

# Toxigenic and Non Toxigenic *Aspergillus Flavus* Strains Isolated from Candlenut (*Aleurites Moluccana*) in North Sumatera

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Abstract: This study was conducted to investigate contamination of *Aspergillus flavus* strains in distribution chains of candlenut (*Aleurites moluccana*). Dried-stored unshelled candlenut were collected from farmers, collectors and distributors at 3 Regencies in North Sumatera (Regency of Karo, Deli Serdang and Langkat) during harvest period (February to April 2019). One and half kilogram of candlenut was taken from each of the distribution chains. Kernel moisture content were determined by distillation. Population each of *A. flavus* strain was enumerated by a dilution method followed by a pour plate in dichloran 18% glycerol agar (DG18) medium. Culture method in agar medium containing 10% coconut milk was used to determine toxigenicity of *A. flavus*. Their toxigenicity also was determined molecularly using specific primers for amplifying regulatory (*aflR*) and structural genes (*nor-1*, *ver-1*, *omt-1*) that determine aflatoxin biosynthesis. Results showed that moisture content of candlenut kernels at the level of farmers, collectors and distributors in Regency of Karo, Deli Serang and Langkat was above 5%. A total of 38 strains of *A. flavus* were isolated and 37 of the strains were toxigenic (aflatoxin producers). The average of *A. flavus* population (cfu/g) on candlenut at farmers was the lowest and the highest population was at distributor chain.

## 1 INTRODUCTION

Candlenut (*Aleurites moluccana*) is one of the important commodity in North Sumatera. The main area of candlenut producers in North Sumatera are at Deli serdang, Langkat, Binjai, Karo, Tapanuli, Dairi, Nias, and Asahan (Sihombing, 2015). Most of the candlenut are produced by farmers. Preharvest and postharvest handling of the nut such as harvesting, drying, cracking and storing were conducted conventionally.

Drying using sun light in open air and high relative humidity during inappropriate storage, therefore, dried-stored candlenut is susceptible infected by fungi and contaminated by mycotoxins. In addition, high lipid content in the nut commonly contaminated by fungi (Krishnawati et al. 2011). Among storage fungi that common contaminate unshelled candlenut are *Aspergillus flavus*, *A. niger*, *A. wentii*, *A. tamaritii*, *Aspergillus rubrum*, *A. chevallieri* and *Penicillium citrinum* (Pitt et al. 1998 ; Lambaga, 2005).

*Aspergillus flavus* is one of toxigenic fungi that commonly contaminated on corn, peanut, spices, nuts, cotton seeds, pistachio, etc. However, not all

strains of *A. flavus* are toxigenic (aflatoxin producer) (Ehrlich (2014). Toxigenicity of *A. flavus* is determined by strains, substrates, geography and culture technique (Perrone et al. 2014). The purpose of the recent study was to enumerate *A. flavus* strains and their toxigenicity that contaminated on shelled candlenut at farmers, collectors and distributors chains in North Sumatera.

## 2 MATERIALS AND METHOD

### 2.1 Sample Collection

As much as 1500 g samples of unshelled candlenut were obtained at farmers, collectors and distributors chain at 3 Regencies in North Sumatera i.e Karo, Deli Serang and Langkat. Tree replicates were conducted for each sample Sample then was placed into a sterile polyethylene bag and stored in a refrigerator at  $\pm 12^{\circ}\text{C}$  for further use.

## 2.2 Determination of *A. flavus* Population

*Aspergillus flavus* population on each sample was enumerated by a dilution method and followed by pour plate method in dichloran 18% glycerol agar (DG18) medium. Each sample was ground and 25 g were ground nut was placed onto a 500 ml flask and suspended with 250 ml of sterile distilled water and then homogenized 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 (9 cm in diameter). Each dilution was replicate 3 times. All plates were incubated for 5 days (29°C). Population of *A. flavus* per gram of candle nut (cfu/g) was determined using the formula:

$$A. \text{ flavus population} = \frac{1}{X \cdot Y} \cdot Z \text{ (cfu/g)}$$

X = volume of suspension transferred to each petri dish (1 ml)

Y = dilution which gives the *A. flavus* colonies separately

Z = average number of colonies of *A. flavus* from 3 petri dishes

## 2.3 Morphological Identification of *Aspergillus flavus*

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

## 2.4 Determination on the Toxigenicity of *A. flavus* Strains

The potential aflatoxin production of each *A. flavus* strains was determined by culture method in 10% coconut agar medium (CAM) in petri dish (9 cm in diameter) according to the procedure of Lin and Dianese (1978). The aflatoxin producers were indicated by the presence of yellow pigment at the reverse side of the medium.

