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Prevalence and identification of *Aspergillus* and *Penicillium* species isolated from peanut kernels in central Myanmar

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Abstract

The objective of the present study was to conduct a survey to evaluate the postharvest handling of peanuts at farm level and to screen and identify the strains of *Aspergillus* and *Penicillium* species from peanut kernels in central Myanmar. A total of 640 samples from the stores of farmers, collectors, and wholesalers were collected and seeded for growth, isolation, and characterization of fungi. Out of 85 isolates, *Aspergillus flavus* (38), *A. niger* (20), *A. terreus* (15), and *Penicillium citrinum* (12) were identified and confirmed by molecular techniques including DNA sequencing using internal transcribed spacer (ITS5/4) and beta-tubulin (Bt2a/2b) primer sets. The study indicates different factors associated with fungal contamination at farm level peanut storage and warrants an immediate attention of food safety regulatory authorities to design and implement strategies for postharvest handling and storage practice in developing countries to minimize fungal contamination.

Practical applications

In developing countries like Myanmar, there are lack of food safety practices and policies regarding the food storage, handling, and farmer education. The current study indicates the different storage methods for peanut kernels at farm level and their influence on prevalence of fungi. The study will give a deep insight to the policy makers to design food safety practices at farm level and educate the farmers to adopt the better practices to assure the consumer health and satisfaction in developing countries.

1 | INTRODUCTION

Myanmar has increased its production of peanuts (*Arachis hypogaea* L.) and ranked as sixth largest producer in the world (Wijnands, Biersteker, Hagedoorn, & Louisse, 2014). Peanuts are mainly used for the production of edible oil and in the fabrication of sweets, candies, spreads, and other food items. Fungal contamination of agricultural commodities leads to huge loss of crop yields resulting in significant economic losses of a country. Fungal contamination of various food commodities and associated hazards are of great concern (Pitt & Hocking, 2009). The prevalence of fungal growth and mycotoxin (fungal secondary metabolites) contamination in foodstuffs remain very high, especially in developing countries, mainly due to improper handling and storage conditions (Mutegi, Ngugi, Hendriks, & Jones, 2009). Mycotoxins are produced by fungi and contaminate food commodities through their supply chain systems, during various stages from production to transportation or marketing. The mycotoxin contamination of peanuts at farm level is difficult to control due to favorable environmental conditions for fungal growth and mycotoxin production (Ghali, Hmaissia-Khlifa, Ghorbel, Maaroufi, & Hedill, 2008; Moss, 1991).

Aflatoxin, a fungal secondary metabolite is mainly produced by toxigenic strains of *Aspergillus* species, especially *A. flavus* and *A. parasiticus* (Klich, Tang, & Denning, 2009). In addition, *A. flavi* has also been reported as the prime fungal colonizers of peanut seeds (Perrone, Gallo, & Logrieco, 2014; Sultan & Magan, 2010). In general, moderate temperature (27–38°C) and high humidity are inductive for the growth of *Aspergillus* and can lead to aflatoxin contamination at

both pre and postharvest levels (Fernández-Cruz, Mansilla, & Tadeo, 2010; Kimatu, McConchie, Xie, & Nguluu, 2012). The major factors for food mycotoxin contamination are higher moisture content and damage either by insects or by other physical means during harvesting, drying, and storage (Hell & Mutegi, 2011; Kaaya & Warren, 2005). Although polypropylene bags are commonly used for storage, even then peanuts are prone to fungal contamination (Hell, Cardwell, Setamou, & Poehling, 2000; Wagacha, Mutegi, Christie, Karanja, & Kimani, 2013).

In Myanmar, Magway province is one of the major peanut production regions of the country. This region has tropical climate, that is favorable for fungal growth in stored peanuts. The major factors for fungal contamination of peanuts in the study area include poor management of postharvest handling, drying practices and storage practices. Various countries have developed strategies to control aflatoxins and fungal contamination in peanuts. However, Myanmar is lacking in the implementation of strategies to ensure safe consumption and marketing of peanuts.

For the proper management and treatment of food fungal contamination it is essential to identify the fungi (Newcombe & Dugan, 2010). The molecular methods for the identification of fungi include 18S rRNA gene, intergenic spacer region, ITS5/4 regions, and beta-tubulin (Bt2a/2b) regions (Aittakorpi et al., 2012; Travis, Iwen, & Hinrichs, 2000).

To the best of our knowledge, there is no published literature yet regarding the prevalence studies on fungal contamination of peanuts in central Myanmar. To overcome the fungal contamination of crops and aflatoxin production, good agricultural practices, selection of insect and drought resistant seeds, postharvest handling have been adopted in many developed countries. However, these practices have not been widely and precisely adopted by small farmers in developing countries. The objective of this study was to screen and identify the strains of *Aspergillus* and *Penicillium* species from peanut kernels in central Myanmar. The molecular detection techniques such as the sequencing of internal transcribed genes and beta-tubulin genes were used to differentiate the *Aspergillus* and *Penicillium* species.

2 | MATERIALS AND METHODS

2.1 | Survey, study site, and sampling

The study area was selected based on high peanut production and located in the State of Magway, central Myanmar (Figure 1). The survey, based on postharvest handling and storage practices questionnaires, was conducted by interviewing 178 respondents that included 140 farmers from 11 villages, 28 collectors, and 10 wholesalers (Tables S1 and S2).

