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Postharvest Fungi Attack on the Candlenut (*Aleurites moluccana*) Distribution Chain in Several Areas at North Sumatra

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Abstract:
Background: *This research was conducted to obtain several informations regarding postharvest fungal infections in candlenut seeds at the farmer, collector and distributor levels in three different distribution chains at the province of North Sumatra. Candlenut seed samples were collected by farmers, collectors and distributors are 0.5/kg*
Materials and Methods: *Analysis was carried out on the mouisture content and isolation of postharvest fungal infections in candlenut seeds.*
Results: *The results has showed that the average mouisture content of candlenut seeds at the level of farmers, collectors and distributors were 5.19%, 5.33%, and 5.49% at the farmer level, 5.09%, 4.69%, and 5.19% at the level of collectors and 4.49%, 5.09 % and 5.02% at the distributor level. Twelve fungal species has been isolated from candlenut seeds were obtained from farmers, collectors and distributors in three different distribution chains.*
Conclusion: *The dominant fungus species are the Aspergillus group that can produce mycotoxins which are harmful to human health.*
Key word: *Candlenut seeds, distribution chain, postharvest fungi attack, Aspergillus*

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I. Introduction

Indonesia is a producer of spices. One type of spices that often used in cooking is candlenut. Candlenut (*Aleurites moluccana*) or candle nut is an industrial plant that is spread in the tropics and subtropics. Candlenut distribution in Indonesia covers East Nusa Tenggara, South Sulawesi, Aceh, North Sumatra and Gorontalo. Candlenut is one of Indonesia's export products to other countries such as Singapore, Malaysia, the Netherlands and Saudi Arabia. The main candlenut producing areas at northern Sumatra including Deli Serdang, Langkat, Binjai, Karo, Tapanuli, Dairi, Nias, and Asahan (Sihombing, 2015). Data from the Ministry of Agriculture (2017) shows that national candlenut production from 2013 to 2016 has increased, as follows 99,467 tons, 97,613 tons, 100,610 tons and 107,154 tons, respectively. According to Sihombing (2011), the demand of candlenut seeds for the domestic and foreign markets continues to increase every year. However, the producers cannot meet the demands of the community or distributors because there is no appropriate technology that can produce large amounts of candlenut in large capacity (Mahlinda, 2010). Constraints on handling post-harvest candlenut by farmers are still traditional. The breaking tool used is still inefficient and ineffective because it requires time and there are still a lot of broken or cracked candlenut seeds so that they are easily attacked by fungi (Sinaga, 2017).The drying and storage process plays an important role in determining the quality of seeds. Candlenut harvesting by farmers is generally done by taking seeds that have been ripe and fall on the ground. Drying is generally done conventionally and unhygienic so that seeds are susceptible to fungi. A tropical climate with humidity >90% causes stored candlenut to increase its mouisture activity so that it is susceptible to fungi.

Besides the high fat content in candlenut, seeds also cause the candlenut seeds to experience a reduction in quality. Candlenut seeds contain 55-56% oil and 60% oil content in the shell. The fatty acids contained in the oil consist of 55% palmitic acid, 6.7% stearic acid, 10.5% oleic acid, 54.5% linoleic acid and 28.5% linolenic acid. Palmitic and stearic fatty acid are referred to as saturated fatty acids, while oleic, linoleic and linolenic acids are classified as unsaturated fatty acids (Krishnawati *et al.*, 2011). Candlenut seeds without shells are easily degraded because of their high fat content, so they are easily oxidized and hydrolyzed to form free fatty acids. High fat content causes candlenut seeds susceptible to fungi (Krishnawati *et al.*, 2011). Among the fungi that often attack candlenut are *Aspergillus flavus, A. niger, A. wentii, A. tamarii, Aspergillus rubrum, A. chevallieri and Penicillium citrinum* (Pitt *et al.*, 1998; Lambaga, 2005). Prevention of fungus attacks and mycotoxin contamination in candlenut can be done with good handling at harvest and postharvest at the level of farmers, collectors and distributors, so that the quality produced can meet food safety standards.

II. Materials and Methods

Sample collection

Candlenut samples were taken from 3 distribution chains in Langkat, Deli Serdang, and Karo districts. Sampling total of 0.5 kg of shelled or shelled candlenut seeds from each distribution chain of farmers, collectors and distributors are used as a sample, the sample is put into a sterile polyethylene bag and stored at 4 ℃ until used.

