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Morphological Characterization and Determination of Aflatoxigenic and Non-Aflatoxigenic *Aspergillus flavus* Isolated from Sweet Corn Kernels and Soil in Malaysia

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Abstract: This study aimed to morphologically characterize and determine the aflatoxigenic and non-aflatoxigenic *Aspergillus flavus* isolates. Forty isolates of *A. flavus* were obtained from sweet corn kernels and soil samples collected from Kampong Raja, Rose Valley, Kea, and Klebang farms in Malaysia. They were cultured on potato dextrose agar (PDA), dichloran rose-bengal chloramphenicol (DRBC), *Aspergillus flavus* and *Aspergillus parasiticus* agar (AFPA), and coconut cream agar (CCA). Macromorphological characteristics were determined by observing the colony color and texture, while the micromorphological characteristics were determined by examining the spore color, size, structure, conidiophore structure, and vesicle shape. The production of aflatoxin was determined by direct visualization of the UV fluorescence of *A. flavus* colonies on CCA. Aflatoxin was qualitatively detected in 18 (45%) isolates of *A. flavus* using UV fluorescence screening while the remaining 22 (55%) isolates did not exhibit any aflatoxin production. The highest incidence of *A. flavus* (30%) and aflatoxin production (15%) was recorded in samples from Kampong Raja. On the other hand, isolates from Rose Valley (17%) and Kea (12%) were non-aflatoxigenic. Klebang recorded a 25% incidence of *A. flavus* in which 15% were aflatoxigenic while 10% were non-aflatoxigenic. The occurrence of aflatoxin-producing *A. flavus* emphasizes the need for the measure to eradicate their presence in food crops. A biological control treatment utilizing the non-aflatoxigenic strains to compete with the aflatoxigenic ones is underway. Validation of aflatoxin production through high performance liquid chromatography is also ongoing.

Keywords: aflatoxin; *Aspergillus flavus*; morphological characterization; UV fluorescence

1. Introduction

Living in the present time is dangerous, not simply because of accidents or ecological threats, but also because of the foodstuff that we consume in our routine life. Most of the foods we consume are contaminated by microorganisms as well as their toxins. The intake of food materials affected by toxigenic fungi causes the induction of mycotoxicosis (toxic syndrome). Mycotoxicosis is a disease

caused by the ingestion of mycotoxins produced by toxigenic fungi on specific foodstuffs. Mycotoxins are secondary metabolites produced by several species of fungi that significantly affect the quality of food products, thus causing serious problems for animals and humans [1]. In agricultural commodities, the most significant and economically relevant mycotoxins are aflatoxins (AF), fumonisins (FUM), deoxynivalenol (DON), ochratoxin A (OTA), zearalenone (ZEA), patulin (PAT), and citrinin (CIT) [2]. Among these mycotoxins, AF, produced by *Aspergillus flavus* and *A. parasiticus*, have been receiving substantial attention because of their sporadic incidence in agricultural commodities, and their health effects in different animals such as poultry, fish, swine, and cattle [3].

AF are extremely potent carcinogens, predominantly found in cereal crops, like corn, rice, nuts, and sorghum [4]. Currently, several AF have been discovered; however, the most significant AF are AFB_1, AFB_2, AFG_1, and AFG_2 [5]. Food contamination with aflatoxigenic *Aspergillus* spp. and the subsequent production of AF could take place pre-, during, and post-harvest [6–10]. Food contamination by AF depends on vulnerability, environmental factors, fungal community, and their capacity to penetrate the food. Humans could be exposed to AF through sporadic intake of adulterated foodstuff, causing nutritional deficiencies, immunosuppression, and hepatocellular carcinoma [11].

Although most of the symptoms are associated with chronic AF exposure, in some exceptional cases, however, a high level of AF could cause various clinical symptoms including bile duct proliferation, edema, anorexia, hepatitis, kidney malfunctioning, and fatigue [12,13]. Despite the developments in food safety, endemic aflatoxicosis still occurs in various developing countries. According to Qian et al. [14], Ross et al. [15], and Wang et al. [16], the exposure of AF could increase the incidence of hepatocellular carcinoma by 2.4 to 5.5 times, especially in those who have been infected by the hepatitis B virus. The exposure of AF can severely affect the human immune system. Currently, information on the effects of the chronic exposure of AF on human immune systems exists.

