2 Mycological analysis

Fungal isolation was performed on PDA by a direct-plating technique [20]. The grain corn was taken from each silo storage, surface-sterilised with 5% (v/v) sodium hypochlorite (NaOCl) for 5 min, rinsed twice with sterile distilled water, and blotted dry on sterile filter paper. Aseptically, the kernels were directly plated onto PDA plates at an equal distance (five kernels per plate). Three replicates of each silo storage were prepared.

Inoculated plates were incubated at 30°C in the dark for 7 d. Following incubation, all fungal colonies present were enumerated, and the isolation frequency (% IF) of each fungal colony was determined using Equation 1. Each fungal colony was sub-cultured by taking an agar plug of the isolated fungi and inoculating it onto fresh PDA prior to incubation at 30°C to obtain axenic cultures for fungal identification.

$$\% \text{ IF} = \frac{\text{total number of corn kernel colonised by a genus}}{\text{total number of corn kernels per plate}} \times 100 \quad (1)$$

A dilution-plating technique was performed to determine the fungal population. Briefly, 10 g of grain corn was aseptically weighed in a sterile stomacher bag, and 90 mL of sterilised 0.1% peptone water was added. The grain corn was immersed in peptone water for 1 h before being homogenised by a stomacher for 2 min. The initial homogenization of grain corn yielded a 10^{-1} dilution. Subsequently, a set of 10^{-2}, 10^{-3}, and 10^{-4} dilutions were made by three serial 10-fold dilutions, also with sterilised 0.1% peptone water. Following this, 100 µL aliquots of the four dilutions were separately and evenly spread onto AFPA and dye-binding capacity (DBC) in triplicates using a sterile bent glass rod, and incubated at 30°C for 48 h [21]. Following incubation, fungal colonies were counted using a colony counter. Plates with 10-100 colonies were chosen to determine the fungal populations as the number of colonies forming units per gramme (CFU/g^{-1}) using Equation 2. The results were expressed as log CFU/g by converting the average of CFU/g from triplicate into log. In total, 288 plates for each AFPA and DRBC were incubated [24 samples × 4 dilutions × 3 replicates].

$$\text{CFU} = \frac{\text{number of colonies appeared} \times \text{dilution factor}}{\text{volume of aliquot}} \quad (2)$$

2.5.3 Qualitative mycotoxigenicity screening of fungal isolates

A qualitative mycotoxigenicity screening of fungal isolates was performed according to Thathana et al. [22] with slight modifications. Using a sterile loop, the spores of mycotoxigenic fungi from axenic cultures were transferred into 1,000 µL of sterile distilled water in microcentrifuge tubes and thoroughly mixed. Next, 100 µL of the spore suspension was aseptically inoculated at the centre of CCA plates. Inoculated plates were incubated at 30°C for 7 days in the dark, following which, the plates were observed for fluorescence (positive for mycotoxins) under UV light in the laminar flow hood.

2.6 Aflatoxin analysis

2.6.1 Chemicals and reagents

All solvents used in the present work were of High-performance liquid chromatography (HPLC) grade and purchased from Merck (Darmstadt, Germany). Mixed aflatoxin standards, i.e., AFB_2 and AFG_2 (at 300 ng/mL^{-1} concentration) as well as AFB_1 and AFG_1 (at 1,000 ng/mL concentration), were purchased from Supelco (Bellefonte, PA, USA) and kept at -20°C prior to usage. AflaTest WB SR (Super Recovery) immunoaffinity chromatography (IAC) columns were purchased from VICAM (Watertown, MA, USA).

