Infection and Population of *Aspergillus chevalieri* on Dried-stored Tropical Spices

K. Nurtjahja[1*], C. F. Zuhra[2], H. Sembiring[2], A. Bungsu[1], J. Simanullang[1], J. E. Silalahi[1], B. N. L. Gultom[1], Sartini[3]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Jln. Bioteknologi no. 1, Medan, North Sumatera, Indonesia 20155*

[2]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Jln. Bioteknologi no. 1, Medan, North Sumatera, Indonesia 20155*

[3] *Faculty of Biology, Medan Area University, Jln. Kolam no. 1 Medan, North Sumatera, Indonesia 20223*

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Abstract: Fourty five samples of 10 dried stored-spices (black pepper, candle nut, cinnamom, cloves, coriander, cordamom, cumin, nutmeg, star anise, white pepper) obtained from 8 to 9 retailers at 5 traditional markets in Medan, North Sumatera was investigated for infection by *Aspergillus chevalieri* (formerly *Eurotium chevalieri*). Population of the fungal species was enumerate using dichloran 18% glycerol agar medium. Moisture content of each spices and fungal characteristics were also observed. Results showed each dried-stored spices has different moisture content, candle nut and nutmeg have the lowest moisture content (4.2 and 8.1 %) and white pepper and cinnamon have the highest moisture content (14.4 and 12.8 %) consecutively. All spices observed infected by *A. chevalieri*, the infection occurred predominantly on coriander followed by nutmeg and white pepper with fungal population 4.58, 4.41 and 4.02 (log CFU g^{-1}) respectively.

1 INTRODUCTION

Molds infection on agricultural commodities particularly on dried stored spices is one of the most common problem during storage. The infection is caused by molds that survive during pre and post-harvest handling (Stankovic et al. 2006; Toma and Abdulla, 2013). Pre-harvest handling is the main inoculum for causing contamination of diverse strains of molds on spices in the field (Kneifel and Berger, 1994). Poor handling practices of drying, transportation and storage can incrase fungal infection.

Xerophilic mold are able to grow at water activity (a_w) ≤ 0.85 (Pitt and Hocking, 2009), the other fungal species have been reported to grow at a_w value 0.64 – 0.75 (Butinar et al. 2005). *Aspergillus chevalieri* (formerly called *Eurotium chevalieri*) characterized by having yellow cleistotecia, uniseriate conidial head and yellow orange hyphae. Hubka et al. (2013) reported that *A. chevalieri* is one of xerophilic and xerotolerant molds that cause predominant spoilage on nut, dried beans, spices etc. [Samson et al. 1995; Pitt and Hocking, 2009). The ability to grow at low a_w or equilibrium relative humidity (ERH) makes *E. chevalieri* increase a_w value and allow other toxigenic molds such as *Aspergillus* and *Penicillium* to grow. The aim of the present study was to investigate infection and population of *Aspergillus chevalieri* isolated from dried stored spices in retailers on traditional markets in Medan, North Sumatera.

2 MATERIALS AND METHODS

2.1 Sample Collection

Fourty five composite samples (200 g for each sample) of 10 kinds of stored-dried spices *i.e.* black pepper, candle nut, cinnamom, cloves, coriander, cardamom, cumin, nutmeg, star anise and white pepper were collected from 8 to 9 retailers at 5 different traditional markets. Each of the sample were packed in steril polyethylene bag and stored in refrigerator (-4 ºC) for further use.

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2.2 Morphology Colony

Colony characteristics were observed macroscopically from cultures grown for 7 days (29±2 ºC) at dichloran 18% glycerol agar (DG18) and malt extract agar (MEA) medium. Microscopic morphology made using light microscope, Olympus CH2 Japan.

2.3 Determination Population of *A. chevalieri*

Population of *A. chavalieri* on each spice was determined using dilution method according to Pitt and Hocking (2009). Each sample (200 g) was ground for 30 seconds using blender (Model RT-04, Taiwan). Triplicate plates were made for each dilution. Each plates were incubated for 7 days at 29±2 ºC. All *A. chevalieri* colonies were counted as colony forming unit (CFU g^{-1}) of sample. Each single separate of the colony was isolated and cultured on czapex yeast extract agar (CYA) or CYA+20% sucrose (CYA20S) and identified according to procedure Pitt and Hocking (2009).

2.4 Moisture Content Analysis

Spice moisture content was determined according to oven drying method [4]. Fifty gram of ground sub sample stored-dried spices were put in aluminum foil dish and dried in oven at 110 ºC for 24 h and reweighed, three replicates per sample. Moisture content was calculated using the following formula:

$$\text{Moisture content (\% wet basis)} = \frac{(M_0 - M_1)}{M_0} \times 100 \qquad (1)$$

M_0 = initial weight, in grams of test portion
M_1 = final weight, in grams of dried test portin

3 RESULTS AND DISCUSSION

Dried-stored spices sold by retailers in traditional markets commonly packed separately in small plastic container or plastic bag. Each sample of the spices studied was presented in Table 1.

3.1 Spice Moisture Content

Moisture content of each dried-stored spices was presented in Figure 1. The spices has different moisture content (4.2-12.8%), except white pepper has moisture content (14.4%) higher than recommended by International food standard that stated safe moisture level that has to be achieved for spices is 12-14%.

Table 1: Ten species of dried-stored spices commonly sold by retailers at traditional markets.