Sampling method to obtain a working sample

The study was conducted at the USU FMIPA Microbiology Laboratory, Mouisture content analysis was carried out at the USU FMIPA Organic Chemistry laboratory. Candlenut samples were grinded using Mill Powder RT-04 no Serie 980923 (Mill Powder 16 Tech. Co. LTD, Taiwan) blender with a speed of 25 000 rpm for 30 seconds (Nurtjahja, 2017). The sample was then divided into 3 parts of working samples for determination of mouisture content, population of each fungal species and reserve samples.

Determination of Moisture content

Moisture content is determined using the distillation method (SNI 1993). A total of 40 g of candlenut samples that had been ground and escaped from a 1 mm sieve were then placed in a 500 mL distillation vessel, then added 75 mL of toluene until the toluene covered the sample. The sample mixture is shaken until the sample is homogeneously mixed. Pumpkin heating is carried out with a distillation speed of 100 drops per minute, and if the mouisture from the pumpkin has been distilled, the distillation speed will be increased to 200 drops per minute.

$$\text{Moisture content } (\%) = \frac{\text{sample volume (mL)}}{\text{sample weight (g)}} \; X \; 100\%$$

Enumeration of fungal population

Fungal population from each sample were enumerated, isolated, and identified using a serial dilution method on dichloran 18% glycerol agar (DG18) plate (Pitt & Hocking 2009). A total of 25 g of crushed candlenut samples were placed in a 500 ml Erlenmeyer flask, then added sterile distilled mouisture until the volume reached 250 ml, thus obtaining a 1: 10 dilution (10-1). The suspension is homogenized using a shaker at 250 rpm for 2 minutes, then 10 ml of the suspension is taken with a pipette and placed in a 250 ml Erlenmeyer flask containing 90 ml of sterile distilled mouisture so that a 1: 100 dilution is obtained (10-2). Furthermore, in the same way a series of dilutions is made up to 1: 1000 (10-3). As much as 1 ml of sample suspension from each dilution factor was inserted into a 9 cm diameter petri dish, then poured 15 mL of DG18 medium (40 ℃), the cup was shaken manually so the suspension of the dilution results was evenly distributed in the medium. Each cup was incubated for 7 days at room temperature (28 ± 2 ℃). Each dilution factor made 3 replications. Colonies of each fungus species were distinguished by their color and growth patterns and were identified based on Pitt and Hocking (2009).

III. Results and Discussions

Moisture Content of Candlenut Seeds

Candlenut seed drying is one of the important post-harvest handling processes to prevent damage during the storage process aimed at reducing the content of candlenut seeds. The moisture content of candlenut seeds at the level of farmers, collectors, and distributors in various distribution chains can be seen in Figure 1. From the observations on the moisture content it is also known that the range of moisture content of the candlenut seeds obtained at the level of farmers, collectors, and distributors in various distributor chains most of the mouisture content is higher than the maximum standard of SNI 01-1684-1998, which is a maximum of 5%. The moisture content of candlenut seeds which is more than 5% causes candlenut seeds more easily attacked by fungi. Zain *et al.* (2009) states that the main environmental factor influencing the development of postharvest fungi is mouisture content. Moisture content of food affects the resistance of food to fungal attacks.

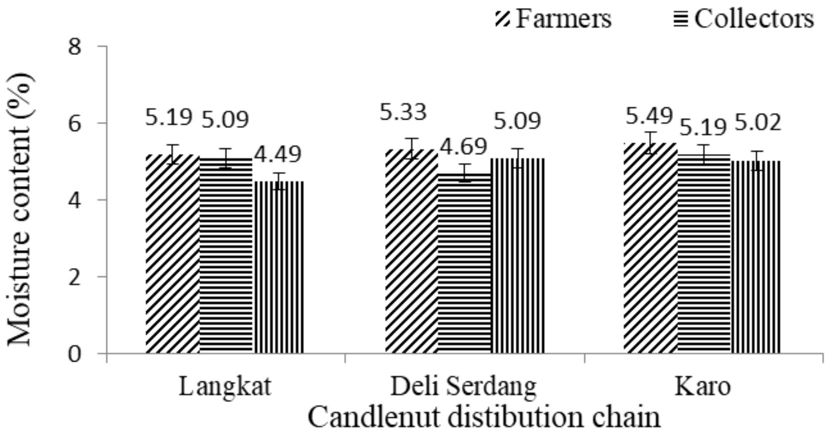

Figure 1. Candlenut Moisture Content in the Distribution Chain of Langkat, Deli Serdang, and Karo Regencies

