Fungal Infection and Toxigenicity *Aspergillus flavus* Isolated from Cacao and Coffee Beans in North Sumatera

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Keywords: Cacao, Collector chain, Fungal infection, *Aspergillus flavus*

Abstract: Cacao (*Theobroma cacao*) and coffee beans (*Coffea* sp,) in Indonesia produced mostly by small-scale plantation by farmers. This study was aimed to investigate fungal infection and toxigenicity of *Aspergillus flavus* strains on dried-stored cacao and coffee beans at collector distribution chain. As much as five kilogram dried-stored of the beans were collected from collector distribution chain at Karo Regency, North Sumatra.. The moisture content were determined by oven drying metthod. The percentage of beans infected by each fungal species was observed by direct plating on dichloran 18% glycerol agar (DG18) medium.. Fungal population was enumerated by a dilution followed by a pour plate in DG18 medium. Cultural method in agar medium containing 10% coconut milk was used to determine toxigenicity of *A. flavus*. Results showed moisture content cacao and coffee beans at collecto distribution chain was above National Indonesia Standard. Eighty eight percent of cacao beans were infected by *A. niger*, whereas, coffee beans were the most infected by *A. flavus*. (78.60%). A total of 14 strains of *A. flavus* were isolated, 4 strains found at cacao and 10 strains at coffee beans. Among of the total *A. flavus*, 3 strains are aflatoxin producers at cacao and 4 strains at coffee beans.

1 INTRODUCTION

Cacao (*Theobroma cacao*) and robusta coffee (*Coffea canephora* L.) are important commodities in North Sumatera. Most of cacao and coffee beans are produced by farmers as small-scale plantation (Direktorat Jenderal Perkebunan 2018). Preharvest and postharvest handling of the commodities such as harvesting, drying, and storing were conducted traditionally. Therefore, quality of the commodities under National Standard (Amaria et al. 2014). Physical damage of the beans caused by insect or inappropriate postsharvest handling accelerate fungal infection and mycotoxin contamination (SNI, 2017; Nurhadi et al. 2019).

Among storage fungi that commonly contaminate cacao beans are *Aspergillus, Botryodiplodia, Mucor, Fusarium, Neurospora, Penicillium* and *Phytophthora* (Fagbohun et al. 2011). Whereas, coffee beans were infected by *Aspergillus niger, Aspergillus flavus*, and *Aspergillus ochraceus* that produce ochratoxin A (Dharmaputra et al. 1999; Klich 2007; Djossuo et al. 2015). The purpose of the study was to enumerate fungal infection and toxigenicity of *A. flavus* strains on cacao and coffe beans at collector distribution chain in North Sumatera.

2 MATERIALS AND METHOD

2.1 Sampling of Cacao and Coffee

As much as four kilogram each of intact, dried-stored cacao and coffee beans as samples were obtained at collector distribution chain at Karo Regency, North Sumatera. Sample then was packed in a sterile polyethylene bag and keep in cold at ±12°C for further use.

2.2 Determination of Fungal Contamination

The percentage of beans infected by fungal species was conducted by direct plating on dichloran 18% glycerol agar medium. (DG18). Each sample was surface disinfected using 1% sodium hypochlorite solution for 1 minute, they were then dried using sterilized filter paper and placed in petri dish

Nurtjahja, K., Hastuti, L., Nurfalah, A. and Ramayani, .
Fungal Infection and Toxigenicity Aspergillus flavus Isolated from Cacao and Coffee Beans in North Sumatera.
DOI: 10.5220/0010613300002775
In *Proceedings of the 1st International MIPAnet Conference on Science and Mathematics (IMC-SciMath 2019)*, pages 558-560
ISBN: 978-989-758-556-2

(diameter. 9 cm) containing DG18. The number of cacao and coffee beans in petri dish was 5 and 10 respectively. Five replication were made for each sample. All plates were incubated at 29°C for 5 days.