At eight storage times, samples were collected for a total of 1 kg per sample. Each 1 kg sample of peanut kernels was obtained from five different sites by aseptic technique and stored in sterile packages for further investigation. The sampling of four storage times (freshly harvested peanut kernels) was performed at 3, 4, 7, and 10 days after sun drying of kernels from the farmer's storage houses. Moreover, the stored peanut kernels (30, 60, 90, and 120 days after storage) from the previous season were also collected from the farmers, collectors, and wholesaler's storage houses. The moisture content of peanut kernels in all cases was less than 13%.

The peanut samples thus were collected from a total of 80 sources that included 40 from farmers (8 storage times × 5 different sites), 20 from collectors (4 storage times × 5 different sites), and 20 from wholesalers (4 storage time × 5 different sites). All kernel samples were collected from different levels of storage structure (top, middle,

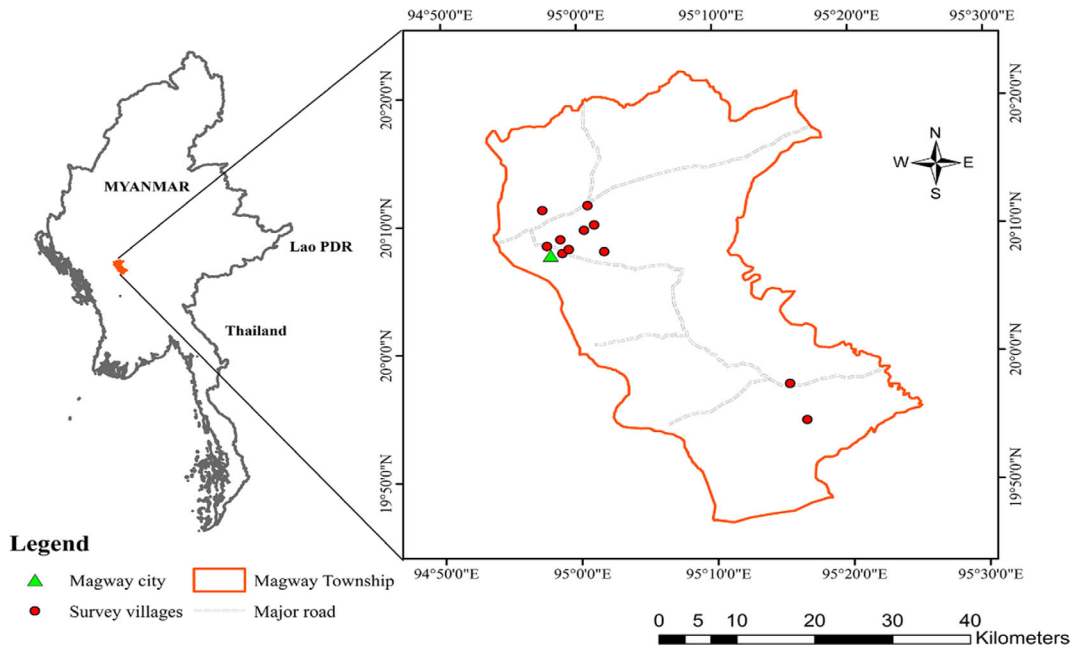

FIGURE 1 The study area with the survey sites in the State of Magway, Myanmar

and bottom) and mixed. Finally, a 1 kg of each kernel sample was stored at 5 ± 2°C in the laboratory for fungal contamination analysis. There were 320 samples (8 storage times × 5 different sites × 8 kernels) for farmers and 160 samples each for collectors and wholesalers (4 storage times × 5 different sites × 8 kernels) resulting in a total of 640 samples. A total of 1,920 peanut kernel samples (640 samples × 3 replications) were analyzed.

2.2 | Moisture content

The moisture content of peanut kernels was determined by grain moisture meter (WILE 55, Finland) at the field level and storage house during the data collection. The moisture content was also determined at the laboratory according to the AOAC Method 925.10 (AOAC, 2000).

2.3 | Isolation and morphological identification of fungi

Each sample (50 g) was disinfected with sodium hypochlorite solution (0.4%, for 3 min) and then washed with sterile distilled water to remove surface contaminants (Nakai et al., 2008). The disinfected kernels (eight kernels/plate) were inoculated in Dichloran Glycerol 18 (DG18) agar medium (HiMedia, Mumbai, India) (Pitt & Hocking, 2009) and incubated at 25°C for 5 days. The results were expressed as total percentage kernels infected with fungi. Colonies showing different morphologies were subcultured on potato dextrose agar medium (HiMedia, Mumbai, India) and Czapek-Dox agar medium (HiMedia, Mumbai, India) at 25°C for 5 days and analyzed for morphological characteristics. Species of *Aspergillus* and *Penicillium* were distinguished based on morphological characteristics, (Klich, 2007) and by comparison with standard strains acquired from the National Science and Technology Development Agency (NSTDA), Thailand. Those belonging to the genus *Aspergillus* and *Penicillium* were further identified to species level using molecular techniques.