## 2.5 Extraction of Genomic DNA

About 40 mg *A. flavus* mycelia, harvested from 4 days old in a microtube containing 600 µl nuclei lytic was homogenised and extracted following procedure of Mini Kit (Promega, Madison, WI, USA). The DNA concentration obtained was determined using nanophotometer (IMPLEN, Munich, Germany) and followed by electrophoresis using 1.2% agarosa gel (SCIE-PLAS, Cambridge,

England), stained by 1µl ethidium bromide (EtBr) and visualised by Gel Doc (Uvitecc, Cambridge, Serial) under UV light (303 nm).

## 2.6 PCR Amplification

Specific primers used to amplify genes determine aflatoxin biosynthesis were regulatory gene (*aflR*) and structural genes (*omt-1*, *nor-1*, *ver-1*,) with the length of the base 1032, 895, 400, 1232 bp respectively. Amplification reaction was conducted according Erami et al. (2007). PCR was performed as follows: as much as 12,5 µL amplification mix [dNTP, Taq DNA polymerase (Promega Corporation Madison WI), MgCl<sub>2</sub>, Sybr Green 1 dye], 1 µM on each primer and 75 ng DNA template were added until the volume up to 25 µL. PCR was conducted 35 cycle consisted of preincubation for 10 min (94 °C), denaturation 1 min (94 °C), annealing for 2 min (65 °C), extension for 2 min (72 °C), final extension for 7 min (72 °C). All amplification process was 3 h 20 min. The PCR products were continued by electrophoresis (SCIE-PLAS. Ltd, Cambridge, England) using 1.5% agarose gels in 1 × TAE [40 mM Tris-acetate, 1 mM EDTA (pH 8)]. Gels were stained with 0.5 µg µl<sup>-1</sup> ethidium bromide and visualized using Gel Doc (Uvitec, Cambridge, Serial no. 13200263) under UV light (260 nm) and 1 kb DNA ladder (Promega, Madison, WI) was used as standard.

## 3 RESULTS AND DISCUSSION

### 3.1 Moisture Content of Candlenut

The moisture content of all candlenut obtained at farmers, collectors and distributors chain in Regency of Karo, Deli Serang and Langkat found that the highest moisture occurred farmers (Table 1). According to Indonesia National Standard (SNI 01-1684-1998) the maximum moisture content of candlenut up to 5%. Most candlenut in all distribution chains above standard, it means that the commodity are potential contaminated by fungi. Most of the storage fungi are xerophilic and grow at low water activity (Pitt and Hocking 2009). Therefore, improper postharvest handling might increase *A. flavus* population.

Table 1: Candlenut moisture content of candlenut obtained at farmers, collectors and distributors at Regency of Karo, Deli Serdang and Langkat

Regencies	candlenut moisture content (%)		
	Farmers	Collectors	Distributors
Karo	5.49	5.19	5.02
Deli Serdang	5.33	4.69	5.09
Langkat	5.19	5.09	5.02

### 3.2 Population of *A. flavus*

All samples of candlenut were contaminated by *A. flavus* (Table 2). In general, the lowest contamination occur at farmers and it become increase at collectors and at distributors the ontamination were highest.

Table 2: Population of *A. flavus* (cfu/g) isolated from candlenut on distribution chains at farmers, collectors and distributors at Regency of Karo, Deli Serdang and Langkat

<i>A. flavus</i> code	Primers				CAM
	<i>aflR</i>	<i>omt-1</i>	<i>nor-1</i>	<i>ver-1</i>	
Af <sub>1</sub> K	+	+	+	+	+
Af <sub>2</sub> K	+	+	+	-	-
Af <sub>1</sub> D	+	+	+	+	+
Af <sub>2</sub> D	+	+	+	+	+
Af <sub>1</sub> L	+	+	+	+	+
Af <sub>2</sub> L	+	+	+	+	+

+ = amplicon were amplified

- = no amplification

+/- = the presence of yellow pigment in coconut agar medium (CAM)

The presence of regulatory and structural genes in each of *A. flavus* determine aflatoxigenicity. The results was similar to the study of Criseo et al. (2001) Erami et al. (2007) and Nurtjahja et al. (2019). It was found that more toxigenic *A. flavus* strains than that of non toxigenic on candlenut.

## 4 CONCLUSION

The presence of toxigenic *Aspergillus flavus* at candlenut on distribution chains were potential to spoil and contaminated by aflatoxin. Good handling practices on candlenut were required to prevent the fungal growth and produce aflatoxins during storage.

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