The exposure of AF is also responsible for stunting in children. Gong et al. [17] found a significant negative relationship between the new-born children growth and AF exposure. Similarly, Shuaib et al. [18] presented a close relationship between AF exposure and the increased incidence of miscarriage and jaundice. Various research have also been conducted on the evaluation of AF toxicity on animals' health. AF exposure causes serious diseases in animals such as pulmonary and tracheal exudates in horses and pulmonary edema, mucosal accretion, capillarity flimsiness, and icterus lesions in pigs. *Aspergillus flavus and A. parasiticus* are ubiquitous filamentous mold, growing under different environmental conditions [19]. They can be both saprophytic and pathogenic, copiously growing on various organic nutrient resources [20]. They are also capable of living in diverse temperatures ranging between 12 °C and 48 °C, with optimum growth temperatures of 28 °C to 37 °C [21,22].

Owing to the tropical climate of Malaysia, the temperature remains around 28 °C to 31 °C [23], and humidity ranges from 60% to 80% [24] all year round, which provides a suitable environment for *A. flavus* to contaminate the food and food products, and produce AF. To the best of our knowledge, no work has been documented thus far on the prevalence of aflatoxigenic and non-aflatoxigenic strains of *A. flavus* isolated from sweet corn kernels and soil samples collected from the Cameron Highlands region. Therefore, this study was aimed to identify and determine the aflatoxin production potential of *A. flavus* isolated from the sweet corn kernels and soil of Cameron Highlands, Malaysia.

2. Materials and Methods

2.1. Description of the Study Site

In this study, the samples were collected from Kampong Raja, Rose Valley, Kea, and Klebang farms of the Cameron Highlands, Malaysia. Cameron Highlands is the smallest district of Pahang, sharing its borders with Kelantan in the north and Perak in the west, and it is known for its plantations of tea, vegetables, and fruits that are supplied nationwide. Like all the tropical hilly regions, the annual rainfall in the Cameron Highlands is around 2500–3000 mm per year. There are two conspicuous rainy phases in the Cameron Highlands each year, one from September to December, and the second from

February to May. The average temperature of Cameron Highlands ranges from 25 °C to 28 °C with the highest being recorded from June to August (33 °C) [25].

2.2. Sample Collection

2.2.1. Sweet Corn Kernels

A total of 80 sweet corn cobs (20 cobs from each farm) were collected, kept in sterile plastic bags, and sealed properly. The cobs were kept at room temperature and examined the day after collection. The sweet corn kernels were sampled from different parts of the cob using closed spear driven through to the top and sides to obtain 300 g of incremental samples [26]. All the samples were randomly sampled.

2.2.2. Soil Sampling

The intermittent design was followed across the sweet corn farm to obtain the points where the soil samples were collected using a trowel. Firstly, 9 m was diagonally measured from one corner, which was the first point from where the topsoil up to 5 cm deep was collected. Then, 9 m horizontally and 9 m vertically were measured, from where the second sample was collected. The process was continued until 16 samples, giving a composite soil sample from 50 to 60 g collected in sterilized plastic bags. Finally, the soil samples were kept at room temperature and analyzed the day after collection.

2.3. Isolation and Identification of Aspergillus flavus

Several growth media including potato dextrose agar (PDA; general medium), dichloran rose bengal chloramphenicol (DRBC; enumeration medium) agar, and *Aspergillus flavus* and *Aspergillus parasiticus* agar (AFPA; selective medium) were used for the isolation of *A. flavus* isolates from sweet corn and soil samples.

2.3.1. Preparation of Growth Media

The growth media (PDA, 39 g/L dH_2O, DRBC, 31.5 g/L dH_2O, AFPA, 45.5 g/L dH_2O) were prepared following the manufacturer's instruction (PDA; Oxoid, UK, DRBC and AFPA; Hardy Diagnostics, USA) supplemented with chloramphenicol (Fisher, UK) and autoclaved (Hirayama, Japan) at 121 °C and 15 psi for 15 min. The sterilized media were left to cool for 20 min and transferred into 90 mm Ø Petri plates. The Petri plates were kept at 4 °C until inoculation.

2.3.2. *Aspergillus flavus* from Sweet Corn Samples

One gram of each sample (sweet corn) was washed with distilled water and sterilized with sodium hypochlorite solution. The samples were then inoculated on PDA plates supplemented with 0.1 mg of chloramphenicol to prevent bacterial contamination. Three replicates for each sample were made. The uninoculated agar plate served as a negative control while the plate inoculated with *A. flavus* American Type Culture Collection (ATCC) 200026 (synonym: NRRL 3357; Northern Regional Research Laboratory) served as a positive control. The uninoculated and inoculated plates were incubated at 28 °C for 7 d and examined daily for growth and spore formation. Following 7 d of incubation, the fungal colonies were sub-cultured on freshly prepared PDA and incubated for 7 d. *Aspergillus* section *Flavi* colonies were observed under a stereo–binocular microscope (Magnus M24). Representative isolates of *Aspergillus* section *Flavi* were then single spored as described by Ho and Ko [27], Choi, Hyde and Ho [28], and Singh, Kumar and Bandyopadhyay [29] to obtain axenic cultures. The axenic cultures of *Aspergillus* section *Flavi* were sub-cultured on PDA and incubated for 7 d to observe the morphological characteristics [30,31].