2.4 | Molecular identification of the isolated fungi

The isolated fungal strains were subjected to DNA extraction by subculturing on Czapek yeast autolysate agar and incubated at 25°C for 7 days according to the modified method of Pitt and Hocking (2009). The fresh mycelia were selected to extract the genomic DNA by using forensic DNA isolation Kit (Omega Bio-Tek, Norcross, GA). The beta-tubulin gene and the ITS regions were amplified by polymerase chain reaction (PCR) using a DNA thermocycler (Bio-Rad Laboratories Inc., Hercules, CA). For beta-tubulin gene, PCR was processed with an initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 53°C for 1 min, and finally 72°C for 2 min. For internal transcribed gene, the PCR was processed with an initial denaturation at 96°C for 2 min followed by 35 cycles of 96°C for 1 min, 53°C for 1 min and finally 72°C for 1.5 min. The final extension was carried out for 10 min at 72°C for each PCR reaction.

The PCR products were further analyzed on 1% (wt/vol) agarose gel and PCR products were subjected to automated DNA sequencer (Macrogen Inc., Korea). The obtained DNA sequence was then blasted at the National Center for Biotechnology Information, Thailand, and accession numbers were obtained. Phylogenetic tree was constructed using MEGA 7.01 software with the sequences retrieved from the GenBank.

2.5 | Statistical analysis

Analysis of variance was carried out by using SAS 9.0 software (SAS Inst, Inc., Cary, NC), to compare the means by using Fisher's least significant difference (LSD) test ($p < .05$).

3 | RESULTS

3.1 | Drying and storage method of peanut kernels in the study area

Peanuts being one of the most important agricultural commodities in Myanmar, have various uses, especially as processed food products. In Myanmar, these products are often exposed to various abiotic factors (moisture and temperature) conductive for the growth of fungi. *Aspergillus* spp. was the prevalent fungal species, isolated from peanuts.

Harvested peanut crops were sun-dried on the bare ground, tarpaulin, and mat. Curing of peanuts was conducted for 3–5 days at the farm before drying and removing the pods from the haulms to reduce kernel moisture. About 42, 21, 20, and 17% of the farmers dried their peanut plants for 3, 4, 7, and 10 days, respectively (Table S1). In Magway district, generally, farmers stored peanuts for 1–7 days before selling to collectors and collectors distributed the peanuts to wholesalers and retailers in the form of dry pods and kernels.

The peanut pods were mainly sun dried by using traditional methods, that is, on the bamboo mat, tarpaulin, and bare ground or even at the roadside. The favorable fungal growth environment and traditional methods of drying are major sources of fungal contamination of peanuts in central Myanmar. At selected study site, 54% of the farmers dried peanuts by spreading on the bare ground, 29% farmers used bamboo mat, and 16% used tarpaulin. Collectors and wholesalers, on the other hand, buy dried peanuts. About 75% of the collectors and 70% of the wholesalers dried again the purchased peanuts, in case the peanut kernels were not properly dried or if mold growth was visible. About 39% of the farmers stored their peanuts for 1–10 days and only 17% of the farmers stored peanuts for 4 months.

3.2 | Moisture content

The percentage of moisture content of the peanut kernels collected from farmer, collector, and wholesaler's storage houses are presented in Tables 1–3. The freshly harvested peanut kernels (3, 4, 7, and 10 days after harvesting) from the farmer's storage houses had 12.8%, 11.5%, 7.5%, and 7.2% moisture content, respectively.

TABLE 1 Frequency of *A. flavus*, *A. niger*, *A. terreus*, and *P. citrinum*, and moisture contents of peanut kernels under different storage times measured at farmer's stores at 33 ± 4°C and 71 ± 2% relative humidity

	Fungal frequencies (%)				
Storage time	*A. flavus*	*A. niger*	*A. terreus*	*P. citrinum*	Moisture content (%)
3 days	27.3 ± 0.8c	20.3 ± 0.8ab	9.1 ± 1.0b	17.9 ± 1.0a	12.8 ± 0.1
4 days	25 ± 2.1c	19.7 ± 2.3ab	5.4 ± 1.4 cd	4.8 ± 0.8a	11.5 ± 0.2
7 days	6.3 ± 1.5b	5.4 ± 1.5a	4.2 ± 0.8a	1.2 ± 0.8de	7.5 ± 0.2
10 days	4.2 ± 0.8a	2.8 ± 1.0a	4.2 ± 0.8a	0.8 ± 1.0a	7.2 ± 0.1
30 days	4.5 ± 0.5a	3.6 ± 1.4a	4.6 ± 1.1a	1.1 ± 0.3de	7.1 ± 0.1
60 days	10.1 ± 2.6b	12.1 ± 3.4b	7.2 ± 1.8bc	6.7 ± 1.0b	8.7 ± 0.1
90 days	32.8 ± 2.3d	17.9 ± 2.1b	7.9 ± 2.0b	2.5 ± 0.5a	9.1 ± 0.1
120 days	35.2 ± 2.0d	22.3 ± 1.0b	14.4 ± 1.5c	4.8 ± 1.0a	9.3 ± 0.1

Notes: Means followed by the same letter in each column do not differ by least significant difference (LSD) at $p < .05$. Data are means ± *SD* of three replications.