2.6.2 Extraction

Association of Official Analytical Collaboration (AOAC) method 991.31 [23] was utilised with slight modifications for the determination of aflatoxins. Firstly, the samples were milled using a Waring blender (Waring, Torrington, CT., USA). Subsequently, 100 mL of methanol:water (80:20, v/v) was added to 50 g of samples and 5 g of sodium chloride (NaCl; Merck), mixed for 1 min at high speed, and filtered through fluted filter paper. Then, 10 mL of the resulting extract was blended with 40 mL of distilled water, followed by another filtering with a glass microfiber filter. Next, 10 mL of filtrate was passed through the AflaTest IAC at a flow rate of 1 to 2 drops sec^{-1}. Subsequently, the column was further washed twice with 10 mL of distilled water at a similar speed. Next, the aflatoxins were eluted by passing 1 mL of methanol through the IAC, and the eluate was collected and diluted with 1 mL of purified water prior to injection into the HLPC system.

2.6.3 HPLC analysis

To determine aflatoxins in grain corn samples, the method described by Arzandeh and Jinap [24] was used with slight modifications. The HPLC system was equipped with a fluorescence detector (Waters 2475, NY) for which the excitation and emission wavelengths were set at 365 nm and 435 nm, respectively, a symmetry XBridge C_{18} column (25 cm length × 4.6 mm width and 5 µm particle size), and a post-column photochemical reactor for enhanced detection (PHRED; Aura Industries, NY). The mobile phase used was

water:methanol:acetonitrile (55:35:10, v/v/v) at a flow rate of 0.6 mL/min^{-1} in isocratic mode. Methanol, acetonitrile, and purified water were filtered using a nylon membrane filter (0.45 µm; Merck) and degassed using a microprocess-controlled bench-top ultrasonic cleaner (Powersonic 420; Hashin Technology, Seoul). The injection volume was 20 µL. The aflatoxin determination was done in triplicates.

To obtain the actual aflatoxin concentration from the HPLC data, standard calibration curves were constructed at six concentrations of 0.5, 1, 2.5, 5, 10, and 20 µg/kg for AFB_1 and AFG_1, and 0.2, 0.3, 0.8, 1.6, 3.2, and 6.4 µg/kg for AFB_2 and AFG_2. The injection into the HPLC system was as previously described. The calibration curves yielded $R^2 > 0.999$, and the limits of detection for AFB_1, AFB_2, AFG_1 and AFG_2 were 0.10 µg/kg, 0.07 µg /kg, 0.19 µg/kg, and 0.62 µg/kg, respectively. In addition, the recoveries in corn samples were in the range of 70-110%, and the precision, as indicated by RSD values, was in the range of 0.4% to 5.7%.

2.7 Statistical analysis

The moisture contents and fungal loads were measured in triplicate and the results were expressed as means ± standard error (SE). Results obtained at the end of experiments for both the outdoor and indoor silos were compared with those of the control using a paired-sample *t*-test by Minitab (v. 16; Minitab, State College, PA). Differences were considered significant at $p \leq 0.05$.

3. Results and discussion

3.1 Temperature and humidity profiles of silos during storage

Figures 2 and 3, respectively, illustrate the mean temperature and humidity profiles of the downscale silos. Larger fluctuations in temperature and humidity were observed in the outdoor silos as compared to those of the indoor silos, which were more stable throughout the day. Generally, the temperature and humidity of the indoor silos (25.1-28.7°C, mean 27.1°C; 64.9-65.7%, mean 65.4%) were slightly lower than those of the outdoor silos (25.3-33.6°C, mean 29.0°C; 65.4-69.6%, mean 67.7%). When compared with the temperature (21-37°C) and humidity (77%) data obtained from a weather station nearby to where the downscale silo experiment was performed [25], it was found that the temperature range of the downscale silo experiment fell within the weather station range, but not for the humidity, where the downscale silo experiment yielded lower readings. This could be due to the fact that the air surrounding the weather station was more humid due to ponds, tall trees, and greenery. The outdoor silos were less humid as the space was enclosed throughout the experiment. In a study by Muga, et al. [13], the grain samples were placed in a controlled environment and artificially inoculated to assess fungal growth and mycotoxin levels. In the present study, the aim was to imitate actual practises of grain corn storage in industrial silos by using downscale silos and to evaluate the risk of natural aflatoxin contamination. Therefore, the silo conditions were not controlled but left to the effect of the actual environmental conditions of Malaysian tropical weather.