2.3.3. *Aspergillus flavus* from Soil Samples

In a 7 mL sterile polystyrene tube, 1 g of soil was suspended in 3 mL of distilled water, mixed for 20 min on a Roto-Shaker prior to plating onto DRBC [32]. Then, 1 mL of soil solution was plated onto the surface of the agar. The uninoculated agar plate served as negative control while the plate inoculated with ATCC 200026 (NRRL 3357) served as a positive control. The uninoculated and inoculated plates were incubated at 28 °C for 7 d. *Aspergillus* section *Flavi* colonies were observed under a stereo–binocular microscope (Magnus M24). The representative isolates of *Aspergillus* section *Flavi* were then single spored as described by Ho and Ko [27], Choi, Hyde and Ho [28], and Singh, Kumar and Bandyopadhyay [29] to get pure cultures. Isolates of *A. flavus* were identified by the colony morphology and morphological keys as described by Klich [33].

2.3.4. Morphological Confirmation of *Aspergillus flavus*

To confirm the identification of A. flavus by colony reverse color, the isolates were cultured on AFPA as described by Pitt, Hocking and Glenn [34] and incubated at 28 °C for 2–3 d.

2.4. Determination of Aflatoxigenic Potential of Aspergillus flavus

Coconut cream agar (CCA) was used to screen the ability of *A. flavus* to produce aflatoxins, thus distinguishing the aflatoxigenic from the non-aflatoxigenic isolates. CCA was prepared in Petri dishes as described by Davis, Iyer and Diener [35] and Fente et al. [36]. Spores from 7-d old *A. flavus* cultures were suspended in dH_2O comprising 0.025% Tween 80. Then, 10 µL of spore suspension was aseptically inoculated at the center of the Petri plates containing CCA and 0.1 mg chloramphenicol [35]. The Petri plates were incubated at 28 °C for 7 d. Following incubation, the plates were examined under UV light (365 nm) for the fluorescence screening of aflatoxins, and the results were recorded.

3. Results

3.1. Detection of Aspergillus flavus

Forty isolates of *A. flavus* were detected from the sweet corn and soil samples (Figure 1). The *A. flavus* isolates were identified through the observation of their morphological characteristics as per the key descriptions by Klich [33] and Clayton [37]. The percentage detection of *A. flavus* in Kampong Raja, Rose Valley, Kea, and Klebang farms was 30% (12), 25% (10), 20% (8), and 25% (10), respectively. Moreover, the recovery of *A. flavus* isolates from sweet corn kernels was 30% (12) while soil samples were 70% (28).

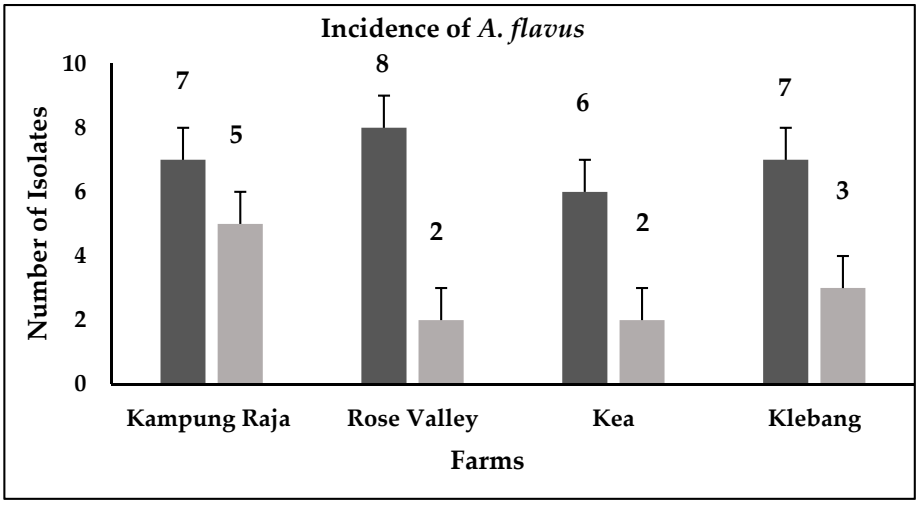
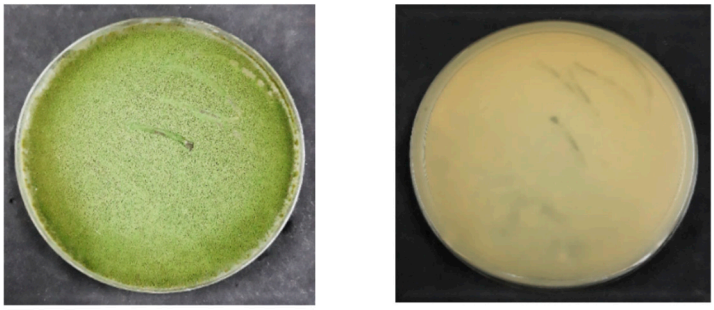