TABLE 2 Frequency of *A. flavus*, *A. niger*, *A. terreus*, and *P. citrinum*, and moisture contents of peanut kernels under different storage times measured at collector's stores at 33 ± 4°C and 71 ± 2% relative humidity

	Fungal frequencies (%)				
Storage time	*A. flavus*	*A. niger*	*A. terreus*	*P. citrinum*	Moisture content (%)
30 days	5.7 ± 0.6a	4.2 ± 1.3a	3.5 ± 0.9a	2.4 ± 1.5a	7.5 ± 0.1
60 days	11.9 ± 1.4b	9.6 ± 1.9b	5.8 ± 1.7a	2.9 ± 0.5a	9.1 ± 0.2
90 days	29.6 ± 1.5c	19.3 ± 2.1c	9.6 ± 2.3b	5.7 ± 1.5b	10.2 ± 0.2
120 days	31.3 ± 3.5c	21.4 ± 2.5c	11.9 ± 1.0b	7.1 ± 1.7b	10.9 ± 0.1

Notes: Means followed by the same letter in each column do not differ by least significant difference (LSD) at $p < .05$. Data are means ± *SD* of three replications.

TABLE 3 Frequency of *A. flavus*, *A. niger*, *A. terreus*, and *P. citrinum*, and moisture contents of peanut kernels under different storage times measured at wholesaler's stores at 33 ± 4°C and 71 ± 2% relative humidity

	Fungal frequencies (%)				
Storage time	*A. flavus*	*A. niger*	*A. terreus*	*P. citrinum*	Moisture content (%)
30 days	5.1 ± 1.7a	3.3 ± 1.2a	3.1 ± 1.0a	1.0 ± 1.0a	7.1 ± 0.2
60 days	9.7 ± 1.5a	4.1 ± 1.7a	5.1 ± 1.7a	3.2 ± 2.0bc	8.7 ± 0.1
90 days	22.1 ± 1.7c	10.9 ± 1.7b	7.9 ± 1.7b	4.9 ± 1.7ab	9.3 ± 0.2
120 days	27.9 ± 2.6c	11.7 ± 0.6b	8.7 ± 1.5b	7.6 ± 1.2b	10.1 ± 0.1

Notes: Means followed by the same letter in each column do not differ by least significant difference (LSD) at $p < .05$. Data are means ± *SD* of three replication.

3.3 | Frequency of fungal isolation from peanut kernels

The prevalence of fungi isolated from peanut kernel samples are presented in Tables 1–3. The moisture content of collected samples was less than 13% at the farm stores. Peanut kernels were more contaminated by *A. flavus* and *A. niger* with 3 and 4 days stored product compared with 7, 10, 30, and 60 days stored peanut kernels (Table 1) indicating the occurrence of fungal contamination under improper drying practice and relatively short storage time at the farm level. Furthermore, 90 and 120 days stored peanut kernels were more contaminated by *A. flavus*, *A. niger*, and *A. terreus* than 30 and 60 days stored peanuts at the farmer, collector, and wholesaler's stores (Tables 1–3). Overall, *A. flavus* and *A. niger* were isolated at higher occurrence than *A. terrreus* and *P. citrinum* in all the collected samples (Figure 2). The kernel samples of the collector's stores had higher occurrence of isolation of *A. flavus* compared with the farmer and wholesaler's stores. *A. flavus* was the highest (19.4%) in its frequency followed by *A. niger* (13.5%) at the collector's stores, while these two species of fungi were the second highest in their frequencies at the farmer's stores (Figure 2). On the other hand, *A. terreus* and *P. citrinum* were the least in their frequencies at each store.

3.4 | Molecular identification

In this study, the peanut kernel samples were contaminated with three different species of *Aspergillus* and one *Penicillium* species. These were *A. flavus*, *A. niger*, *A. terreus*, and *P. citrinum*. A total of 73 isolates of *Aspergillus* species and 12 isolates of *Penicillium* species were isolated from the peanut kernel samples collected from Magway

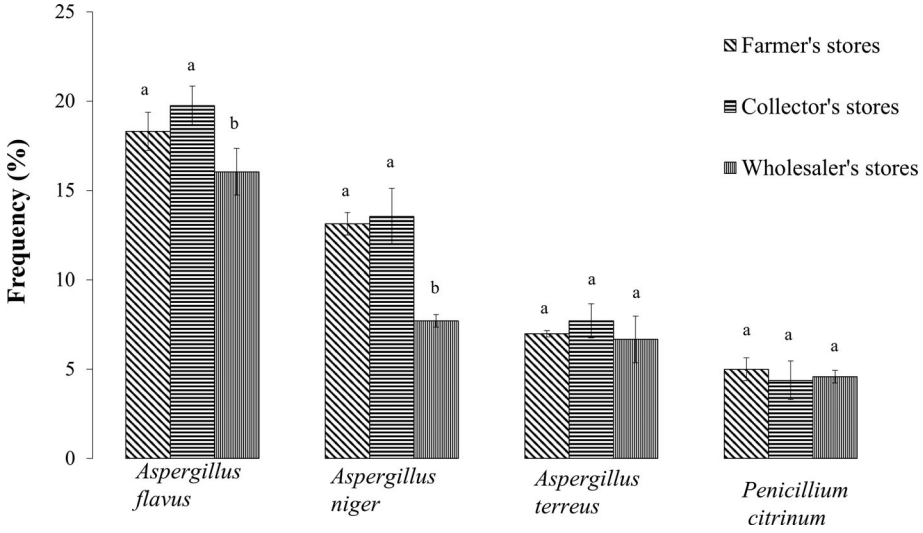

FIGURE 2 Overall occurrence (%) of fungal contamination in peanut kernel samples in farmer, collector, and wholesaler's stores. Means with same superscript letter are not significantly different ($p < .05$) and different superscript letters above the bars indicate significant differences in frequency of fungal occurrence during storage at farmer, collector, and wholesaler's stores

