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# FUNGAL CONTAMINATION AND AFLATOXIN $B_1$ ON POSTHARVEST COFFEE BEANS IN NORTH SUMATERA, INDONESIA

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## ABSTRACT

The aim of the paper is to observe aflatoxin contamination and toxigenicity of *Aspergillus flavus* in relation to soil at plantations and coffee beans during drying and storage at smallholder plantations in Berastagi, Karo Regency, North Sumatra. Serial dilution and a direct plating method were used to determine the fungal population and the percentage of beans infected by fungal species. Toxigenicity of *A. flavus* was tested using a culture method and thin-layer chromatography. The results showed a total of 18 species of fungi were isolated from the soil plantation. *Aspergillus niger* was the most dominant (log 3.69 cfu/g), followed by *A. flavus* (log 3.47 cfu/g) and *A. tamarii* (log 3.43 cfu/g). *Cladosporium cladosporioides*, *Rhizopus stolonifer*, and *Mucor* sp. contaminated the coffee beans during drying, while *Aspergillus chevalieri*, *A. niger*, *A. repens*, *A. terreus*, and *Penicillium citrinum* contaminated the beans during storage. The highest percentage (15%) of beans contaminated during drying was caused by R. *stolonifer* and *Mucor* sp., whereas during storage, *A. niger* (15.5%) was the dominant contaminant, followed by *A. flavus* (14%). A total of eleven *A. flavus* strains were isolated, consisting of three strains from the plantation, seven strains from coffee beans during drying, and one strain from storage. Based on toxigenicity, 10 strains of *A. flavus* were aflatoxin producers, with three strains isolated from the soil, six strains isolated from coffee beans during drying, and one strain isolated during storage.

**Keywords:** Aflatoxin; *Aspergillus flavus;* Coffee beans; Fungal population; Toxigenicity

## 1. INTRODUCTION

Robusta coffee (*Coffea canephora* L.) is one of the most important commodities in Indonesia. However, most of the coffee is produced by smallholder farms, where harvest and postharvest handling are insufficiently controlled. The drying process by smallholder farmers is done under the sun using tarpaulin on the ground, leading to coffee beans being susceptible to fungal infection and mycotoxin contamination [1,2]. In addition, environmental and storage conditions with high relative humidity favour the growth and development of mycotoxigenic fungi [3]. According to Dharmaputra *et al*. [4] *Penicillium citrinum*, *Cladosporium cladosporioides*, *Fusarium solani*, *Aspergillus ochraceus*, *Endomyces fibuliger*, *A. niger*, *A. flavus*, and *A. tamarii* were the most predominant species found on coffee beans in the delivery chain. Green coffee beans during the pre-roasting stage were most contaminated by *Aspergillus* section *Nigri* and *Aspergillus* section *Circumdati* [5]. Yani [6] reported that coffee beans were contaminated during primary processing by strains of *A. flavus*. Lilia *et al*. [7] reported that more fungal contamination occurred on coffee beans stored in gunny sacks than that in polystyrene bag. Noonim *et al*. [8] stated that the diversity of the fungal population on coffee beans is correlated with the geographical origin and processing method. Yu [9] and Samuel *et al*. [10] reported that *A. flavus* is commonly found in temperate and tropical regions in soil, plant debris, compost piles, and agricultural areas such as maize, cotton, and ground nut farms. Environmental and geographical conditions in the soil determine the biodiversity characteristics and contamination of *Aspergillus* species on agricultural products [11,12]. During the harvest and postharvest period, coffee beans are highly susceptible to contamination by soil fungi. Therefore, this study investigates the fungal population in soil at smallholder coffee plantations in relation to fungal contamination and toxigenic *A. flavus* on coffee beans during drying and storage.

## 2. MATERIALS AND METHODS

### 2.1 Soil sampling at coffee plantation

Soil samples were taken from a ten-hectare smallholder coffee plantation at Berastagi, Karo Regency, North Sumatera during dry season from April to October 2021. First, 100 sampling plots (each plot 1×1 $m^2$) were determined randomly, and each plot was divided into 10 points. A 20-g soil sample was obtained from each point. The composite soil samples were placed in a sterile plastic bag and kept in a cool box for further use.