Figure 1. Prevalence of *A. flavus* isolates in Kampong Raja, Rose Valley, Kea, and Klebang farms; black bars = soil samples; grey bars = maize samples.

3.2. Morphological Characterization of *Aspergillus flavus*

3.2.1. Macroscopic Characteristics of *Aspergillus flavus* on PDA

The morphology of *A. flavus* colonies on PDA is presented in Figure 2. In the beginning, the mycelial color of *A. flavus* was white. Following 3 d of incubation, the *A. flavus* colony formed olive-green conidia that dominated the colony's appearance. Generally, the colonies were flat at their borders while raised in the middle. On PDA, all the *A. flavus* isolates produced exudates (droplets) that are brown in color or colorless. In some isolates, a compact mass of fungal mycelia (sclerotia) was produced, which were dark brown. Moreover, the colony diameter was ranged from 65 to 75 mm, surrounded by a white circle. The reverse of the colonies was pale in color.

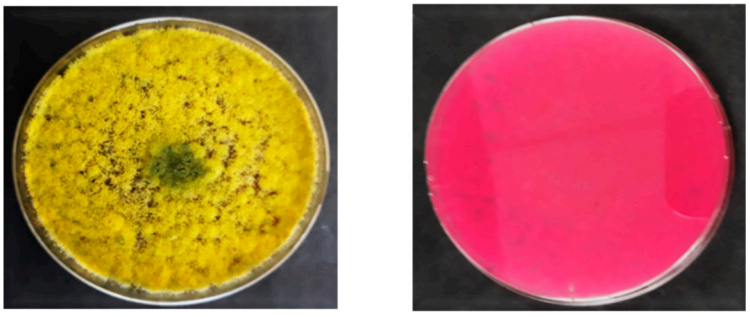


(**A**) (**B**)

Figure 2. Colony morphology of *A. flavus* on the potato dextrose agar (PDA); (**A**) = obverse, (**B**) = reverse.

3.2.2. Macroscopic Characteristics of *Aspergillus flavus* on DRBC

On DRBC (Figure 3), the *A. flavus* isolates initially produced white mycelia that were smooth and upraised. Following 3 d of incubation, they produced dark yellow conidia, which transformed into the olive green after 6 d. No sclerotia were produced by *A. flavus* isolates on DRBC. The exudates (liquid droplets) produced were colorless and embedded in the mycelia. The *A. flavus* isolates did not yield any soluble pigments on DRBC. The reverse sides of *A. flavus* colonies were colorless. The light purple is the original color of DRBC. The colonies' diameter of *A. flavus* ranged from 50 to 70 mm.



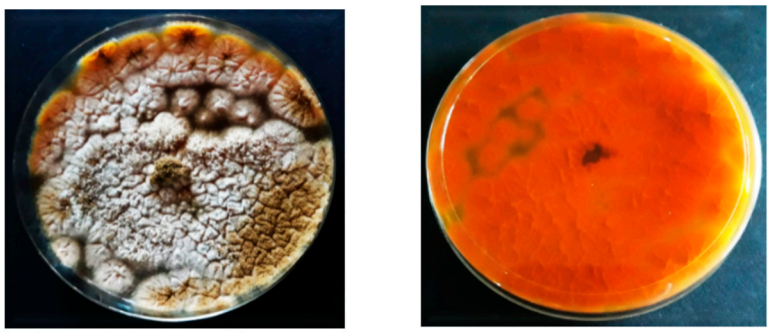
(**A**) (**B**)

Figure 3. Colony morphology of *A. flavus* on dichloran rose bengal chloramphenicol (DRBC); (**A**) = obverse, (**B**) = reverse.