The moisture content of candlenut seeds at the farm level is relatively higher compared to the mouisture content at the distributor and collector level, this is likely to occur because most collectors and distributors re-dry the candlenut seeds they buy from farmers. The high moisture content in candlenut seed samples can be caused by an imperfect drying process, drying the candlenut seeds using the sun's heat, which results in the drying process being very susceptible to weather changes. Balitbang (2009) stated that drying with the source of sunlight cannot be relied upon in the rainy season because it takes a long time and yield losses are quite high.

Fungus population

The results of isolation and identification of fungi obtained from Langkat District can be seen in Figure 2. The species of fungi isolated at the farmer level are *Aspergillus flavus, A. terreus A. tamarii, A. niger, Penicillium sp*., The fungus that dominates at the farm level is *A niger.* At the collection level, there was an increase in the population of 6 fungi which were isolated, namely *A. flavus, A. tamarii, A. niger, A. terreus, A. versicolor, Penicillium sp.*. From the collection level in the Langkat distribution chain, it is known that *Aspergillus niger* is a fungus that dominates. In the distribution chain Langkat at the distributor level as many as 8 fungi have been isolated namely, *A. flavus, A. tamarii, A. niger, A. terreus, A. chevalieri, A. versicolor, fusarium sp. and Pennicilium* sp., the predominant fungi are *A. niger*.

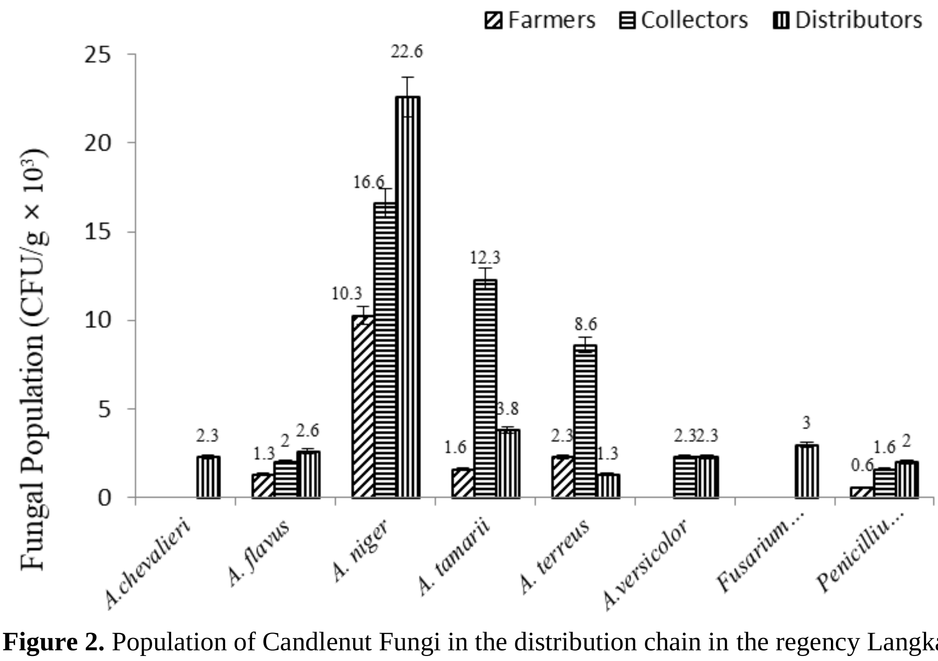

Figure 2. Population of Candlenut Fungi in the distribution chain in the regency Langkat

From Figure 2. shows that the population of *Aspergillus niger* is a postharvest fungus that dominates at the level of farmers, collectors and distributors in the Langkat distribution chain. The high diversity of fungi found was caused by Indonesia's tropical climate, thus supporting the growth of fungi both mold and yeast (Djaenudin, 2010). *Aspergillus niger* is more commonly found in warmer climates, black spores provide protection from sunlight and UV rays (Pitt and Hocking, 2009). This species is cosmopolitan in the tropics and subtropics, and is easily isolated from soil, air, water, spices, cotton, fruit, wheat, rice, corn, sugar cane, coffee, tea, chocolate, and leaves. According to Diba *et al.* (2007) who found that *A. niger* was the most dominant fungus isolated from the environment. According to Yani (2008) *Aspergillus niger* is also the dominant fungus on coffee beans obtained from farmers, traders in Bengkulu province.