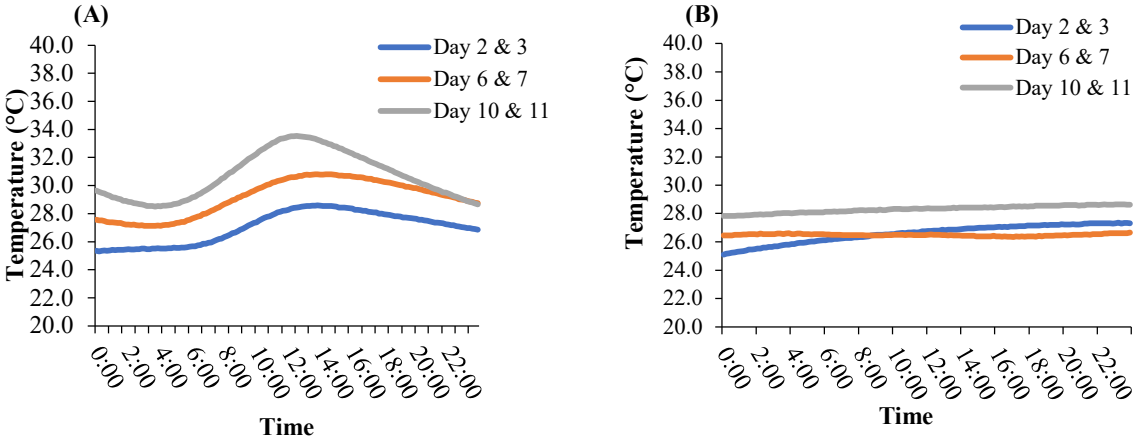

Figure 2 Mean temperature profiles for (A) the outdoor silo and (B) the indoor silo.

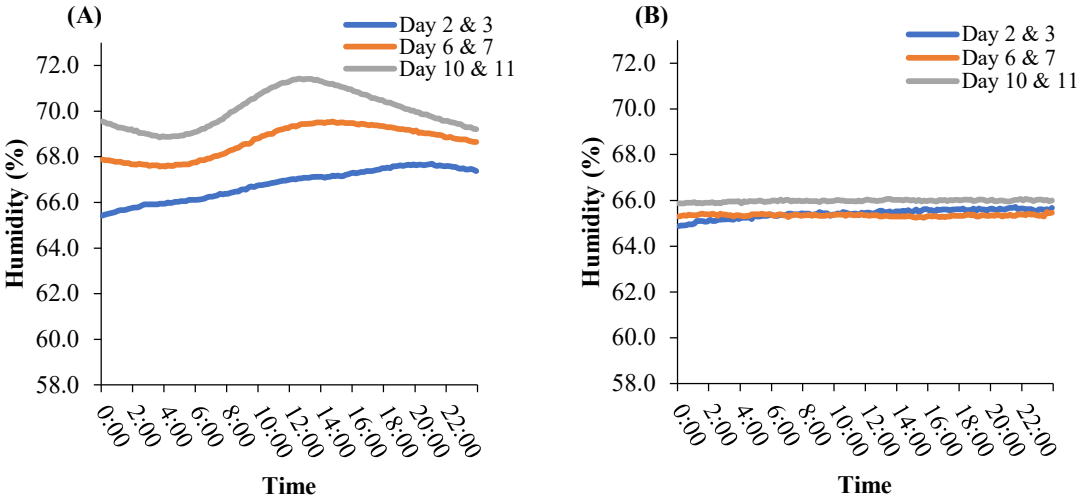

Figure 3 Mean humidity profiles for (A) an outdoor silo and (B) an indoor silo.

3.2 Moisture content of grain corn pre- and post-storage

The mean moisture content of grain corn pre-storage was significantly lower (2.86 ± 0.03%) than post-storage for both outdoor (3.523 ± 0.012%) and indoor (3.908 ± 0.002%) silos. The increase in grain moisture content of around 1% in the downscale silo during the two-week storage period is consistent with Angelovič et al. [26], who monitored the moisture content of grain corn and reported a difference of 1.1% throughout a one-year storage period. Grain corn hygroscopicity would contribute to the increase in grain corn moisture content observed in the present work, especially when the storage was in a tropical climate with mean ambient humidity of 65.4% and 67.7%.