  

**2.2 Determination of population and characterization of soil fungi**

A 20-kg of soil sample was mixed thoroughly, and 25 g was placed in a 1000-ml Erlenmeyer flask. Sterilized distilled water was added until the volume was 250 ml. The suspension was homogenized, and 1 ml was placed in a Petri dish (9 cm in diameter) and pour plate with 18% dichloran glycerol agar medium (DG18, NEOGEN®, Lansing, MI, USA). Dilution was done up to $10^{-4}$. Three replications were made for each plate. All plates were incubated for 5 days at 29℃. Each species of fungal colonies was counted as a colony forming unit (cfu/g), isolated, and identified morphologically using potato dextrose agar (PDA) for Mucorales, Czapek yeast agar (CYA), and Czapek Doc yeast agar with 20% sucrose (CYA20S) for *Aspergillus* and *Penicillium* according to Pitt and Hocking [13].

**2.3 Coffee bean sample and parameter determination**

Next, 3500 g of coffee beans from the drying stage (2 days after harvesting) and storage stage (one month storage after sun-drying) were purchased from twenty five smallholder farmers at the area of a coffee plantation at Berastagi, Karo Regency, North Sumatera-Indonesia. The samples were divided into parts for parameter analysis of moisture content, the percentage of beans contaminated by fungi, and fungal population.

**2.3 Moisture content**

The bean moisture content was calculated on a wet basis using an oven drying method [14]. Samples of ground coffee beans (40 g) were dried in an oven at 130ºC for 2 hours with three replicates per sample.

**2.4 Percentage of coffee beans contaminated by fungi**

Next, 200-g samples of coffee beans in drying and storage stages were surface sterilized separately in a 500-ml beaker by immersion in 1 % sodium hypochlorite for one minute and then thoroughly washed three times with distilled water. The beans were then placed in a Petri dished containing sterilized filter paper to remove the rest of the water. 10 coffee-bean samples were cultured by direct plating on a Petri dish (9 cm in diameter) containing DG18. All plates were incubated for 5 days at 29℃.  Each plate was replicated 5 times. Each fungal colony growing on the beans was counted to determine the contamination percentage. Each fungal species was then isolated and identified based on morphological characteristics using PDA, CYA, and CYA20S. The percentage of coffee beans contaminated by each fungal species was determined by the following formula:

ISSN: 2455-6939

Volume: 09, Issue: 03 "May-June 2023"

$$M/N \times 100\%$$

M = Number of coffee beans contaminated by fungal species
N = Total number of coffee bean samples

**2.5 Fungal population on coffee beans**

The fungal population on coffee beans obtained during drying and storage was found by dilution. 2000-g samples of beans were powdered using a blender (Mill Powder RT-04 Series No. 980923, Mill Powder Tech. Co LTD, Taiwan) at 25,000 rpm for 30 seconds. Distilled water was added to 25 g of the powder in a 1000-ml Erlenmeyer flask until the volume was 250 ml. The suspension was then homogenized, and 1 ml of the suspension was placed in a Petri dish (9 cm in diameter) and pour plate with DG18 medium. Dilution was done until $10^{-4}$. Three replications were made for each dilution. All plates were incubated for 5 days at 29℃. Each separate colony of fungi was counted as a colony forming unit per gram sample (cfu/g).

**2.6 Toxigenicity of *Aspergillus flavus***

Aflatoxins produced by *A. flavus* strains were determined qualitatively using 10% coconut agar medium (CAM) according to Lin & Dianese [15]. Each isolate of *A. flavus* was inoculated at the centre of the medium in a Petri dish (9 cm in diameter). The plate was then incubated for 5 days at 29˚C. The presence of yellow pigment on the reverse side of the Petri indicated it was toxigenic (an aflatoxin producer). Quantitatively, the toxigenicity of *A. flavus* strains was determined using thin-layer chromatography. All isolates of *A. flavus* were cultured in 10% CAM for 5 days at 29˚C in a Petri dish (9 cm in diameter). The medium was placed in a 500-ml Erlenmeyer flask and extracted using 50 ml of methanol. The suspension then was homogenized for 30 minutes and filtered using Whatman no. 1 filter paper. 250 ml of the filtrate in the separating funnel was extracted twice with 50 ml of n-hexaneand cleaned up with 50 ml of chloroform. The extract was dehydrated in a vial and filtered using an hydrate sodium sulphate ($Na_2SO_4$). Using a micro syringe, 10 µl of the residue were dotted on a thin-layer chromatography plate (Merck No.1.05554, Silica gel 60, F254), and chromatography was run for 20 minutes. The developing solvent used was chloroform: acetone (9:1). Commercially available aflatoxins (Sigma-Aldrich Chemical Company, USA) were used as standards.