3.2.3. Macroscopic Characteristics of *Aspergillus flavus* on AFPA

The inoculation of *A. flavus* on AFPA resulted in the colonies having various profiles and dimensions (Figure 4). The colonies' color of *A. flavus* on AFPA was whitish brown with dispersed conidia. On AFPA, the isolates of *A. flavus* did not produce exudates or soluble pigments. *A. flavus*

produced a bright orange or golden color on the reverse due to the reaction between aspergillic acid with ferric ammonium citrate contained in the medium.

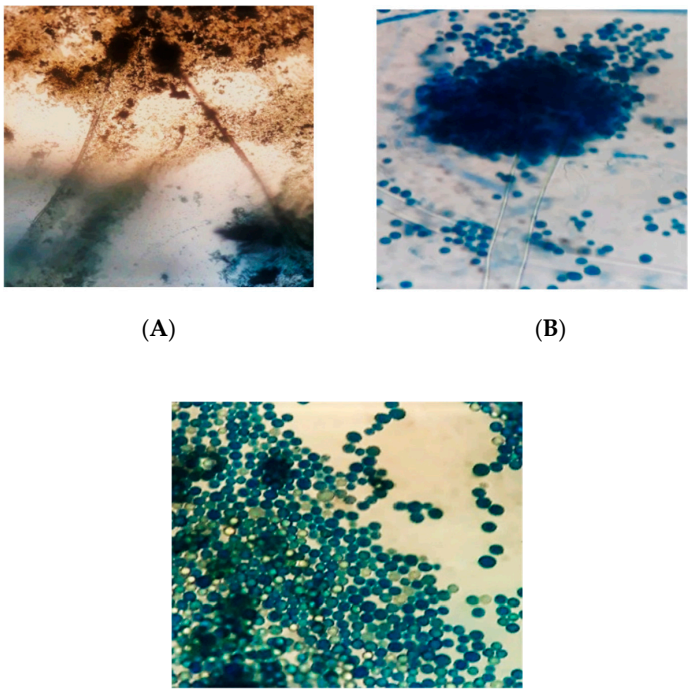


(**A**) (**B**)

Figure 4. Colony morphology of *A. flavus* on *Aspergillus flavus* and *Aspergillus parasiticus* agar (AFPA); (**A**) = obverse, (**B**) = reverse.

3.3. Microscopic Characteristics of Aspergillus flavus

The microscopic characteristics of *A. flavus* are shown in Figure 5. Under the microscope, the conidiophores of *A. flavus* isolates were colorless, thick walled, roughed, and bearing vesicles. The diameter of the conidiophores ranged from 800 to 1200 µm. The vesicle shape of *A. flavus* isolates was globose to sub-globose. The diameter of the vesicles ranged from 1800 to 2000 µm. The cells were uniseriate or biseriate. In the biseriate cells, the phialides grew on the metulae whereas, in uniseriate cells, they grew on the vesicles. The metulae enclosed the vesicles' surface and emitted in all directions. The conidia were globose, thin walled, slightly roughed, and ranged from 250 to 450 µm in diameter.



(**A**) (**B**)

(**C**)

Figure 5. Microscopic characteristics of *A. flavus*; (**A**) = conidiophores and conidia under 10× objective; (**B**) = conidiophore and conidia under the 100× objective; (**C**) = conidia under the 100× objective of the stereo–binocular microscope (Magnus M24).

3.4. Screening for Aflatoxin Production

Figure 6 represents the findings obtained from the UV light screening of *A. flavus*, displaying the fluorescent and non-fluorescent isolates of *A. flavus* on CCA. It was found that 18 (45%) out of 40 isolates fluoresced blue on CCA once they were exposed to UV light (365 nm), thus indicating the ability of aflatoxin production (Table 1). On the other hand, the remaining 22 (55%) isolates did not exhibit blue fluorescence in UV light, thus classified as non-aflatoxigenic isolates of *A. flavus*. The distribution of the aflatoxigenic and non-aflatoxigenic isolates of *A. flavus* in four different farms is presented in Figure 7. Of the 12 isolates from Kampong Raja, six isolates (15%) were tested positive for aflatoxin production while the remaining six (15%) were tested negative for aflatoxin production. For Rose Valley, aflatoxin was detected in three isolates (7.5%) and the remaining seven (17.5%) isolates did not produce aflatoxin. Similarly, of the eight isolates of Kea, three (7.5%) isolates were aflatoxigenic while 12% (five) of the isolates were non-aflatoxigenic. Finally, six (15%) out of 10 isolates of Klebang were aflatoxigenic whereas the remaining four (10%) isolates were found to be non-aflatoxigenic.