The moisture content of grain corn is one of the main factors that regulates aflatoxin contamination; when grain has more moisture, the temperature will increase, and fungal growth will be promoted, which subsequently will lead to aflatoxin development [27]. However, the increase in grain corn moisture content post-storage observed in the present work was still lower than 12%, which is the value for grain corn storage stability [26]. In the present work, a gradual increase (≈1%) in the moisture content of grain corn was observed after 14 days at a mean ambient humidity of 67.7% in the outdoor silos. This result is in line with that of Volenik et al. [28], who reported an increase of only 1.7% in grain moisture content at 20°C and 93% humidity after a 34-day storage period. Although high humidity positively affects fungal load and aflatoxin production, our results suggest that the ambient humidity in the present work, which ranged from 65.4 to 69.6%, had minimal impact on increasing the moisture content of the grain corn in the silos.

3.3 Mycological analyses on grain corn pre- and post-storage

Figure 4 depicts the isolation frequency (% IF) of fungi isolated from grain corn from pre-storage, indoor silo, and outdoor silo on PDA following a 7-days incubation at 30°C. Initially (pre-storage), *Aspergillus* spp. (15%), *Fusarium* spp. (30%), and *Penicillium* spp. (20%) were present in the grain corn samples. At post-storage, *Aspergillus* spp. were found to diminish in both indoor and outdoor silos, while *Fusarium* spp. yielded 28% and 33% IF from indoor and outdoor silos, respectively, and *Penicillium* spp. yielded 35% and 38% IF from indoor and outdoor silos, respectively. The present work isolated common field fungi such as *Aspergillus*, *Penicillium*, and *Fusarium*, and this is consistent with other researchers who have also isolated these fungal species in grain corn [29]. This could be due to the fact that feed ingredients such as grain corn, which have a high content of carbohydrates, serve as a good medium for fungal proliferation, thus increasing the feeds likelihood of fungal contamination [30]. In addition, the absence of *Aspergillus* spp. from both indoor and outdoor silo samples was also confirmed by negative results on AFPA (no growth; AFPA is a selective growth medium for *Aspergillus* spp. Absence of growth on this medium indicates the absence of *Aspergillus* spp. from the samples). Competitive interactions between fungal community structures (biotic factors) in a host are a plausible reason for the absence of *Aspergillus* spp. from the silo samples. Temperature and water availability (abiotic factors) also influence fungal growth in a host [31]. As per Chatterjee et al. [32], they opined that fungal competitive fitness of *Fusarium* spp. might have hindered *Aspergillus* spp. growth, as evidenced in the present work. Fungal interaction is quite complex, with temperature and moisture affecting the interaction differently, resulting in different fungal dominances over others [33].

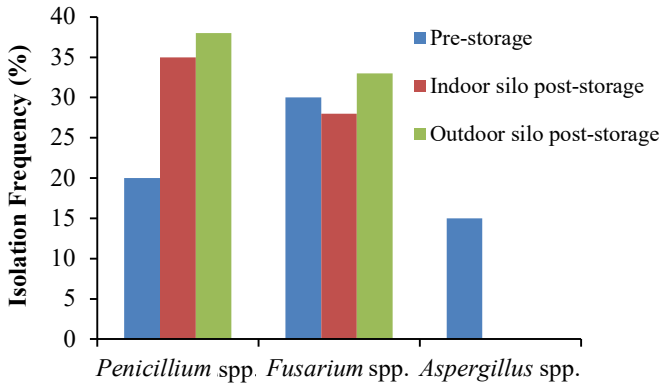

Figure 4 Isolation frequency (% IF) of fungi isolated from grain corn on PDA following a 7-d incubation at 30°C for the downscale silos at different storage conditions.

