Enumeration of Fungal Population Isolated from Dried-Stored Spices at Retailers in Traditional Markets in Medan, North Sumatera

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Keywords: Filamentous Fungi, Isolation, Spices, Traditional Market

Abstract : Fungal population on dried spices sold by retailers at traditional market in Medan was studied, the aim was to investigate the number of postharvest fungi on each spice. A total of ten kinds of dried spices i.e. white and black pepper, coriander, candle nut, cloves, cinnamon, star anise, nutmeg, cardamom and cumin were obtained from retailers at five traditional markets in Medan. As much as 250 g of the each spice was taken from five retailers at each traditional market. Fungal population was determined using a dilution method and followed by a pour plate method on dichloran 18% glycerol agar medium. Each fungal species was isolated and identified using czapex yeast agar and malt extract agar. Results showed that dried spices sold by retailers in traditional markets were infected by storage fungi. Spice infection in traditional markets such as Pringgan, Setiabudi and Sikambing was more predominant than that in Padang bulan and Simalingkar. Among the fungi aspergilli was the most common found and Aspergillus chevalieri has the highest population and almost found in all kinds of spice samples.

1 INTRODUCTION

Spices are one of Indonesia's main export commodities. This commodity plays an important role because it is used daily as a mixture of traditional food, drinks and medicines. Most tropical spices was harvested conventionally, the spices were dried using sunlight and stored in gunny or polypropylene sacks that permeable to water favor. Among microorganisms, fungi are the main cause of spice damage (Kneifel and Berger, 1994; Dimic et al, 2000; Romagnoli et al, 2007). The increase of water content during storage can cause fungal infection. Postharvest fungi such as *Aspergillus* and *Penicillium* are able to grow in water activities 0.78 (Koci-Tanackov et al., 2007). Fungal infection on spices causes a decrease in quality such as physical damage, loss of aroma and contain mycotoxins (McKee, 1995; Koci-Tanackov et al., 2007). Dried stored spices in traditional markets are generally sold by retailers in open air. High humidity can cause an equilibrium relative humidity between dried spice and the surrounding air, as a result in increase in spice water content. The purpose of this study was to investigate the number of fungal population in spices sold by retailers at traditional markets in Medan.

2 MATERIALS AND METHODS

2.1 Sample Collection

A total of 250 g samples from ten kinds of dried spices such as white and black pepper, coriander, candle nut, cloves, cinnamon, star anise, nutmeg, cardamom and cumin were obtained from 5 retailers at five traditional markets, namely Simalingkar, Sei Sikambing, Setiabudi, Pringgan and Padang bulan. Each sample was put into a sterile polyethylene bag and stored in a refrigerator at ± 12°C for further use.

Gultom, B., Nurtjahja, K., Bungsu, A., Simanullang, J., Silalahi, J. and Riyanto, .
Enumeration of Fungal Population Isolated from Dried-stored Spices at Retailers in Traditional Markets in Medan, North Sumatera.
DOI: 10.5220/0010199600002775
In *Proceedings of the 1st International MIPAnet Conference on Science and Mathematics (IMC-SciMath 2019)*, pages 407-412
ISBN: 978-989-758-556-2

2.2 Determination Fungal Population

The population of each fungus was determined using a dilution method followed by pour plate method on the DG18 medium. Each 250 g sample was ground using a Mill Powder RT-04 No. blender Series 980923 (Mill Powder Tech. Co. LTD, Taiwan) with a speed of 25000 rpm for 30 seconds. As much as 25 g were put into a 500 ml erlenmeyer and suspended with 250 ml of sterile distilled water and then homogenized to obtain a 10^{-1} suspension. Furthermore, dilution is carried out on 10^{-2}, 10^{-3} and 10^{-4}. Every 1 ml of dilution suspension was cultured by pouring method using DG18 medium. Each dilution was repeated 3 times. All plates were incubated for 5 days (29°C). Fungal population in each gram of spice (cfu/g) was determined using the formula:

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

X = volume of suspension transferred to each petri dish (1 ml)
Y = dilution which gives the fungus colonies separately
Z = average number of colonies of each fungal species from 3 petri dishes

2.3 Fungal Identification

Each fungus was cultured on the medium of malt extract agar (MEA) and czapex yeasr agar (CYA) and then identified according to Pitt and Hocking (2009).