**2.7 Statistical analysis**

Data of fungal populations on soil and coffee beans were analysed using an analysis of variance (ANOVA) and Duncan's multiple range test at the 5% probability level for significant differences. For statistical analysis, SPSS software version 22 was used.

ISSN: 2455-6939

Volume: 09, Issue: 03 "May-June 2023"

## 3. RESULTS AND DISCUSSION

### 3.1 Fungal population on soil and coffee beans

A total of 18 genera of fungi were isolated in the coffee plantation area, which were dominated by *Aspergillus* (Table 1).

**Table 1: Population of soil fungi (cfu/g) at coffee plantation and on coffee beans during drying and storage**

| Fungal species | Fungal population (log cfu/g) | | |
|---|---|---|---|
| | Soil at coffee plantation | Coffee beans during drying | Coffee beans during storage |
| *Acremonium* sp. | 3.11[b] | 0.00[e] | 0.00[e] |
| *Aspergillus clavatus* | 3.36[ab] | 0.00[e] | 0.00[e] |
| *Aspergillus chevalieri* | 3.14[b] | 2.00[cd] | 2.30[c] |
| *Aspergillus flavus* | 3.47[a] | 2.00[cd] | 2.30[c] |
| *Aspergillus niger* | 3.69[a] | 2.00[cd] | 2.51[bc] |
| *Aspergillus repens* | 0.00[e] | 2.00[cd] | 2.51[bc] |
| *Aspergillus sydowii* | 3.30[b] | 0.00[e] | 0.00[e] |
| *Aspergillus tamarii* | 3.43[ab] | 0.00[e] | 0.00[e] |
| *Aspergillus terreus* | 3.30[b] | 0.00[e] | 1.30[d] |
| *Aspergillus* sp. | 3.41[ab] | 0.00[e] | 0.00[e] |
| *Cladosporium cladosporioides* | 0.00[e] | 2.51[bc] | 0.00[e] |
| *Fusarium* sp. | 3.00[b] | 0.00[c] | 0.00[e] |
| *Mucor* sp. | 0.00[e] | 1.00[d] | 0.00[e] |
| *Penicillium citrinum* | 0.00[e] | 1.00[d] | 1.52[cd] |
| *Penicilium* sp. | 3.00[b] | 0.00[e] | 0.00[e] |
| *Rhizopus stolonifer* | 0.00[e] | 2.51[bc] | 0.00[e] |
| *Trichoderma* sp.1 | 3.00[b] | 0.00[e] | 0.00[e] |
| *Trichoderma* sp.2 | 3.00[b] | 0.00[e] | 0.00[e] |
| Average | 2.35[c] | 0.83[d] | 0.69[de] |

Numbers followed by the same letters are not significantly different ($P<0.05$) according to Duncan's Multiple Range Test (DMRT). cfu/g = colony forming unit/gram

Among *Aspergillus*, *A. niger* was the most common (log 3.69cfu/g), followed by *A. flavus* (log 3.47 cfu/g), *A. tamarii* (log 3.43cfu/g), *Aspergillus* sp. (log 3.41 cfu/g), and *A. clavatus* (log

  

ISSN: 2455-6939

Volume: 09, Issue: 03 "May-June 2023"

3.36cfu/g). Some species such as *A. chevalieri*, *A. flavus,* and *A. niger* contaminated beans during drying, and their population increased during storage. *Cladosporium cladosporioides* was the predominant species during drying, followed by *Rhizopus stolonifer* and *Mucor* sp. The contamination by *A. repens*, *C. cladosporioides*, *P. citrinum*, *R. stolonifera,* and *Mucor* sp. during drying was probably due to the practice of sun-drying. In line with this study, Yassin *et al*. [16] reported that *R. stolonifer* had the second highest distribution on coffee beans, whereas *Cladosporium cladosporioides* is a field fungus and contaminates beans during preharvest or at harvest. Many studies revealed that *C. cladosporioides* was the most predominant in the collector delivery chain when the bean moisture content is still high in *Coffea arabica* [4] and at the beginning of storage of "physic nut" (*Jatropha curcas*) [17] and nutmeg (*Myristica fragrans*) [18]. *Cladosporium cladosporioides* isan aerial and phyllosphere fungus [19]. The fungi contamination occurs in the field or during harvesting, and their conidium grows during drying while the bean moisture content is still high.

