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Isolation and Identification of Mold Contaminants in Peanut Paste from the Cengkareng Market of West Jakarta

Alfriana Margareta[1], Anis Setyowati[2]
Program Studi Teknologi Laboratorium Medis, Sekolah Tinggi Ilmu Kesehatan Kesetiakawanan Sosial Indonesia, Jakarta, Indonesia[1,2]

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ABSTRACT

The filamentous molds associated with peanut paste produce biochemicals known as mycotoxins. The major mycotoxins are aflatoxins produced by molds from the genera *Aspergillus* and *Penicillium*. Mold contaminants can be occurred due to poor hygiene storage. The purpose of this study was to determine the presence of molds in peanut paste sold at the Cengkareng market in order to assist people in being attentive to the storage conditions of peanut paste. Samples were collected randomly with a total of 15 packages of peanut paste from Cengkareng market, West Jakarta, Indonesia. Potato Dextrose Agar was used for the isolation and identification of molds. The molds were observed based on the morphological features through macroscopic and microscopic examination. The frequencies of 15 samples contaminated with molds were computed on a percentage. From the observed morphological features and colony colour of molds, those were *A. niger*, *A. flavus*, *Penicillium* sp, and *Mucor* sp. This research also revealed that of the 15 samples identified, *A. niger* and *A. flavus*, 40% respectively. Other species *Penicillium* sp. 27% and *Mucor* sp. 13% were the least common. Therefore, this study confirmed that *Aspergillus* is the highest risk of contaminating peanut paste compared to other genera, which can cause a health problem for humans.

Email :
alfrianamargareta@gmail.com
anisyayank@gmail.com

1. INTRODUCTION

Peanut is a food product that contains 25-30% protein, 40-50% fat, and 12% carbohydrates [1], [2]. In addition, there are 2 grams of fiber in every 100 grams of peanuts, which means it fulfills 10% of the daily fiber requirement [3]. Peanuts are processed and produced in various food products, such as butter, oil, flour, tempeh, and peanut paste [4]. The Indonesian people have a great demand to consume food of peanut products because of their high nutritional value [5]. However, food made from peanuts is very susceptible to contamination by types of molds that cause health problems in humans [6], [7].

Filamentous molds associated with food can produce biochemicals known as mycotoxins. Mycotoxins are mostly produced by molds from the genus *Aspergillus*, such as aflatoxins, ochratoxins, patulin, citrinin, aflatrem, secalonic acids, cyclopiazonic acid, terrein, sterigmatocystin, gliotoxin, and other characteristic molecules [8], [9]. Aflatoxins are thermostable, genotoxic, hepatotoxic, mutagenic, teratogenic, and carcinogenic. Aflatoxins B1, B2, G1, and G2 contaminate various agricultural and food products that cause health issues in humans and animals. Aflatoxin B1 is metabolized as aflatoxin M1 in humans, and it causes cancer in the liver, prostate, and other human organs. Aflatoxins G1 and B1 metabolize Aflatoxins G2 and B2 after ingestion. Ochratoxin and citrinin are the causative agents of Balkan Endemic Nephropathy (BEN), which attenuate RNA synthesis in the kidney. Aflatrem is reported to induce staggers syndromes and neurodegenerative disorders in both animals and humans. Furthermore, patulin produced by *Penicillium* and *Aspergillus* contaminates various foods. Patulin precipitates ulcers, inflammation, and intestinal bleeding. Similarly, gliotoxin, cyclopiazonic acid, terrein, and sterigmatocystin synthesized by *Aspergillus* genus exhibit extensive toxicity in humans and animals [9].

Molds contamination, especially *A. flavus* in food products, often occurs in tropical and subtropical areas, such as Indonesia, which has an air temperature of around 20˚ – 30˚C and high humidity. These conditions have provided the growth of *Aspergillus* [10]. Poor storage can also contribute to mold growth [11]. *A. flavus* is commonly found in processed peanut products, such as peanut paste [4], [12]. Peanut paste distributed in traditional markets is generally stored at room temperature so that it can gain the risks of food contamination by molds. It was proven in research on Palembang local market in Indonesia revealing that out of 17 samples, 8 Aspergillus sp, 4 Aspergillus sp and Penicillium each, and 2 Penicillium sp from total samples [4]. Based on research [13] there were also samples of peanuts infected with *A. flavus* (45%) collected from several Banjarnegara local markets in Indonesia. Therefore, this research aims to identify the types of molds in peanut paste at the Cengkareng market, Indonesia, as a means to maintain the hygienic storage conditions of food products-made peanut that is susceptible to mold contamination.