Meanwhile the results of isolation and identification of fungi obtained in the distribution chain of Deli Serdang district can be seen in Figure 3. Where at the level of isolated fungi farmers *are A. flavus, A. tamarii, A. niger, A. terreus, A. chevalieri, A. candidus, Penicillium* sp. The predominant fungus at the farm level is *A.chevalleri*. Meanwhile at the collection level in the Deli Serdang distribution chain, 8 isolates were isolated, *A. terreus, A. chevalieri A. candidus, A. flavus, A. tamarii, A. niger, Penicillium* sp., *Fusarium* sp., meanwhile the fungus that dominates at the collection level in the Deli Serdang distribution chain is *Pennicilium sp.*. In the Deli Serdang distribution chain, 8 mushrooms were isolated, namely *A. flavus, A. tamarii, A. niger, A. terreus, A. chevalieri, A. candidus, Fusarium* sp. and *Pennicilium* sp. the fungus that dominates is *Aspergillus tamarii*.

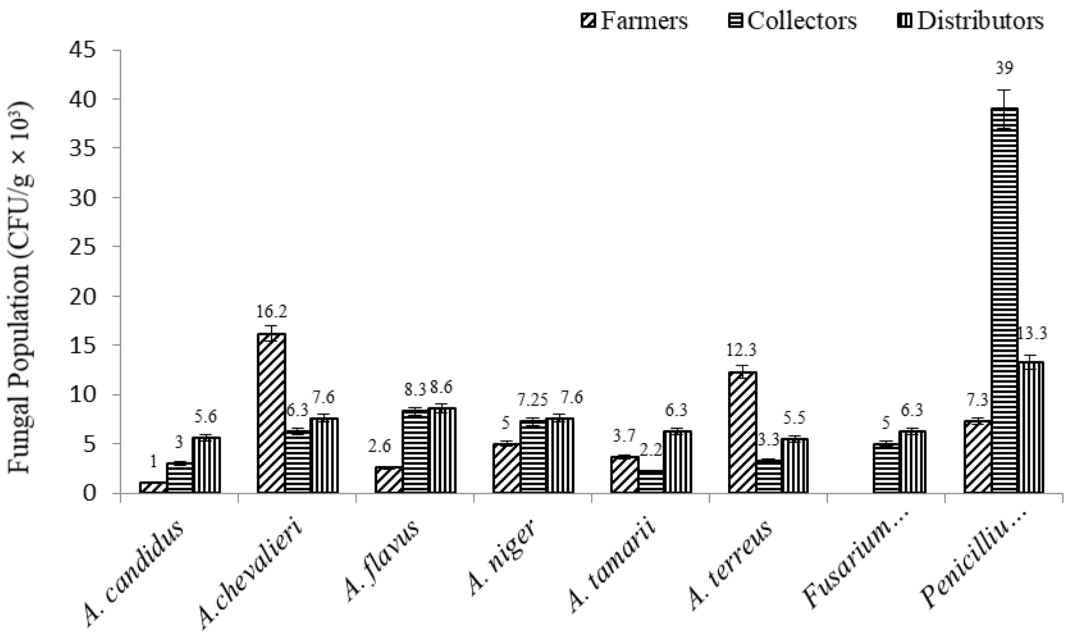

Figure 3. Population of Candlenut Fungi in the Distribution Chain in Deli Serdang regency

In the Deli Serdang distribution chain that can be seen in Figure 3. the dominant fungus that attacks is *A. chevalieri* at the farm level, and *Penicillium* sp. at the level of collectors and distributors. *A. chevalieri* itself is a xerophilic fungus or fungus that grows and develops at low mouisture content. According to Pitt and Hocking (2009), Askospora of this species contaminates nuts, candlenut, salted fish, rice flour, corn and vegetables. *Penicillium* sp. is a postharvest fungus that often attacks spices. The attack has been reported to attack pepper and coriander (Pitt and Hocking, 2009), black cumin and black pepper (Bokhari, 2007), nutmeg (Dharmaputra *et al.*, 2015), cumin (Kulshrestha *et al.*, 2014). Candlenut seeds that fall and come into contact with the soil at harvest become the main source of fungus attacks, then the population increases during storage.