During storage, fungal contamination on beans was dominated by *A. niger*, *A. repens*, and *P. citrinum* (log 2.51cfu/g), followed by *A. chevalieri* (log 2.30cfu/g), *A. flavus* (log 2.30cfu/g), and *A. terreus* (log 1.30cfu/g). Our results are consistent with the findings of Viegas *et al.* [5] that *Aspergillus* and *Penicillium* are the most predominant genera on pre-roast beans. A high population of *A. niger* at the plantation (log 3.69cfu/g) leads to the highest contamination on beans during storage. Alvindia & Acda [20] reported that *A. niger* was frequently isolated after drying coffee beans. Yassin *et al.* [16] found that among 12 fungal species isolated, *A. niger* was the most frequently isolated. Viegas *et al*. [5] and Al Attiya *et al*. [21] reported that *Aspergillus* section *Nigri* was the most common on pre-roast coffee beans. Among mycotoxigenic fungi, *A. niger,* toxigenic *A. flavus,* and *Penicillium* on coffee beans during storage have potential to produce mycotoxins. Noonim et al. [8] revealed that 13% of *A. niger* isolates from dried *Coffea arabica* samples produced achratoxin A (OTA). Some strains of toxigenic *A. flavus* have the potential to produce aflatoxins. *Aspergillus* and *Penicillium* are natural contaminants on coffee beans [22, 5] and they can infect them in fields and warehouses [23]. It seems that the presence of fungi on coffee beans during drying and storage is related to fungal species on the plantation. In harvesting practice by subsistence farmers, coffee berries commonly fall to the ground and are mixed with clean berries. Thus, subsistence farmer's crop yields are susceptible to contamination by field and storage fungi [24,25].

### 3.2 Coffee bean moisture content and the percentage of the beans contaminated by fungi

The moisture contents of coffee beans observed at drying and storage were 13.1 and 10.75%, respectively. According to SNI [26] the maximum moisture content for coffee beans is 12.5%. Moisture content of 13.3% in coffee beans during drying has potential to promote fungal growth.

  

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Volume: 09, Issue: 03 "May-June 2023"

The percentage of coffee beans contaminated by fungi showed that *R. stolonifer* was the most common contaminant during drying (50%), followed by *A. flavus* (14%) and *P. citrinum* (6%). Moisture content of 10.75% in stored beans does not inhibit fungal growth. As xerophilic fungi, both *A. flavus* and *P. citrinum*can grow with low pH and water activity [27,13]. As shown in Figure 1, during storage, *P. citrinum* showed the highest percentage of contamination (22%), followed by *A. niger* (4%) and *A. flavus* (2%).