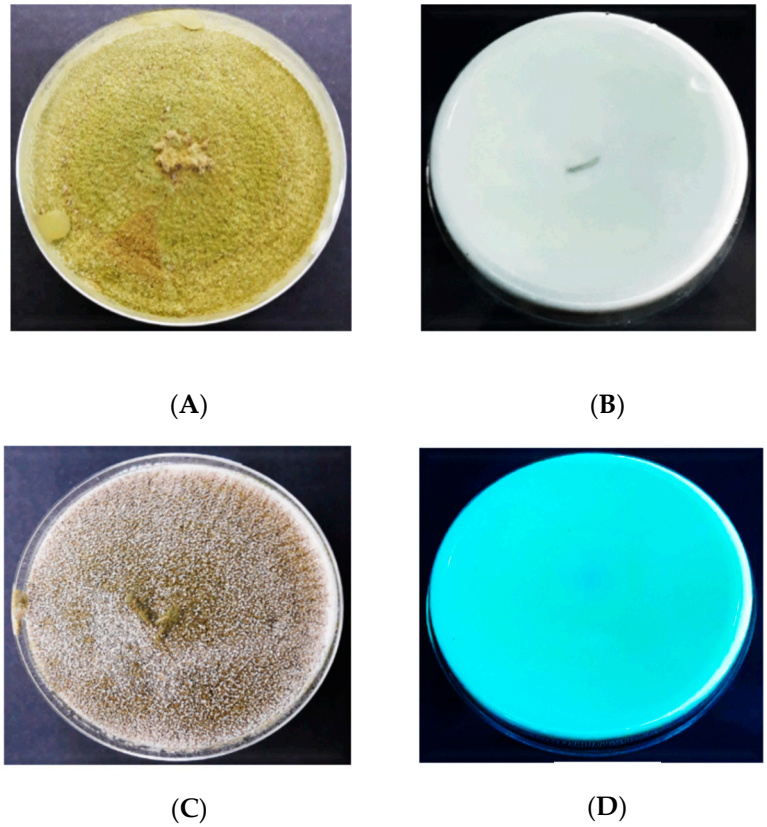


Figure 6. Colonies of the aflatoxigenic and non-aflatoxigenic isolates of *A. flavus* observed under UV light (365 nm); (**A**) = aflatoxigenic obverse, (**B**) = aflatoxigenic reverse, (**C**) = non-aflatoxigenic obverse, (**D**) = non-aflatoxigenic reverse.

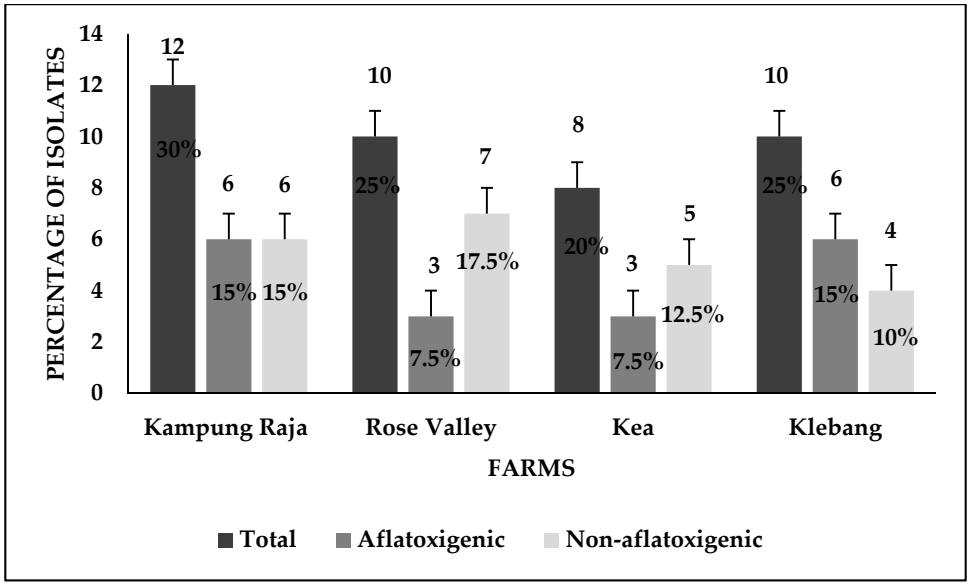


Figure 7. The distribution of the aflatoxigenic and the non-aflatoxigenic *A. flavus* isolates in four sweet corn-growing farms in Malaysia. Black bars = number of *A. flavus* isolates and their respective percentage figure.