Figure 5 depicts the total fungal loads (log CFU/g) of fungi isolated from pre-storage, indoor silo, and outdoor silo grain corn on DRBC following a 2-d incubation at 30°C. Initially (pre-storage), *Fusarium* spp. and *Penicillium* spp. loads were log 3.56 CFU/g and log 4.30 CFU/g, respectively. At post-storage, *Fusarium* spp. fungal loads were log 3.65 CFU/g and log 4.17 CFU g from indoor and outdoor silos, respectively; while *Penicillium* spp. fungal loads were log 4.39 CFU/g and log 4.66 CFU/g from indoor and outdoor silos, respectively. Outdoor silo samples were found to yield higher fungal loads as compared to those of indoor silo samples for both *Fusarium* spp. and *Penicillium* spp. Higher moisture content as well as larger fluctuations in temperature and humidity that were observed in the outdoor silos as compared to those of the indoor could have contributed to the higher fungal loads for both *Fusarium* spp. and *Penicillium* spp. Researchers associated the deterioration of corn with high fungal loads with moisture content, temperature, and relative humidity besides insect pests [34]. Astoreca et al. [35] also observed similar data where they reported grain corn as the most contaminated ingredient in feed (i.e., fungal load ranged from log 4.4 CFU/g to log 4.8 CFU/g). Furthermore, the FAO and the UN World Food Program have guidelines that indicate a maximum of log 5 CFU/g in dry feed, although in the US these levels are considered safe [36]. However, the highest loads of *Fusarium* spp. and *Penicillium* spp. reported in this study were lower than the maximum guidelines.

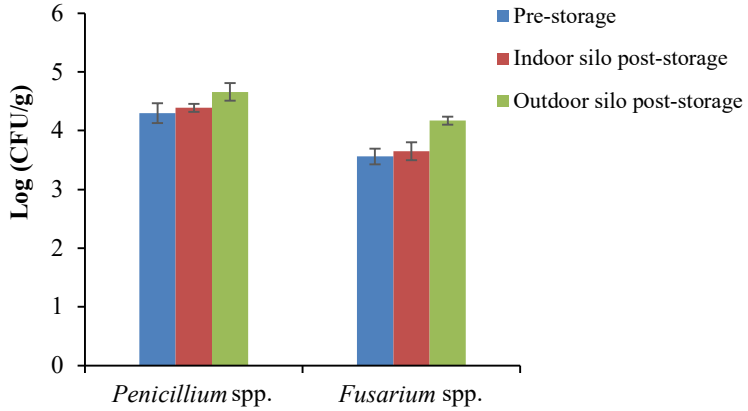

Figure 5 Total fungal population (log CFU/g) isolated from grain corn plated on DRBC following a 2-d incubation at 30°C for the downscale silos at different storage conditions. The data are the means of triplicate (n = 3), with the bars indicating the standard error (± SE).

In the present study, fungal mycotoxigenicity screening using CCA revealed no fluorescence on the reverse sides when exposed to UV light, thus qualitatively indicating no mycotoxin was produced. Nevertheless, an HPLC-FLD analysis was performed to conclusively confirm the absence of aflatoxins.

3.4 Aflatoxin contamination of grain corn pre- and post-storage

Following mycological analyses, a high performance liquid chromatography with fluorescence detection (HPLC-FLD) analysis was conducted on grain corn samples pre- and post-storage. It was found that the aflatoxin levels were absent or present below the detection limit. This corresponded well with the absence of

Aspergillus spp. in the silo samples, and that CCA also yielded negative results (absence of mycotoxins). Based on visual and morphological observation, the *Aspergillus* spp. initially present (pre-storage) could be *Aspergillus* section *Nigri* (*A. carbonarius*, *A. niger*; producers of ochratoxin A; black-brown conidiospores) instead of *Aspergillus* section *Flavi* (*A. flavus*, *A. parasiticus*; producers of aflatoxins; yellow-green conidiospores). Thus far, there is no publicly available report on the aflatoxin level in industrial grain corn silos for animal feed in Malaysia to compare with.