2.3 Enumeration of Fungal Population

Fungal population on each sample was enumerated by a dilution and followed by pour plate in dichloran 18% glycerol agar (DG18) medium. Each sample bean was powdered and 25 g of which was placed in to a 500 ml flask and homogenate with 250 ml of sterile distilled water to obtain a 10^{-1} suspension. The dilution was carried out on 10^{-2}, 10^{-3} and 10^{-4}. One ml of the suspension was placed on DG18 medium in petri dish (diameter 9 cm).. Each dilution was triplicates. All plates were incubated at 29°C for 5 days.

2.4 Moisture Content Determination

Bean moisture content was determined by oven drying method according to Standard Nasional Indonesia (SNI, 2017).

2.5 Identification of *A. flavus*

Each of *A. flavus* was isolated on potato dextrose agar (PDA) medium then identified according to Pitt and Hocking (2009).

2.6 Toxigenicity of *A. flavus*

The toxigenicity of each A. flavus was determined by culturing in 10% coconut agar medium (CAM) in petri dish (Lin and Dianese 1978). The presence of yellow pigment at the reverse side of the medium indicate as aflatoxin producer.

3 RESULTS AND DISCUSSION

3.1 Moisture Content and Percentage of Fungal Contamination

Cacao and coffee beans at collectors distribution chains stored at room temperature (23 to 25°C) and packed in a 50 kilogram polyethylene and gunny bags. The moisture content of cacao and coffee beans was 8.2% and 12.5% respectively. The moisture content was higher than that maximum stndard moisture content for cacao (7.5%) (BSN 2017) and coffee beans (12 %) (SNI 2017). High relative humidity during storage might the dried stored cacao and coffee beans absorb water favor, and might promote fungal growth (Godet and Munaut 2010).

The percentage of the beans infected by fungal species (Table 1) showed that three fungal species were isolated. It was found that 88% of cacao beans was infected by *Aspergillus niger* followed by *A. flavus* (33.33%) and *A. rubrum* (28.88%. The results was similar to the study by Wangge et al. (2012), they found that cacao beans were common infected by *A. flavus* and *A, niger*. In similar, coffee bean was dominated by *A. flavus* (78.60%) followed by *A. niger* (6%).

Table 1: The percentage of cacao and coffee beans infected by fungi

Fungal species	% Beans infected by fungal species	
	Cacao bean	Coffee bean
Aspergillus flavus	33.33	78.60
A niger	88.88	6.00
A rubrum.	28.88	0

Most storage fungi that contaminate agricultural products was soil fungi, they contaminate during harvesting Agricultural products that fallen on the ground or contaminate to the soil during harvesting were susceptible infected by fungi (Dharmaputra et al. (2018). We assumed the infection of the *Aspergillus* on cacao and coffee beans at collector distribution chain occured during postharvest handling.

3.2 Fungal Population

A total of six fungal species were isolated (Table 2). Cacao beans more infected than that of coffee beans. *Aspergillus flavus* and and *A. niger* were the most found.

Table 2: Fungal population (cfu/g) isolated from dried-stored cacao and coffee beans collected from collectors distribution chain at Karo Regency, North Sumatera

	Fungal population (cfu/g)	
Fungal species	Cacao bean	Coffee bean
Aspergillus flavus	1×10^4	13×10^2
A fumigatus	0	1×10^2
A nidulan	0	0.3×10^2
A niger	1×10^4	0
A rubrum.	0.3×10^4	0
Penicillium sp..	0	0.3×10^2
Yeast	0.3×10^4	0

High fungal population at moisture content 8.2% in cacao and 12% in coffee beans indicate that most storage fungi were able to grow at low moisture content. The presence of yeast in cacao beans might the single cellular yeast still viable after fermentation stage before drying process. In addition, a total of 14 *A. flavus* strains were isolated from cacao and coffee beans. Higher moisture content in coffee beans might more *A. flavus* was found than that of cacao beans. The cacao bean was colonized by 3 strains of aflatoxigenic *A. flavus* while 4 strains on coffee beans.

4 CONCLUSION

The presence of fungal infection at cacao and coffee bean at collector distribution chain was potentially reduced quality and mycotoxin contamination. Appropriate postharvest handling of cacao and coffee beans was required to prevent fungal growth during storage.

ACKNOWLEDGEMENT

The research was funded by Universitas Sumatera Utara, contract DRPM Reseach grant no. 152/UN5.2.3.1/PPM/KP-DRPM/2021.

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