3 RESULTS AND DISCUSSION

All the dried spices sold by retailers in traditional markets were infected by various species of fungi and Aspergilli was the most common found. The fungal infection on each spice was varies depending on the kind of spice. Black pepper is the most infected, whereas, star anise was the lowest. Fungal population (cfu/g) in each spice sold by retailers in 5 traditional markets was shown as follows:

White Pepper. Fungal infection on white pepper sold in the Sikambing market has the highest population. Eight species of of the fungi were found in white pepper and *Aspergillus sydowi* was the highest population followed by *A. candidus* (Table 1).

Table 1. Fungal population (cfu/g) isolated from white pepper sold by retailers at traditional markets in Medan, North Sumatera

Fungal species	Traditional markets / fungal population (cfu/g)				
	1	2	3	4	5
Aspergilllus flavus	0.3×10^3	0	0.3×10^3	3.3×10^3	0.6×10^3
A. niger	0	1×10^3	1.6×10^3	2×10^3	0.3×10^3
A. sydowii	100×10^3	32.6×10^3	66.6×10^3	79.3×10^3	79.6×10^3
A. tereus	0	0	0.3×10^3	0	0
A. tamarii	0	0	0	1.3×10^3	0
A. candidus	12×10^3	17×10^3	0	22.6×10^3	2×10^3
A. chevalieri	12.6×10^3	7×10^3	13.3×10^3	1×10^3	17×10^3
Penicillium sp.	6.3×10^3	1×10^3	1.3×10^3	0.6×10^3	15.6×10^3

Simalingkar (1), Padang bulan (2), Pringgan (3), Setiabudi (4), Sikambing (5).
cfu = colony forming unit

Black Pepper. Thirteen species of fungi were successfully isolated by black pepper, *A. niger* had the highest population in black pepper particularly in the Padang Bulan market, followed by *A. ustus* in Sikambing (Table 2). The infection of *A. fumigatus*, *A. ustus* and *A. chevalieri* are found in all markets.

Table 2. Fungal population (cfu/g) isolated from black pepper sold by retailer at traditional markets in Medan, North Sumatera

Fungal species	Traditional markets / fungal population (cfu/g)				
	1	2	3	4	5
Aspergillus flavus	0	2×10^3	0.3×10^3	0.3×10^3	0.3×10^3
A. niger	0	22×10^3	2.3×10^3	5×10^3	1.6×10^3
A. oryzae	0	0.3×10^3	0	0	0
A. sydowii	0	0	0.6×10^3	0	0
A. fumigatus	2.3×10^3	3×10^3	0.6×10^3	19×10^3	2×10^3
A. tereus	0	0.3×10^3	0.3×10^3	1×10^3	0
A. ustus	5.6×10^3	1×10^3	1.6×10^3	2×10^3	16.6×10^3
A. tamarii	0	0	0	0	0.3×10^3
A. candidus	1×10^3	0	5×10^3	0	1.3×10^3
A. chevalieri	3×10^3	1.3×10^3	6×10^3	2.6×10^3	6×10^3
Fusarium sp.	0	0	0.6×10^3	0	0
Mucor sp.	0	0	0	1.6×10^3	0.3×10^3
Penicillium sp.	0.6×10^3	0	0	0	0

Simalingkar (1), Padang bulan (2), Pringgan (3), Setiabudi (4), Sikambing (5).
cfu = colony forming unit

Coriander. Seven species of postharvest fungi isolated from coriander were found, Aspergillus chevalieri has the highest population and is found in all markets followed by *Penicillium* sp. and *Cladosporium cladosporioides* (Table 3).

Table 3. Fungal population (cfu/g) isolated from coriander sold by retailer at traditional markets in Medan, North Sumatera

Fungal species	Traditional markets / fungal population (cfu/g)				
	1	2	3	4	5
Aspergillus flavus	0	0.3×10^3	0	0	0
A. candidus	0	4.3×10^3	0	0	0
A. fumigatus	0	0	1×10^3	0	0
Cladosporium cladosporoides	$0,3\times10^3$	0	$0,3\times10^3$	0	0
A. chevalieri	$22,3\times10^3$	109×10^3	19.6×10^3	39.3×10^3	0.6×10^3
Penicillium sp.	1.3×10^3	2×10^3	0	0.6×10^3	12×10^3

Simalingkar (1), Padang bulan (2), Pringgan (3), Setiabudi (4), Sikambing (5).
cfu = colony forming unit

Candle nut. Eight species of postharvest fungi isolated from candlenut were found and *Aspergillus chevalieri* was the most predominant fungi (Table 4). Among the traditional markets candle nut in Pringgan was the most infected (six species) by the filamentous fungi.