Ambient temperatures of over 24°C and relative humidity of over 17.5% are optimum for the growth of *Aspergillus* spp. [6]. Therefore, it was hypothesised that the aflatoxin levels would be higher in outdoor silos due to favourable humidity and temperature conditions for fungal growth. However, our results showed that the level of aflatoxin pre- and post-storage for all silos was below detection limits.

Elsewhere, Muga et al. [13] reported that aflatoxin B_1 (11,179.7 µg/kg) was detected in corn samples artificially inoculated with *A. flavus* and stored at a constant temperature of 30 °C and humidity of 90% for ten days. However, that study was conducted in a controlled environment, whereas the present work was conducted naturally under the fluctuating Malaysian weather. Grain moisture contents varied between 14% and 20% in the current study, with the minimum 14% moisture content resulting in a the value of detected AFB_1 as low as 0.8 µg/kg at temperatures and humidity of 20°C and 60%, respectively, and as high as 4,999.0 µg/kg at temperatures and humidity of 30°C and 90%, respectively [13]. In contrast, in the present work, for the comparable range of temperature of downscale silos, which was from 24.0 to 32.0°C, and the range of humidity of outdoor silos, which was from 64 to 70%, the aflatoxin level in the silos was below the detection level. Differences in the results between the two studies are speculated to arise from the following: First, in contrast to the present study, the grains were artificially inoculated with *A. flavus* in Muga's. Therefore, future work should consider using artificially inoculated grains, and the results should be compared with those of the present study. Second, dynamic storage conditions were observed in the downscale silos in the present study, where the daily storage temperature and humidity naturally fluctuated throughout the day in comparison with the constant temperature and humidity in Muga's to provide for an optimum environment. Third, the grain moisture content in the present study was four times lower than that of Muga's. Gardisser et al [37] stated that fungal growth is greatly reduced at moisture content levels of 15% or less. In the present study, the moisture content of grain corn post-storage was approximately 4%, which could have restricted fungal growth in the assessed silos.

Fungal identification through morphological and/or molecular methods is also warranted to conclusively identify the actual fungal community structure. Multi-mycotoxin analysis through HPLC is also suggested because although only aflatoxins have been proven carcinogenic towards humans and animals, the presence of other mycotoxins in animal feeds has also been shown to contribute to various clinical symptoms in animals (e.g., ruminants, poultry) such as feed refusal, abortion, and immune suppression. The present work aimed to quantify the levels of aflatoxin contamination in grain corn stored in downscale silos and establish the relationship between temperature and humidity profiles using a remote monitoring system inside the silos to the aflatoxin levels. Although the present study revealed that the risk of *Aspergillus* spp. contamination and aflatoxin production in downscale silos was low and below the detection limit, the presence of other fungal contaminants such as *Fusarium* spp. and *Penicillium* spp. warrants further investigation as these three are well-known mycotoxigenic genera, and their presence in a crop commodity/sample/matrix could indicate the production of mycotoxins other than aflatoxins such as fumonisins, ochratoxins, and trichothecenes.

4. Conclusion

In the present study, the dynamics of temperature and relative humidity profiles were remotely monitored and recorded in the downscale silo. The grain corn, which was stored in the downscale silos and subjected to dynamic storage temperatures of 25.1°C to 33.6°C and storage humidity of 64.9% to 69.6%, was shown to have levels of aflatoxins below the detection limits. It was further observed that the moisture content of the grain corn did not exceed 4%. Isolation frequencies and fungal loads showed dynamic fungal contamination. The present work also demonstrated that under dynamic ambient temperature and humidity in a downscale silo storage condition and in uninoculated grain corn, where the moisture content of grain corn did not exceed 4%, the risk of aflatoxin contamination was low. Although the aflatoxin levels were below detection limits, more studies are needed to understand the interactions between the fungal communities under silo storage conditions so that the risk of aflatoxin contamination can be fully assessed.