In the Karo district distribution chain, 9 farmers were isolated, namely *A. candidus, A. flavus, A. tamarii, A. niger, A. terreus, A. chevalieri, A. versicolor, A. fumigatus, Pennicilium* sp .. From the data collected it can be seen that the fungus that dominates at the farm level is *Aspergillus tamari* and *A. versicolor*. At the level of collector, the population of fungi increased by 11 fungi was isolated from collectors in the Karo distribution chain, namely *A. candidus, A. flavus, A. tamarii, A. niger, A. terreus, A. chevalieri, A. versicolor, A fumigatus, A. sydowi, Fusarium* sp., and *Pennicilium* sp.. The predominant fungi in the Karo distribution chain at the collector level are *A. tamari, A. chevalleri, A. versicolor and A. candidus*. Meanwhile, 12 fungi were isolated from distributors in the Karo distribution chain, namely *A. candidus, A. flavus, A. tamarii, A. niger, A. terreus, A. chevalieri, A.versicolor, A. fumigatus, A. carbonarius, A. sydowi, Fusarium* sp. and *Pennicilium* sp.. The fungus that dominates the distribution chain in Karo at the distributor level is *A. candidus*.

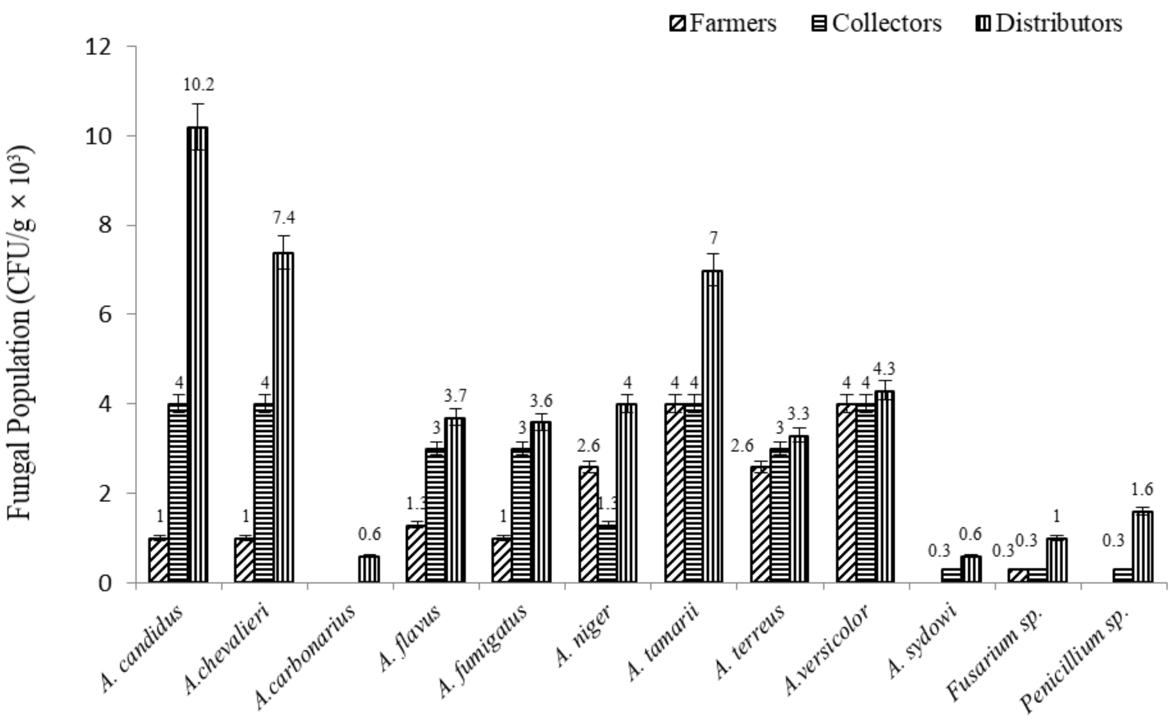

Figure 4. Population of Candlenut Fungi in the distribution chain in Karo District