district, Myanmar. Out of 85 isolated samples, 38 isolates (44%) were *A. flavus*, 20 isolates (24%) *A. niger*, 15 isolates (18%) *A. terreus*, and 12 isolates (14%) *P. citrinum* (Figure 3). As illustrated in Figure 3, the macroscopic and microscopic pictures of isolated molds from peanut kernels are clearly resemble to *A. flavus* (Figure 3a,b), *A. niger* (Figure 3c,d), *A. terreus* (Figure 3e,f), and *P. citrinum* (Figure 3g,h).

DNA sequences were evaluated in GenBank using BLAST to match the DNA sequence of the existing fungal species from the GenBank database. Identification of the four selected isolates was based on the ITS and beta-tubulin regions. Thus, a set of primers, (ITS5 and ITS4) and (bt2a and bt2b), was used to amplify the rDNA region (Table 4, Figure S1). The BLAST data showed that the selected isolates were *A. flavus*, *A. niger*, *A. terreus*, and *P. citrinum*.

The isolates of *A. flavus* G1/A (Figure 4) were identified by molecular analysis of their beta-tubulin (B2ta/B2tb) gene. Based on phylogenetic tree, *A. flavus* isolate G1/A was closely related to HQ 285463, HF 570030, and KX 455740 with beta-tubulin gene similarity of 100%. The isolates of *A. niger* B1/A were identified by molecular analysis of their ITS5/ITS4 gene. *A. niger* isolate B1/A was closely related to AY 656630 and KC 354375, with ITS gene similarity of 99% and FJ 878651 with ITS gene similarity of 100%. In addition, *A. terreus* isolate Y1/A was closely related to *A. terreus* KU 687809 and KR 610363 with ITS gene similarity of 99% and 97%, respectively, and *P. citrinum* isolate G1/P was classified into clade that included *P. citrinum* EU 128582, LT 559015, and KP 235300 (Figure 5). The Y1/A isolate was closely related to EU 128582, LT 559015, and KP 235300 with beta-tubulin gene similarity of 100%.

4 | DISCUSSION

4.1 | Drying and storage methods of peanuts

Effective drying is critical for controlling mold growth during storage. It is generally recommended that farmers should dry their peanuts for 7–12 days. Due to lack of the standard criteria, farmers aim to sell the pods with higher weight having higher moisture content. Farmers may have an economic advantage to sell their damaged nuts that they suspect are more likely to be contaminated with aflatoxin, as reported for maize (*Zea mays* L.) in Kenya (Hoffmann, Mutiga, Harvey, Milgroom, & Nelson, 2013). The current study suggested that lowering the moisture content to 8% or below significantly reduced the fungal contamination during storage of peanuts. Therefore, lowering the moisture content can be used as an effective strategy to reduce the fungal contamination in food commodities.

Field and bare-ground sun drying are the most commonly used traditional drying methods of peanuts; however, these drying methods could expose peanuts to fungal contamination. This may cause injury to the peanuts and thus, increase the susceptibility to fungal contamination. In addition, it increases the chance of cross-contamination as different crops are generally dried together at the same ground (Okello, Biruma, & Deom, 2010).

Farmers from Magwae, Myanmar often sell their pods in wet condition without proper drying and packaging in polypropylene bags to obtain higher weight of their produce. The most commonly used storage materials in the study area were polypropylene bags. Gachara (2015) reported that most farmers in Eastern and Rift valley regions of Kenya first packed their maize in polypropylene bags followed by storage at the granaries. The fundamental reason behind storing peanuts in dry condition is to enhance storability and prevent growth of storage fungi (Kaaya, Warren, & Adipala, 2000). Storage structure commonly used in Myanmar is traditional and may not maintain an even, cool, and dry internal atmosphere resulting in an inadequate protection of the stored products.

4.2 | Moisture content

Moisture and temperature are the main factors that influence post-harvest contamination of stored commodities by *A. flavus* (Hell & Mutegi, 2011). In this study, the maximum temperature and relative humidity during the data collection were 33 ± 4°C and 71–73%, respectively. Fernández-Cruz et al. (2010) reported a temperature range of 32 ± 5°C and a relative humidity of 85% as optimal