No.	Spices (scientific name)	English name	Used parts
1.	*Piper nigrum* L.	black pepper	seeds
2.	*Aleurites moluccana* L.	candle nut	kernels
3.	*Cinnamomum zeylanicum* Blume	cinnamon	bark
4.	*Syzygium aromaticum* L.	Cloves	flowers
5.	*Coriandrum sativum* L.	coriander	seeds
6.	*Amomum cardamomum* L.	cordamom	seeds
7.	*Cuminum cyminum* L.	Cumin	seeds
8.	*Myristica fragrans* Houtt.	nutmeg	kernels
9.	*Illicium verum* Hook.	star anise	fruit
10.	*Piper nigrum* L.	white pepper	seeds

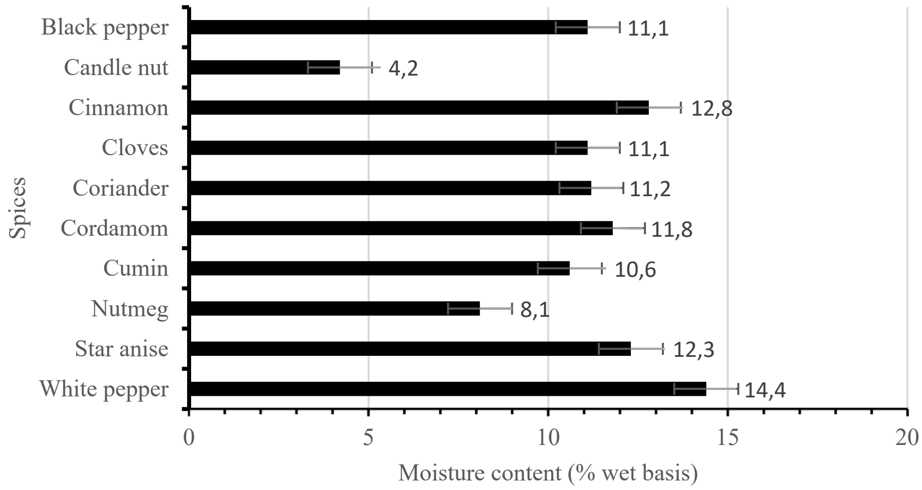

Figure 1: Moisture content (% wet basis) of dried-stored spices obtained from retailers in traditional Markets.

3.2 Morphological Characteristics of *A. chevalieri*

Aspergillus chevalieri, formerly *Eurotium chevalieri*, is characterized forming a yellow cleistotecia (teleomoph) in DG18 medium. Previuos study by Hubka et al. [6] reported that the ascospores *A. chevalieri* were smooth. Andrew and Pitt [9] described the ascospore with prominent crests like pulley wheels, with two prominent, narrow, longitudinal flanges. Conidial head (anamorph) uniseriate with blue or yellow orange.

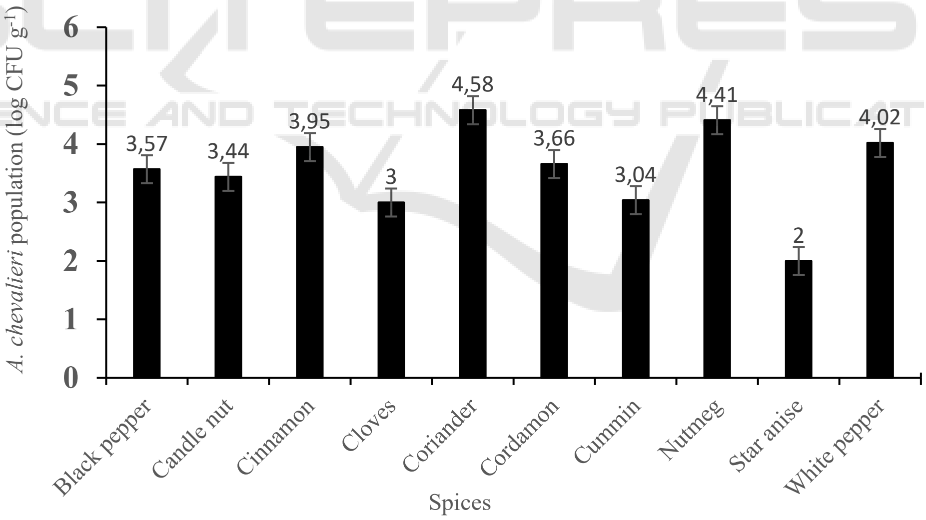

Figure 2: Population of *A. chevalieri* (CFU g^{-1}) at different dried-stored spices isolated on DG18 medium at ambient temperature (29±2 ºC).

3.3 Population of *A. chevalieri*

All spices studies were infected by *A. chevalieri* with different population (Figure 2). Coriander was the most infected (4.58 log CFU g^{-1}) and star anise was the less (2 log CFU g^{-1}). The presence of *A. chevalieri*
in substrate with low moisture content levels was studied at physic nut, pepper and garden thyme (Hashem and Alamri, 2010), nutmeg (Dharmaputra et al. 2015). Yazdani et al. (2009) reported that genus *Eurotium* sp. was obligately xerophilic that growth at a_w range 0.93-0.68. Low moisture level on spice (in Figure 1) not reduce the fungal population (in Figure 2). However, star annise with moisture higher content (12.3%) than nutmeg (8.1) and coriander (11.2%) infect by low population of *A. chevalieri*. The presence of antifungal avtivity of star anise (*Illicium verum*) on *A, niger* was previously studied by Yazdani et al. (2009). We assumed that the growth of *A. chevalieri* was inhibited by antifungal in the spices.

4 CONCLUSIONS

Dried-stored spices sold by retailers in traditional markerts were infected by *A. chevalieri*. Among of the spices, coriander was the most infected followed by nutmeg kernels and white pepper. Reducing the mold growth is required to prevent deterioration of the spices during storage.

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