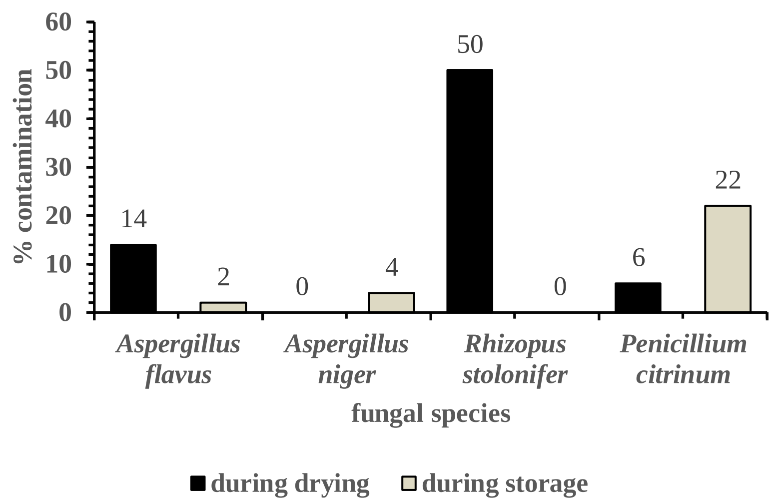


**Figure 1: The percentage of coffee beans contaminated by fungi during drying and storage**

### 3.3 Toxigenicity of *A. flavus* isolated from soil and coffee beans

As a saprobe fungus, *A. flavus* was the second most predominant in the coffee plantation. It resides in the soil and colonizes organic matter as sources of carbon and nitrogen [12]. A total of 11 *A. flavus* strains were isolated from soil at the plantation and coffee beans during drying and storage (Table 2). Based on the culture method on 10% CAM, each strain of *A. flavus* had a specific character with different colony diameter and diverse aflatoxin $B_1$ production, which was shown by different yellow pigment produced on the reverse side of the CAM medium in a Petri dish. Hussein and Brasel [28] and Vaamonde et al. [29] showed similar results that different strains of *A. flavus* produce aflatoxin in different proportions. We found that most of the *A. flavus* found on the soil at coffee plantations and coffee beans are toxigenic (aflatoxin producer). They consist of three strains isolated from the plantation ($TKO_1$, $TKO_2$, $TKO_3$), seven strains isolated from coffee beans during drying ($KPJ_1$, $KPJ_2$, $KPJ_3$, $KPJ_4$, $KPJ_5$, $KPJ_6$, $KPJ_7$), and one strain ($KPS_1$) isolated on beans during storage. Among the strains, $TKO_2$ and $TKO_3$ produced the highest amounts of aflatoxin $B_1$ ($AFB_1$) at 42.4 and 38.7 µg/kg, respectively. The other toxigenic strains produce $AFB_1$ at <3.01 µg/kg. One strain, $KPS_1$, does not produce aflatoxin. *Aspergillus*

  

ISSN: 2455-6939

Volume: 09, Issue: 03 "May-June 2023"

*flavus* had the second highest percentage of contamination on beans during drying. We found more toxigenic *A. flavus* on coffee beans during drying than on plantation soil and stored beans.

**Table 2: The toxigenicity of *A. flavus* strains isolated from soil at coffee plantation and the coffee beans during drying and storage**

| Sources of *A. flavus* | Code of strains | Culture on CAM | Colony diameter on CAM (mm) | Toxigenicity | Aflatoxin $B_1$ (µg/kg) |
|---|---|---|---|---|---|
| Soil at coffee plantation | $TKO_1$ | + | 50.75 | toxigenic | <3.01 |
| | $TKO_2$ | + | 54.25 | toxigenic | 42.4 |
| | $TKO_3$ | + | 44.20 | toxigenic | 38.7 |
| Coffee beans at drying | $KPJ_1$ | + | 33.40 | toxigenic | <3.01 |
| | $KPJ_2$ | + | 50.15 | toxigenic | <3.01 |
| | $KPJ_3$ | + | 53.10 | toxigenic | <3.01 |
| | $KPJ_4$ | + | 30.10 | toxigenic | <3.01 |
| | $KPJ_5$ | + | 19.90 | toxigenic | <3.01 |
| | $KPJ_6$ | - | 48.75 | non-toxigenic | 0.00 |
| | $KPJ_7$ | + | 48.65 | toxigenic | <3.01 |
| Coffee beans at storage | $KPS_1$ | + | 27.00 | toxigenic | <3.01 |
| Average | | | 41.84 | | |

CAM = coconut agar medium 10%; + = produces yellow pigment on the reverse side of Petri dish; - = no yellow pigment after 5 days of incubation at 29℃

Aflatoxin biosynthesis is determined by environmental factors such as moisture content [30] and fungal development [31]. Therefore, the presence of toxigenic *A. flavus* in plantation soil and on coffee beans requires proper pre-harvest and post-harvest approaches to prevent growth of the fungus.

## CONCLUSION

Fungi at a coffee plantation have the potential to contaminate coffee beans during drying and storage. Providing agricultural extension to smallholder coffee farmers is necessary to minimize fungal growth and aflatoxins contamination.

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