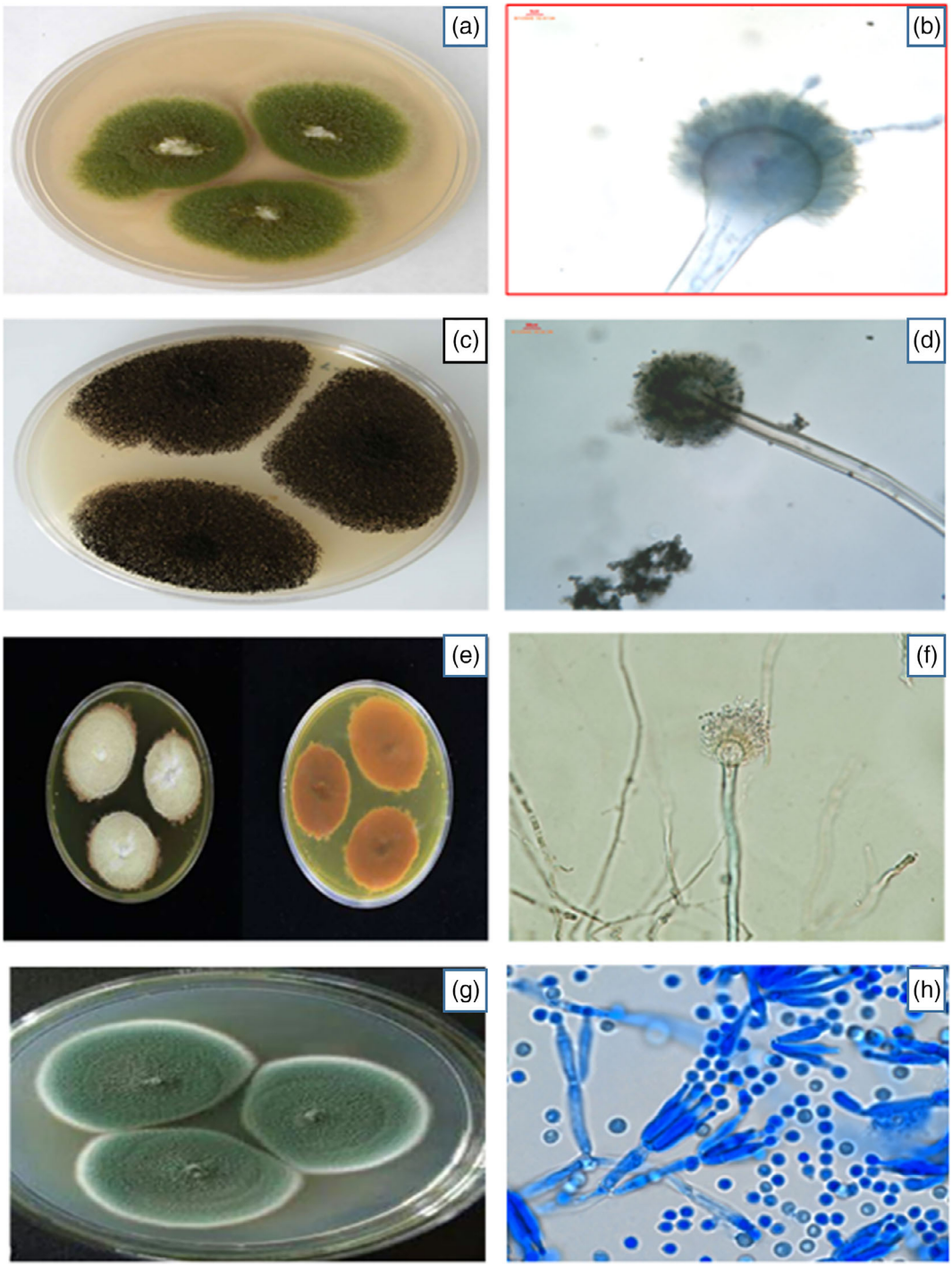

FIGURE 3 Macroscopic (a, c, e, g) and microscopic (b, d, f, h) views of 7 day-old isolates of *A. flavus* (a, b), *A. niger* (c, d), *A. terreus* (e, f) and *P. citrinum* (g, h) grown of Potato Dextrose Agar and Czapek-Dox Agar

environmental conditions for the growth of *Aspergillus* in the field. The minimum moisture content for *A. flavus* growth on peanut is 8–10% at around 82% relative humidity (Nakagawa & Rosolem, 2011; Torres, Barros, Palacios, Chulze, & Battilani, 2014). Integrity of the shell is also important to protect nuts from fungal contamination and the resulting mycotoxins. It is then necessary to maintain that safe storage moisture content until peanuts are processed. This can also be difficult to accomplish because of environmental conditions during harvest as well as during the storage period.

4.3 | Prevalence of fungi in peanuts

Pitt, Hocking, Bhudhasamai, Miscamble, and Wheeler (1993) reported that the predominant fungi infecting peanuts collected from the

farmer storage, middlemen and retailer in Thailand was *A. flavus*, followed by *A. niger*. Among the contaminating fungi, *Aspergillus* spp. was isolated from almost all the samples used in this study. This result correlates with the result of many investigations on seed pathology (Sejiny, Thabet, & Elshaieb, 1989). In addition, *Aspergillus* and *Penicillium* spp. predominately occur in the stored nuts (Khosravi, Shokri, & Ziglari, 2007; Suleiman, 2010). The result of the present study revealed the occurrence of four fungi namely *A. flavus*, *A. niger*, *A. terreus*, and *P. citrinum* in Magway, Myanmar. Furthermore, the occurrence of *Aspergillus* and *Penicillium* was similar to the findings of other investigators studying fungal contamination of peanuts in Brazil and India (Bhattacharya & Raha, 2002; Rossetto, Silva, & Araujo, 2005). Xing et al. (2016) reported that *Rhizopus* and *Eurotium* were predominant in stored peanut kernels and their abundance was higher than *Aspergillus* due to their xerophilic nature and ability to grow on substrates with low moisture content. Ding et al. (2015) reported a significant variation in fungal diversity in stored peanuts based on storage areas and storage time, their findings indicated that abundance of *Aspergillus* spp. and *Emericella* spp. was increased with increase in storage time of peanut kernels.