Meanwhile in the Karo distribution chain that can be seen in Figure 4. at the farmer level is dominated by *A. tamarii* and *A. versicolor* while at the collector is dominated by the fungus *A. tamarii*, *A. candidus*, *A.* chevalieri and *A. versicolor,* and at the distributor level is dominated by the fungus *A. tamarii, A. candidus*, *A. chevalieri and A. versicolor,* and at the distributor level is dominated by the fungus *A. tamarii, A. candidus, A. chevalieri* and *A. versicolor, A. candidus*. According to Maryam (2014), *A. candidus* is a warehouse fungus because it is often found in dry cereals that are stored in large quantities in warehouses. According to Widowati *et al.* (2017), *A. tamarii* is one of the fungi that most attacks ground pepper, is a fungus that is able to grow at aw <0.78, and at 33 ℃. This species is a very common tropical fungus, and is found in many spices. Meanwhile, *A. versicolour* produces mycotoxins, namely sterikmatocystin. Its spread is generally found in cheese, cereals, soil, nuts, spices and dried meat, often also found in the air around the environment *A. versicolour* can tolerate at low pH and grow at low temperatures compared to other aspergillus species (Li *et al.*, 2014).

From the research results obtained 12 fungi that attack the candlenut seeds from various distribution chains can be seen in Table 1. In the candlenut seeds of postharvest fungi group of several species of Aspergillus. Pitt and Hocking (2009), reported that in the tropics the fungus which destroys a lot of food is from the Aspergillus group. In the postharvest management pathway, farmers have a very important role in suppressing fungal contamination, because at the earliest level farmers handle candlenut seeds both pre-harvest and post-harvest. If at the farm level the post-harvest fungus is attacked, the post-harvest fungus produced will attack at the level of collectors and distributors.

The large population of fungi at the distributor level compared to the level of collectors and farmers can be attributed because the candlenut from distributors comes from farmers then to collectors. Interaction between various types of fungi on candlenut seeds in storage locations, can affect the population of fungi that attack candlenut seeds. In addition, the presence of contaminant fungi during storage can reduce the quality of candlenut seeds. Krishnan *et al.* (2009) reported that environmental conditions with low temperature and humidity can trigger the growth of fungi that can contaminate food. This is supported by Bryden (2012), that contaminant fungus is able to damage the structure of food commodities physically, chemically, and biologically during storage. Duration of storage on candlenut seeds will affect the physical properties of the foodstuff.The quality of food stored will decrease if it exceeds a certain time limit. According to Ratnani *et al.* (2009) this is caused by the relationship between the temperature and humidity conditions of the air contained in the storage room, so that high temperatures and humidity will cause an increase in mouisture content, this is because when humidity is relatively low then the mouisture content in food will evaporate. This statement is reinforced by further studies conducted by Ratnani *et al.* (2010) that the relatively low humidity during storage will support the growth of fungi which will hydrolyze fat.

The longer the storage, the increased mouisture content, this causes the higher attack of microorganisms that produce lipase enzymes that can break down lipids. However, the greater percentage of damaged candlenut seeds at the collector and distributor level compared to the farmer level in the three distribution chains causes a negative correlation on the percentage of mouisture content of candlenut seeds, because high-moisture candlenut seeds are candlenut seeds at the farm level, while the percentage of seeds candlenut is attacked by the lowest fungus at the farmer level, this might be caused by the occurrence of candlenut seeds from the distributor and collector level, which is a long-stored candlenut seeds. Where at the level of collectors and distributors there is a longer variation in storage time compared to farmers, in addition to the sample of candlenut seeds at collectors and farmers also come from many farmers, which allows greater chance of fungal contamination.

Table 1. Fungi population in the distribution chains of Langkat, Deli Serdang and Karo Districts

	The percentage of candlenut seeds is fungal								
	Langkat			Deli serdang			Karo		
	farmer	collecting	Distributor	farmer	collecting	Distributor	farmer	collecting	Distributor
A. candidus	-	-	-	+	+	+	+	+	+
A.chevalieri	-	-	+	+	+	+	+	+	+
A.carbonarius	-	-	-	-	-	-	-	-	+
A. flavus	+	+	+	+	+	+	+	+	+
A. fumigatus	-	-	-	-	-	-	+	+	+
A. niger	+	+	+	+	+	+	+	+	+
A. tamarii	+	+	+	+	+	+	+	+	+
A. terreus	+	+	+	+	+	+	+	+	+
A.versicolor	-	+	+	-	-	-	+	+	+
Fusarium sp.	-	-	+	-	+	+	+	+	+
Penicillium sp	+	+	+	+	+	+	-	+	+
A.sydowi	-	-	-	-	-	-	-	+	+

Candlenut seeds that are stored are derived from ripe fruit that falls on the ground leaving them vulnerable to contamination. Hastuti (2010) reported that the damage caused by fungi on seeds is successive involving various species. Respiration by postharvest fungi can increase the temperature, carbon dioxide and seed moisture content. Improper storage can worsen fungal contamination. Chehri and Hasani (2017) reported that environmental conditions with low temperature and humidity can trigger the growth of fungi so that they can contaminate food. This is supported by Bryden (2012) that contaminant fungi are able to damage the structure of food commodities physically, chemically, and biologically during storage.