2. METHOD

Sampling Technique

A total of 15 peanut paste samples were collected randomly from traders at Pasar Cengkareng, West Jakarta, Indonesia. The samples were packed in plastic bags to avoid contamination, labeled, and sent to the microbiology laboratory, Sekolah Tinggi Ilmu Kesehatan Kesetiakawanan Sosial Indonesia. This study also used a control sample, PDA media without peanut paste.

Preparation of Culture Media

The mold growth media using PDA media followed [14]. Twenty-gram Potato Dextrose Agar was weighed and dissolved into 500 ml of distilled water in a conical flask. The preparation was afterward heated using a hot plate to dissolve completely. Following it, the solution was autoclaved at 121 °C for 15 minutes, allowed to cool to about 50°C, and dispensed into the Petri plate in aseptic conditions.

Isolation and Identification of Fungi

The mold A total of the fifteenth peanut paste samples were cultured onto a PDA and incubated for seven days at 28 °C. After seven days, mold colonies grew on Petri dishes. A small portion of the moldy colony was placed on a glass object and stained with Lactophenol Cotton Blue. It was observed under the microscope, starting with a lower magnification (10x) and later with a higher magnification (40x), and the corresponding images took with a mobile camera. Furthermore, Mycotoxigenic molds were identified on the morphological features and appearance of their colonies on Petri dishes as described by [15], and [16], [17]. Recording of the frequency of samples contaminated with mold species was calculated based on the following formula (Kocic et al., 2013).

$$Frequency\ (\%) = \frac{Number\ of\ samples\ in\ which\ a\ particular\ molds\ occured\ \times 100}{Total\ number\ of\ samples\ examined}$$

3. RESULTS AND DISCUSSION

Distribution of Mycotoxin Mold

Mycotoxins are an assorted group of toxic compounds produced by several molds, known to cause poisonous effects on the health of humans and animals. Food safety is periodically risked by mycotoxins emerging in food, especially grains [11]. Based on the research conducted, there were several molds were found in peanut paste samples. Species of *A. flavus*, and *A. niger* (40%), each respectively followed by two others *Penicillium* sp. (27%) and *Mucor* sp. (13%) were distributed from the total peanut paste sample (Table 1).

Table 1. Mycotoxin Molds in Peanut Paste Samples Collected from Cengkareng Market

Source of sample isolate	Molds species isolates	Results	N	%
Peanut paste	*A. niger*	+	6	40%
		-	9	60%
	A. flavus	+	6	40%
		-	9	60%
	Penicillium sp.	+	4	27%
		-	11	73%
	Mucor sp.	+	2	10%
		-	13	90%

The major molds isolated from peanut paste samples collected from the Cengkareng market indicated the most common *Aspergillus* species (Table 1). *Aspergillus* species were known to produce mycotoxins which contaminate grains particularly, such as peanut paste. Its contamination mainly occurs in countries with tropical climates like Indonesia [18], Africa, and South America [8]. In addition, the emergence and spread of Aspergillus depend on several factors, including environmental, social, and economic conditions.

Cultural and Microscopic Characteristics of Isolated Mold

Mycotoxigenic molds were identified in the peanut paste samples based on the characteristic macroscopic or colonies feature and microscopic characteristics that could see in Figures 1, 2, 3, and 4. Their characteristics were clarified in Table 2 as described by [17].

Figure 1. Molds colonies on PDA (macroscopic) and microscopic 40x. A) *A. flavus*, B) *A. niger*, C) *Penicillium* sp., D) *Mucor* sp.