Table 1. Identification of the aflatoxin production through UV fluorescence screening.

Isolate	No.	CCA
AKR1	1	+
AKR2	2	-
AKR3	3	+
AKR4	4	+
AKR5	5	+
AKR6	6	-
AKR7	7	-
AKR8	8	+
AKR9	9	+
AKR10	10	-
AKR11	11	-
AKR12	12	-
ARV13	13	-
ARV14	14	+
ARV15	15	-
ARV16	16	-
ARV17	17	+
ARV18	18	+
ARV19	19	-
ARV20	20	-
ARV21	21	-
ARV22	22	-
AK23	23	+
AK24	24	+
AK25	25	-
AK26	26	-
AK27	27	-
AK28	28	+
AK29	29	-
AK30	30	-
AKL31	31	+

Table 1. *Cont.*

Isolate	No.	CCA
AKL32	32	-
AKL33	33	-
AKL34	34	+
AKL35	35	+
AKL36	36	-
AKL37	37	+
AKL38	38	+
AKL39	39	-
AKL40	40	+

AKR: *A. flavus* isolates from Kampung Raja. ARV: *A. flavus* isolates from Rose Valley. AK: *A. flavus* isolates from Kea. AKL: *A. flavus* isolates from Klebang. (-) = did not fluoresce or non-aflatoxigenic. CCA: coconut cream agar.

4. Discussion

4.1. Prevalence of Aspergillus flavus across Sweet Corn Farms in Malaysia

In Malaysia, sweet corns are grown in different environments. In unfavorable environmental conditions, sweet corn can be infected by various fungi. *A. flavus* has been recognized as among the most important fungal pathogens of corn. *A. flavus* contaminates corn both in the field as well as in storage, and the rate of infection could be different amid farms of the same region [38,39]. In this study, 40 isolates of *A. flavus* were identified from the fungal cultures formerly isolated from the sweet corn and soil of Kampung Raja, Rose Valley, Kea, and Klebang farms in Malaysia.

The highest occurrence (30%) of *A. flavus* was found in the sweet corn and soil of Kampung Raja while the lowest (20%) incidence of *A. flavus* was recorded for Kea farm. Rose Valley and Klebang farms comprised 25% and 25% incidence of *A. flavus*, respectively. The difference in the occurrence of *A. flavus* in the different farms could be owed to the diversity in environmental conditions between those farms. Crops grown in hot environments are more susceptible to aflatoxin-producing *A. flavus* and in some areas, contamination simply takes place as soon as the temperature rises with high humidity [39]. Kampung Raja is characterized by temperatures of about 25 to 33 °C with 80% humidity, which favors aflatoxin infection of sweet corn by *A. flavus*. While the other three farms (Rose Valley, Kea, and Klebang) are characterized by temperatures of 12–21 °C with 46–60% humidity.

4.2. Morphological Characterization of Aspergillus flavus

The morphological characterization of fungi via observing the main macro and micromorphological features cultured on diverse media is an extensively used method for fungal identifications. In this study, the morphological characterization was done to give importance to such basic identification methodologies for the fast screening of isolates in most of the developing countries, where the accessibility of unconventional tools is a great challenge. Consistent identification of *A. flavus* isolates was done by observing the key morphological characteristics. The physical characteristics of *A. flavus* including the colony color, texture and edges, as well as the exudates, soluble pigmentations, and sclerotia were studied on four different cultural media.

Overall, the *A. flavus* had olive-green, yellowish-green, or dark green colonies surrounded by a white circle that was eventually covered by conidia. The colonies' textures were usually velvety or woolly with a floccose center. The *A. flavus* colonies produced exudates on two of the four culture media utilized in this study. Similarly, sclerotia were detected in the majority of *A. flavus* isolates, as it is a specific characteristic of aflatoxigenic *A. flavus*. In the interpretation of the taxonomic descriptions by Klich [33], the isolates' colony morphology in this study bore a resemblance to *A. flavus* as shown in Figures 2 and 3. On the other hand, some other *Aspergillus* species have the same phenotypical characteristics and which makes it difficult to differentiate them from *A. flavus*. It is known to have the same colony color as *A. oryzae*. This renders the need for comprehensive scrutiny using microscopic

and macroscopic characteristics for accurate identification and classification. However, there is a consensus that, even microscopically, some characteristics are common among *Aspergillus* isolates [40]. The microscopic features of *A. flavus* such as conidiophore, vesicles, sterigmata and conidia were examined in this study.