Figure 5. The results of isolation of fungi on DG 18 media to see the fungus population on candlenut with a row dilution method followed by pouring method, 7 days after incubation at ± 28 °C.

Storage in the open air and unhygienic can cause an increase in seed moisture content which triggers the growth of various species of fungi that have attacked the candlenut seeds. Prevention of fungus attacks during storage is done by reducing the maximum moisture content of candlenut seeds. However, during storage in open air or using airtight packaging, stored candlenut seeds absorb moisture from the air so that the moisture content of the seeds is balanced with the relative humidity of the air around the storage area. Lambaga (2005) stated that peeled candlenut seeds that are stored in a controlled atmosphere can reduce the mouisture content of peeled candlenut seeds and fungus populations that attack peeled candlenut seeds. Control Moisture content in candlenut seeds is needed after harvesting before seeds are stored to reduce population, prolong shelf life, prevent damage and possible attacks by species of toxin-producing fungi that are stressed when drying seeds. Attacks by other species are thought to occur when postharvest handling occurs.

Various species of field and postharvest fungi that attack candlenut seeds have been carried out by several researchers. The results of Hastuti's research (2010) showed that damaged candlenut seeds obtained from several markets in Malang were contaminated by several contaminant fungi. Among these species of contaminant fungi, some species are mycotoxin-producing, namely *Aspergillus flavus, A. ochraccus, A. clavatus, A. parasiticus, A. rugulosum, A. versicolor, Penicillium citrinum*. Meanwhile lambaga (2005) has isolated 13 fungi from peeled candlenut seeds that have been distorted for 90 days in controlled atmospheres, namely Aspergillus flavus, *A. candidus, A.penicillioudies, A. wentii, Cladoporium clasdoporidies, A. chevalier, A Repens, Hypopichia burtonii, Penicillium citrinum, P. rugolosum, Synchepalastrum racemosum, Wallemia sebi*.

Of the 12 fungi that have been isolated from the entire distribution chain, it can be seen that some of these fungi can produce mycotoxins which are harmful to human health. As *A. fumigatus* produces mycotoxins, namely gliotoksin, fumigaplatines, fumitremorjims, ferukulogen. *A. carbonarius* can also produce mycotoxins in the form of octratoxin. *A. terreus* itself produces mycotoxins, namely citreofiridin. *A. niger* produces mycotoxins which can produce some or all of the extolytes of cotanine, fumonisin and octratoxin, fumonisin b2 and b4 and okratoksin A. *A. versicolour* produces mycotoxins, namely sterikmatocystin. While *A. flavus* can produce aflatoxin which is also dangerous for health.

IV. Conclusion

The moisture content of candlenut seeds obtained from farmers and distributors in three distribution chains namely Langkat, Karo, and Deli Serdang in North Sumatra has a mouisture content exceeding SNI 01-1684-1998, which is a maximum of 5%. Of the three levels of the distribution chain, most fungi are found in the Karo distribution chain, which are 12 species of fungi. *Aspergillus niger* is a postharvest fungus that dominates at the level of farmers, collectors and distributors in the Langkat distribution chain. In the Deli Serdang distribution chain the dominant fungi were *A. chevalieri* at the farm level, and *Penicillium* sp. at the level of collectors and distributors. the Karo distribution chain at the farm level is dominated by *Aspergillus tamarii* and *Aspergillus versicolor* while the collectors are dominated by the fungus *Aspergillus tamarii, Aspergillus candidus, A. chevalieri* and *Aspergillus versicolor*, and at the distributor level is dominated by the fungus *Aspergillus tamarii, Aspergillus candidus, A. chevalieri* and *Aspergillus versicolor*, and at the distributor level is dominated by the fungus *Aspergillus tamarii, Aspergillus candidus, A. chevalieri* and *Aspergillus versicolor*. Of the 12 fungi that have been isolated are dominated by the aspergillus group which can produce mycotoxins which are harmful to human health.

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