In this study, the prevalence of *P. citrinum* in peanut kernels was lower than the isolated *Aspergillus* spp. The increase in the occurrence of *Aspergillus* spp. can be explained by the fact it is considered as

TABLE 4 Primer sets and corresponding amplification targets

Target gene	Primer	Primer DNA sequence	PCR product size
ITS	ITS5	5' GGAAGTAAAAGTCGTAACAAGG 3'	601
	ITS4	5' TCCTCCGCTTATTGATATGC 3'	
	ITS5	5' GGAAGTAAAAGTCGTAACAAGG 3'	548
	ITS4	5' TCCTCCGCTTATTGATATGC 3'	
β-Tubulin	Bt2a	5' GGTAACCAAATCGGTGCTGCTTC 3'	520
	Bt2b	5' ACCCTCAGTGTAGTGACCCTTGGC 3'	
	Bt2a	5' GGTAACCAAATCGGTGCTGCTTC 3'	468
	Bt2b	5' ACCCTCAGTGTAGTGACCCTTGGC 3'	

Abbreviation: PCR, polymerase chain reaction.

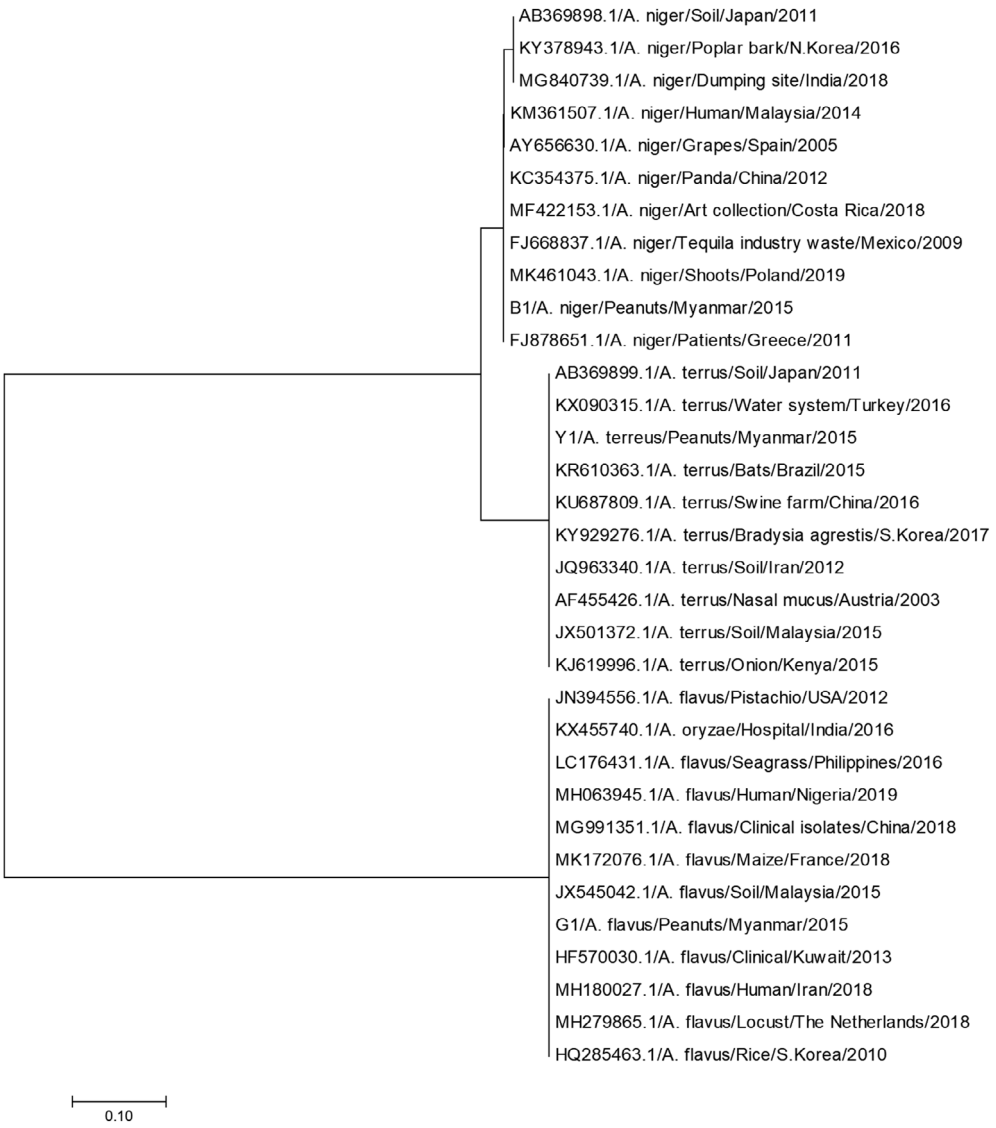

FIGURE 4 Phylogenetic tree of *A. flavus*, *A. niger*, and *A. terreus* based on ITS gene and beta-tubulin gene