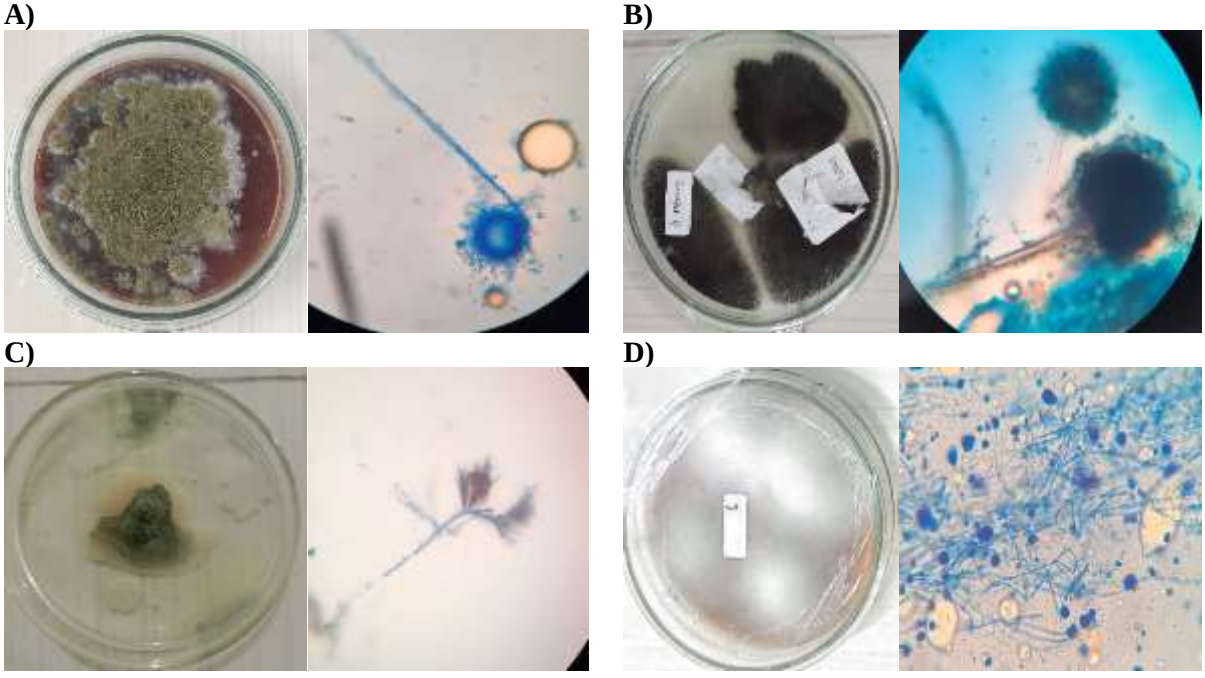

Table 2. Identification of Molds in Peanut Paste [17]

	Molds speccies	Macroscopic Features	Microscopic Features
1	*A. flavus*	*A. flavus* had olive-green, or dark-green colonies surrounded by a white circle that was eventually covered by conidia.	The conidiophores of *A. flavus* isolates were colorless, thick-walled, roughed, and bearing vesicles. The vesicle shape of *A. flavus* isolates was globose.
2	*A. niger*	Microscopic observation of *A. niger* revealed that their growth was to begin with white but they altered to black after a few days producing conidial spores.	Microscopic observation of *A. niger* revealed that *A. niger* had smooth-colored conidiophores and conidia.
3	*Penicillium* sp.	Colonies observation revealed fast-growing, in shades of green and composed of dense conidiophores.	Septate hyphae, the mycelium was branched and colorless. The hype where the spores were attached had a distinctive shape similar to a broom.
4	*Mucor* sp.	*Mucor* sp. had a fast growth speed on PDA, with a white colony initial colour, that changed slowly to grayish brown	Microscopic observation, revealed broad aseptate hyphae, with the extension of columella into sporangium and accumulation of sporangiospores.

Based on the research results, the most common contaminated samples were *A. flavus* and *A. niger*, followed by *Penicillium* sp. and *Mucor* sp. (Figure 1). Similar results by [4] in the local market in Palembang, Indonesia showed that the genera Aspergillus were the most common molds that contaminated peanut paste. Research of [18] also revealed that the species of *Aspergillus* was the most commonly isolated mold followed by species of Fusarium, Mucor, Penicillium, and Rhizopus. Contamination of A. flavus and aflatoxin contamination may occur either during storage or marketing/distribution. Specifically, higher contamination levels were reported for peanut samples obtained from retailers in local markets [13]. Mold growth in traditional markets can occur due to poor storage conditions, mostly occurring in hot and humid regions where high temperature and humidity are optimal for mold growth. This situation is often found in tropical areas such as Indonesia [19].

A. flavus is the most common cause of superficial infection. It produces several mycotoxins one of which is aflatoxins, the most toxic and potent hepatocarcinogenic natural compounds ever characterized. Aflatoxin attacks the respiratory system and causes cancer, hepatotoxic, and allergic reactions due to exposure to mold spores [8], [9]. *A. niger* is one of the most abundant species of *Aspergillus* in nature as it can grow on large kinds of substances. It can also even grow in environments with barely any nutrients or water available. *A. niger* produces a mycotoxin called gliotoxin which causes serious lung disease and otomycosis [20]. *Penicillium* sp. causes superficial infections (keratitis and otomycosis) and allergic lung disease commonly [21].

4. CONCLUSION

The peanut paste samples collected from the Cengkareng market zone, Indonesia were found to be contaminated with Aspergillus genera the most. This research revealed a potential threat to food and consumption. Therefore, it is important to control food safety regulatory systems and to distinguish the peanut paste samples whether suitable for human consumption or not, particularly in local markets. Additionally, it is important to develop and strengthen an effective food safety regulatory system, as well as provide education to producers, traders, and consumers about the risks of fungal contamination and ways to prevent it.

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