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6. References

[1] Chulze SN. Strategies to reduce mycotoxin levels in maize during storage: a review. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2010;27(5):651-657.

[2] Warr S, Rodriguez G, Penm J. Changing food consumption and imports in Malaysia: opportunities for Australian agricultural exports. 1st ed. Canberra: Australia; 2008.

[3] Salay E, Zerlotti Mercadante A. Mycotoxins in Brazilian corn for animal feed: Occurrence and incentives for the private sector to control the level of contamination. Food Control. 2002;13(2):87-92.

[4] IARC. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. 56th ed. Lyon: IARC Publication; 1993.

[5] Dolezal AL, Shu X, Obrian GR, Nielsen DM, Woloshuk CP, Boston RS, et al. *Aspergillus flavus* infection induces transcriptional and physical changes in developing maize kernels. Front Microbiol. 2014;5:384.

[6] Trenk HL, Hartman PA. Effects of moisture content and temperature on aflatoxin production in corn. Appl Microbiol. 1970;19(5):781-784.

[7] Nasaruddin N, Jinap S, Samsudin NIP, Kamarulzaman NH, Sanny M. Prevalence of mycotoxigenic fungi and assessment of aflatoxin contamination: a multiple case study along the integrated corn-based poultry feed supply chain in Malaysia. J Sci Food Agric. 2021;101(5):1812-1821.

[8] Yazid SNE, Ng WJ, Selamat J, Ismail SI, Samsudin NIP. Diversity and toxigenicity of mycobiota in grain corn: a case study at pioneer grain corn plantations in Terengganu, Malaysia. Agric. 2021;11(3):1-22.

[9] Wilson DM, Payne GA. Factors affecting *Aspergillus flavus* group infection and aflatoxin contamination of crops. In: Eaton D, Groopman J, editors. The toxicology of aflatoxins. 1st ed. San Diego: Academic Press; 1993, p. 309-325.

[10] Sulaiman MR, Chye FY, Hamid A, Yatim AM. The occurrence of aflatoxins in raw shelled peanut samples from three districts of Perak, Malaysia. Elec J Env Agricult Food Chem. 2007;6(5):2045-2052.

[11] Yazid SNE, Jinap S, Ismail SI, Magan N, Samsudin NIP. Phytopathogenic organisms and mycotoxigenic fungi: why do we control one and neglect the other? A biological control perspective in Malaysia. Compr Rev Food Sci Food Saf. 2020;19(2):643-669.

[12] Hejri AL, Jinap S, Son R. Occurrence of aflatoxins and aflatoxigenic *Aspergillus* in peanuts. Food Agric Environ. 2013;11(3&4):228-234.

[13] Muga FC, Marenya MO, Workneh TS. Effect of temperature, relative humidity and moisture on aflatoxin contamination of stored maize kernels. Bulgarian J Agric Sci. 2019;25(2):271-277.

[14] Mulyati A, Sutanto H, Warnasih S, Herlina E. Determination of the aflatoxin levels in corn (*Zea mays* L.) during storage process. J Phys Conf Ser. 2021;1882(1):012115.

[15] Liu Z, Gao J, Yu JH. Aflatoxins in stored maize and rice grains in Liaoning Province, China. J Stored Prod Res. 2006;42(4):468-479.

[16] Keller LAM, Gonzalez Pereyra ML, Keller KM, Alonso VA, Oliveira AA, Almeida TX, et al. Fungal and mycotoxins contamination in corn silage: monitoring risk before and after fermentation. Stored Prod Res. 2013;52:42-47.

[17] European Commission. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed- Council statement, https://eur-lex.europa.eu/eli/dir/2002/32/oj [accessed 1 November 2021]

[18] AOAC. Official method of analysis of the association of analytical chemists. 18th ed. Washington DC: AOAC; 2005.

[19] Dyer SK, McCammon S. Detection of toxigenic isolates of *Aspergillus flavus* and related species on coconut cream agar. J Appl Bacteriol. 1994;76(1):75-78.