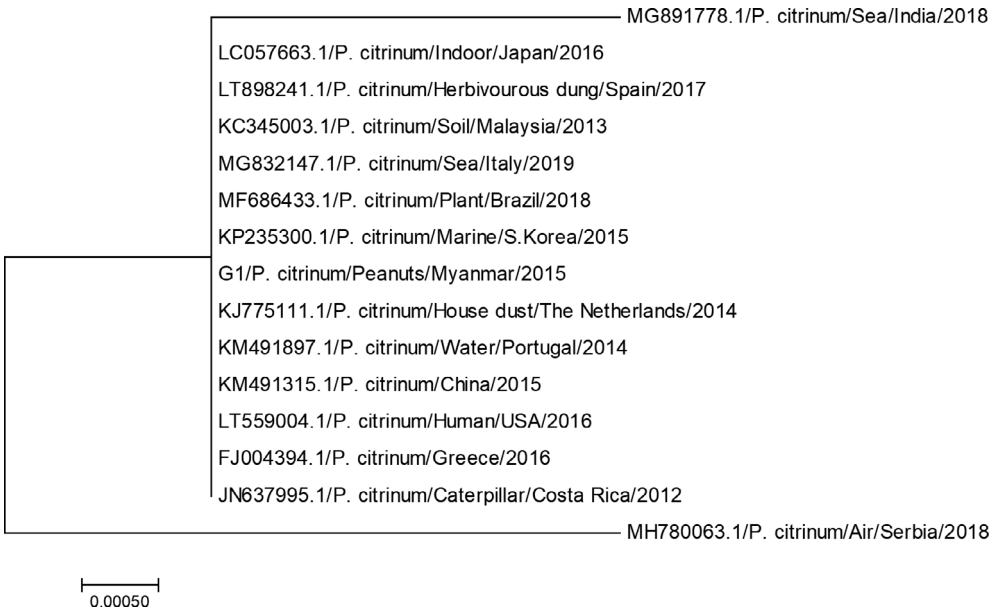

FIGURE 5 Phylogenetic tree of *P. citrinum* based on beta-tubulin gene

storage fungi (Krijgsheld et al., 2013). The growth of *Penicillium* decreases in favor of the growth of *Aspergillus* and the rate of contamination by *Penicillium* is inversely proportional to the duration of storage; therefore, the dominance of the genus *Aspergillus* was higher than *Penicillium*, probably favored by high grain moisture and long storage period (Tahani, Elamrani, Serghini-Caid, Oouzouline, & Khalid, 2008).

Rossetto et al. (2005) identified the fungi responsible for contamination of peanut kernels after 12 and 18 months of storage as *A. flavus* and *Penicillium* spp. Their presence is abundant in the microflora extant in the soils and it is not possible to predict or entirely prevent their occurrence during cultivation, harvest, storage, and processing operations by current manufacturing practices (Dorner, 2008). Environmental factors such as rainfall, humidity, temperature, and respiration are likely to accelerate growth of aflatoxigenic fungi on peanuts, edible nuts, and other food commodities (Mutegi et al., 2009; Soubra, Sarkis, Hilan, & Verger, 2009). The isolates of *A. flavi* were present in all seed samples analyzed. Peanuts are often invaded before harvesting by *Aspergillus* and *A. flavus* is the predominant fungi isolated from peanut samples (Ibiam & Egwu, 2011). Aflatoxigenic strains of *A. flavus* and *A. parasiticus* have been reported in peanut and peanut products in Africa (Sultan & Magan, 2010). *A. niger* is considered as an extremely dangerous pathogen and recognized as a producer of ochratoxin A (Hocking, Su-lin, Kazi, Emmett, & Scott, 2007). The developing countries like Myanmar should opt for the economically feasible and effective biocontrol techniques to address fungal contamination as described by Sriwattanachai, Sadiq, and Anal (2017), who reported the control of fungal contamination in rice (*Oryza sativa* L.) grains using essential oils and *Lactobacillus* cell free supernatant.

4.4 | Molecular identification

Although molecular identification of fungi is expensive, labor, and time intensive, it has become the most common tool for rapid identification of *A. flavus*, *A. niger*, and other types of fungi (Iheanacho et al., 2014). The species of fungi identified are not morphologically and molecularly similar; however, further identification of these species of fungi can be made as a variety of closely related species. Phylogenetic analysis using ITS (El Khoury et al., 2011; Varga, Frisvad, & Samson, 2011) and using beta-tubulin genes were used to analyze differential relationship between closely related species of fungi like these.

5 | CONCLUSION

The present study is the first of its kind to evaluate fungal contaminations in peanut kernels in central Myanmar. The findings emphasize the need for routine monitoring of peanuts (as a source of potential mycotoxin contamination) used for food applications including raw, processed, and value-added products. Most of the samples were contaminated with *Aspergillus* spp. indicating the need to improve the postharvest handling and storage practice to prevent fungal contamination during storage. Molecular technique is regarded as a rapid and an easy method to identify *A. flavus A. niger*, *A. terreus*, and *P. citrinum* isolated from peanut kernels in central Myanmar. However, further research is in progress on the occurrence of mycotoxigenic fungi, species identification, and mycotoxin evaluation in the peanut kernels across other regions in Myanmar.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

S.H.C. performed the main experiments. M.B.S. and A.D. helped in designing the experiments and data analysis. A.K.A. supervised the research and drafted the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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