The key microscopic features of *A. flavus* were rough and thick-walled conidiophores, globose vesicles with radiated sterigmata and thin walled finely rough conidia. The macroscopic and microscopic characteristics presented in Figures 2–5 are similar to that of *A. flavus* characteristics described by Rodrigues et al. [41] and Diba et al. [42]. The use of PDA, DRBC, and AFPA media in this study allowed for sufficient growth and sporulation that permitted a satisfactory examination of the macroscopic and microscopic features of *A. flavus*. A review of the recent literature presented that these selective growth media have frequently been used for the identification of *A. flavus* [34]. Many *A. flavus* isolates isolated from corn in North-Eastern China have been identified after their morphological characterization on SZA and MEA [43].

Likewise, Diba et al. [42] characterized the *Aspergillus* species from medical and environmental samples through observing their macroscopic and microscopic features and stated that the utilization of PDA, DRBC, AFPA, and other growth media may stimulate the growth and sporulation process of *Aspergillus* species. Additionally, morphological characterization approaches have also been used on *Aspergillus* species in the Fars and Kerman provinces of Iran [44] and the Larkana district of the Sindh province, Pakistan [45]. Based on the size of sclerotia, *A. flavus* can be divided into L-strain and S-strain. The L-strain of *A. flavus* produces large-sized sclerotia while the S-strain *A. flavus* produces small sclerotia.

The relationship between the sclerotial size and aflatoxin production has been studied but the published data are unreliable. Cotty and Mellon [46] have reported a close relationship between small sclerotia and aflatoxin production. On the other hand, Abbas et al. [47] found the highest level of aflatoxins in isolates having large sclerotia. Some studies did not even find any correlation between the sclerotial size and aflatoxin production [48,49]. Thus, the sclerotial size and aflatoxin quantity have not been calculated, due to which no correlation between aflatoxin production and sclerotial type could be made.

4.3. Discrimination between Aflatoxigenic and Non-aflatoxigenic Aspergillus flavus

The screening of *A. flavus* isolates, based on their fluorescence characteristics under UV light at 365 nm was carried out. Once the aflatoxigenic isolates of *A. flavus* were exposed to UV light, they showed a blue fluorescence on the reverse of the colonies, while the non-aflatoxigenic *A. flavus* did not show any fluorescence (Figure 7). In this study, the use of CCA media made it possible to distinguish the aflatoxigenic *A. flavus* from non-aflatoxigenic ones very rapidly, albeit just qualitatively. Fluorescence was found in 18 out of 40 *A. flavus* (45%), thus demonstrating their aflatoxin-producing potential. In most of the developing nations, where aflatoxin determination may be delayed due to the unavailability of advanced detection instruments, culture-based techniques could be beneficial for the rapid screening of isolates.

Although the culture-based techniques are comparatively simple and economical, for aflatoxin detection however, these techniques are usually employed in combination with chromatographic methods [47]. For the precise quantification of aflatoxin, a more advanced method such as high-performance liquid chromatography equipped with fluorescence detection (HPLC-FLD) would be useful. Similarly, it has been reported that strains of *A. flavus* could produce B and G aflatoxins [50], a recent classification of *Aspergillus* section *Flavi* noted that such strains, though morphologically similar to *A. flavus*, were completely novel species [51,52]. These outcomes pointed out the fact that modern molecular approaches are mandatory to be used for *A. flavus* isolates' characterization.

5. Conclusions

In conclusion, both aflatoxigenic and non-aflatoxigenic strains of *A. flavus* were present in the sweet corn kernels and soil of Cameron Highlands, Malaysia. Cameron Highlands being a primary source of agricultural produce could take measures to ensure that aflatoxin contamination is minimized. Non-aflatoxigenic strains indigenously present could be further harnessed into a biological control agent that can reduce the dependency on chemically synthesized pesticides. Culture media and morphology are rapid tools for the characterization of *A. flavus* in this study. The morphological characteristics of these isolates were similar to those explained in the published identification manuals. The screening of *A. flavus* isolates for aflatoxin detection under UV light (365 nm) is a fast and reliable method to distinguish the aflatoxigenic and non-aflatoxigenic isolates. Although the culture-based techniques are comparatively simple and economical, however, for the precise quantification of aflatoxin, a more advanced method such as high-performance liquid chromatography equipped with fluorescence detection (HPLC-FLD) would be useful. This further reinforces the need for measures to prevent the fungal contamination of sweet corn and emphasizes the importance of field interventions aimed at curbing agricultural soil contamination by these fungi.

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