[20] Pitt JI, Hocking AD. Fungi and food spoilage. 3rd ed. New York: Springer; 2009.

[21] Pitt JI, Hocking AD, Glenn DR. An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus*. J Appl Bacteriol. 1983;54(1):109-114.

[22] Thathana MG, Murage H, Abia ALK, Pillay M. Morphological characterization and determination of aflatoxin-production potentials of *Aspergillus flavus* isolated from maize and soil in Kenya. Agric. 2017;7(10):80.

[23] Latimer GW. Official methods of analysis of AOAC international. 21st ed. Washington: Association of Official Analytical Chemists; 2019.

[24] Arzandeh S, Jinap S. Effect of initial aflatoxin concentration, heating time and roasting temperature on aflatoxin reduction in contaminated peanuts and process optimisation using response surface modelling. Int J Food Sci Technol. 2011;46:485-491.

[25] Weather2. Local weather forecast: unit golf Universiti Putra Malaysia 2020, https://www.myweather2.com/City-Town/Malaysia/Unit-Golf-Universiti-Putra-Malaysia/climate-profile.aspx?month=3 [accessed 1 November 2021].
[26] Angelovic M, Kristof K, Jobbagy J, Findura P, Krizan M. The effect of conditions and storage time on course of moisture and temperature of maize grains. BIO Web Conf. 2018;10:1-6.
[27] Brewbaker JL. Corn production in the tropics: the Hawaii experience, https://www.ctahr.hawaii.edu/oc/freepubs/pdf/corn2003.pdf [accessed 1 November 2021]
[28] Volenik M, Rozman V, Kalinovic I, Liska A, Kis D, Simic B. Influence of relative humidity and temperature on the changes in grain moisture in stored soybean and maize. Agric Conspec Sci. 2007;72(3):215-219.
[29] Ekwomadu TI, Gopane RE, Mwanza M. Occurrence of filamentous fungi in maize destined for human consumption in South Africa. Food Sci Nutr. 2018;6(4):884-890.
[30] Sugiharto S. A review of filamentous fungi in broiler production. Ann Agric Sci. 2019;64(1):1-8.
[31] Picot A, Marcolla HD, Barreau C, Gadais PL, Caron D, Forget RF, et al. Interactions between *Fusarium verticillioides* and *Fusarium graminearum* in maize ears and consequences for fungal development and mycotoxin accumulation. Plant Pathol. 2012;61(1):140-151.
[32] Chatterjee S, Kuang Y, Splivallo R, Chatterjee P, Karlovsky P. Interactions among filamentous fungi *Aspergillus niger*, *Fusarium verticillioides* and *Clonostachys rosea*: fungal biomass, diversity of secreted metabolites and fumonisin production. BMC Microbiol. 2016;16(1):1-13.
[33] Leggieri CM, Giorni P, Pietri A, Battilani P. *Aspergillus flavus* and *Fusarium verticillioides* interaction: modeling the impact on mycotoxin production. Front Microbiol. 2019;10:2653.
[34] Suleiman RA, Rosentrater KA, Bern CJ. Effects of deterioration parameters on storage of maize. The 2013 ASABE Annual International Meeting; 2013 Jul 21-24; Missouri, United States. Missouri: ASABE; 2013, p. 1-34.
[35] Astoreca AL, Dalcero AM, Pinto VF, Vaamonde G. A survey on distribution and toxigenicity of *Aspergillus* section *Flavi* in poultry feeds. Int J Food Microbiol. 2011;146(1):38-43.
[36] Adams RS, Kephart KB, Ishler VA, Hutchinson L, Roth GW. Mold and mycotoxin problems in livestock feeding, https://extensionpsu.edu/mold-and-mycotoxin-problems-in-livestock-feeding [accessed 1 November 2021].
[37] Gardisser D, Huitink G, Cartwright R. Grain storage and aflatoxin in corn. In: Espinoza L, Ross J, editors. Corn production handbook. 1st ed. Arkansas: University of Arkansas; 2011, p. 79-85.