Table 4. Fungal population (cfu/g) isolated from candle nut sold by retailer at traditional markets in Medan, North Sumatera

Fungal species	Traditional markets / fungal population (cfu/g)				
	1	2	3	4	5
Aspergillus flavus	0	1×10^3	0	0	0
A. niger	0	0	0	0.6×10^3	0
A. sydowii	0	1×10^3	0	0.3×10^3	0
A. wentii	0	1.3×10^3	0	0	0
A. fumigatus	0	0	0	0.3×10^3	0
A. tamarii	0	0.3×10^3	0	0	0
A. chevalieri	0	4.6×10^3	8.6×10^3	0	0.6×10^3
Penicillium sp.	0	0.6×10^3	0.3×10^3	0	0

Simalingkar (1), Padang bulan (2), Pringgan (3), Setiabudi (4), Sikambing (5).
cfu = colony forming unit

Cloves. Only 4 species of filamentous fungi isolated from cloves and *Cladosporium cladosporioides* is the highest population in cloves (0.6 × 104 cfu / g) (Table 5).

Table 5. Fungal population (cfu/g) isolated from cloves sold by retailer at traditional markets in Medan, North Sumatera

Fungal species	Traditional markets / fungal population (cfu/g)				
	1	2	3	4	5
Aspergillus flavus	0	0	0.3×10^4	0	0
A. chevalieri	0	0	0.3×10^4	0	0
Cladosporium cladosporoides	0.6×10^4	0	0	0	0
Penicillium sp.	0	0.3×10^4	0	0	0.3×10^4

Simalingkar (1), Padang bulan (2), Pringgan (3), Setiabudi (4), Sikambing (5).
cfu = colony forming unit

Cinnamon. A total of 4 species of fungi that attack cinnamon from all markets. *Aspergillus chevalieri* has the highest population, found in Simalingkar market and Setiabudi market (Table 6).

Table 6. Fungal population (cfu/g) isolated from cinnamon sold by retailer at traditional markets in Medan, North Sumatera

Fungal species	Traditional markets / fungal population (cfu/g)				
	1	2	3	4	5
Aspergillus flavus	0.3×10^3	0	0	0	0
A. carboniferous	0	0.3×10^3	0	0	0
A. fischerianus	0	0.3×10^3	0	0	0
A. chevalieri	0.6×10^3	0	0	0.4×10^3	0

Simalingkar (1), Padang bulan (2), Pringgan (3), Setiabudi (4), Sikambing (5).
cfu = colony forming unit

Star anise. Among the spices, star anise is the least infected by fungi (Table 7). Three species of storage fungi were isolated from star anise. No fungal infection on star anise sold in Pringgan and Sikambing.

Table 7. Fungal population (cfu/g) isolated from star anise sold by retailer at traditional markets in Medan, North Sumatera

Fungal species	Traditional markets / fungal population (cfu/g)				
	1	2	3	4	5
Aspergillus fumigatus	0	0	0	0.3×10^3	0
A. chevalieri	0.3×10^3	0	0	0	0
Penicillium sp.	0.3×10^3	0.3×10^3	0	0	0

Simalingkar (1), Padang bulan (2), Pringgan (3), Setiabudi (4), Sikambing (5).
cfu = colony forming unit

Nutmeg kernels. A total of 10 species of fungi were isolated from nutmeg. The infection was dominated by *Aspergillus chevalieri* followed by *Aspergillus flavus*. Nutmeg kernels in Padang bulan was the highest infected by fungi followed by Setiabudi market (Table 8).

Table 8. Fungal population (cfu/g) isolated from nutmeg kernels sold by retailer at traditional markets in Medan, North Sumatera

Fungal species	Traditional markets / fungal population (cfu/g)				
	1	2	3	4	5
Aspergillus flavus	0	0.3×10^3	80×10^3	2.6×10^3	30×10^3
A. niger	0.3×10^3	0	0	0	0
A. tamarii	0	0	0	0	11.6×10^3
A. candidus	2.6×10^3	2×10^3	0	0	0
A. ustus	24.6×10^3	4×10^3	0	0	0
A. wentii	0	0	2.6×10^3	0	0
A. chevalieri	7×10^3	67×10^3	0	56.6×10^3	0
Fusarium sp.	0	1.6×10^3	0	0.3×10^3	0
Penicillium citrinum	0	2.3×10^3	6×10^3	0.3×10^3	6.6×10^3
Rhizopus stolonifer	0.3×10^3	0	0	0	0

Simalingkar (1), Padang bulan (2), Pringgan (3), Setiabudi (4), Sikambing (5).
cfu = colony forming unit

Cardamom. All cardamom sold by retailers in traditional markets have infected by fungi. A total of 12 species of fungi isolated and *A. westerdijkiae*, *A. niger* and *A. chevalieri* were the most perdominant found followed by *A. restrictus* and *A. tamarii*. (Table 9).

Table 9. Fungal population (cfu/g) isolated from cardamom sold by retailer at traditional markets in Medan, North Sumatera

Fungal species	Traditional markets / fungal population (cfu/g)				
	1	2	3	4	5
Aspergillus flavus	1.6×10^3	0	0	0	0
A. niger	17.3×10^3	1.3×10^3	0	0	0
A. restrictus	0	0	0.3×10^3	0	16×10^3
A. tereus	0	0	0	0.3×10^3	0
A. tamarii	4.3×10^3	13.3×10^3	0	0	0
A. ustus	2.6×10^3	0.6×10^3	3×10^3	0	0
A. westerdijkiae	38×10^3	0.3×10^3	0	0	0
A. fumigatus	0	11×10^3	0	0	0
A. japonicus	0	0	0.6×10^3	0	0
A. chevalieri	0.3×10^3	1.6×10^3	0.3×10^3	17.3×10^3	3.6×10^3
A. repens	0	0	0	3×10^3	0
Penicillium sp.	0	0	0	0	0.3×10^3

Simalingkar (1), Padang bulan (2), Pringgan (3), Setiabudi (4), Sikambing (5).
cfu = colony forming unit

Cumin. All cumin sold by retailers in traditional markets was infected with postharvest fungi. Setiabudi market has the highest infection. *Aspergillus chevalieri* was the most common found on Setiabudi followed by *A. ustus* on the Simalingkar market (Table 10).

Table 10. Fungal population (cfu/g) isolated from cumin sold by retailer at traditional markets in Medan, North Sumatera

Fungal species	Traditional markets / fungal population (cfu/g)				
	1	2	3	4	5
Aspergillus fumigatus	0	0.3×10^3	0	0	0
A. niveus	1×10^3	0	0.3×10^3	0	0
A. ustus	2.3×10^3	0	0	0	0
A. chevalieri	0	0.3×10^3	1.6×10^3	2.6×10^3	1×10^3
A. repens	0	0	1×10^3	0.3×10^3	1×10^3
Penicillium sp.	1×10^3	0	0.3×10^3	0	0

Simalingkar (1), Padang bulan (2), Pringgan (3), Setiabudi (4), Sikambing (5).
cfu = colony forming unit

Stored-dried spices sold by retailers in traditional markets in Medan were commonly infected by storage fungi. We assumed that the infection occurred during pre and post-harvest handling. Stored in open air and high relative humidity at traditional markets lead to stored-dried spices absorbed water vapor from the surrounding air as a result spice water content increase during storage. Nurtjahja et al. (2017) reported that dried nutmeg kernels stored in various water activity (a_w) showed at a_w 0.75-0.97 were dominated by storage fungi such as *Aspergillus chevalieri* and *Penicillium citrinum*. However, some spices such as cinnamon, star anise and cloves less infected than the other. The presence of antifungal compounds in the spices might inhibit fungal growth. Research conducted by Freire et al. (2011) showed star anise (*Illicium verum*) contain chemical compounds such as limonene, terpineol, α-pinene, methyl-chavicol and trans-anetol. The activity of antifungal of the compounds at a dose of 1 µL / mL inhibit *A. flavus* mycelia, whereas *A. parasiticus* is inhibited at 2.0 µL / mL. The ability of postharvest fungi to live at a_w (<0.85) lead to increase their population. Dried spices sold by relatilers at traditional markets were packed in gunny sack or polypropylene that permeable to water vapor. Balesevic-Tubic *et al.* 2005 reported that fungal infection on agricultural product including spices can not be avoided. Preventing fungal growth on dried spices during storage is required.

4 CONCLUSIONS

Dried spices are infected by fungi. The pre and posthandling spices were required more optimally to

get the quality of spices that are in line with food safety standards.

ACKNOWLEDGEMENTS

The research was supported by Directorate General Higher Education, Ministry of Research and Technology Republic of Indonesia contract DRPM Reseach no. 67/UN5.2.3.1/PPM/KP-DRPM/2018.

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