

# Assessment and Identification of Fungi from Raw Peanuts (*Arachis hypogaea* L.) sold in Public Market of Southern Iloilo

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## ABSTRACT

Recent studies of fungal infections in crops have been a popular research topic since they may bring several health hazards especially for food contamination. This study aimed to identify, characterize, and assess raw peanut kernels that were collected from five stalls in public market of Southern Iloilo and subjected to fungal assessment. Peanut kernels were homogenized, serially diluted, spread-plated on Potato Dextrose agar and were incubated for 1-2 weeks. Macroscopic and microscopic observations of filamentous fungal isolates based on colonial morphology, vegetative and spore characteristics were used to describe and identify the fungal isolates' genera. Findings showed that all peanut samples in the stalls had fungal contamination. *Rhizopus* sp. was present in all stalls while *Aspergillus* sp. was present only in four other stalls. Another *Aspergillus* sp. was present in one stall only. This investigation pointed out that fungal contamination of mycotoxin from raw peanuts sold in Public Market of Southern Iloilo could be possible.

## KEYWORDS

fungi, raw peanuts, *Arachis hypogaea* L., Southern Iloilo

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## INTRODUCTION

The Philippines is one of the tropical countries blessed with diverse agricultural products from its fertile soil. These agricultural crops are very important in maintaining the needs and demands of its growing population. Some of these crops that are produced by the country are an export quality sold in the international market creating more livelihoods for the local farmers and helping boost the economy through agriculture. The Philippines as one of the developing countries in the world sometimes suffer from food shortage especially in staple products like rice, corn, and peanuts (Oñate, 1965). Based on a Social Weather Station (SWS) survey which Cabanilla (2006) reported that due to lack of economic access to food many Filipino families would become hungry. It is not a surprise that there are times in a year that the prices of these staple foods rise up and some citizens of the country cannot afford it, especially those who fall under the poverty line.

Cabanilla (2006) pointed out that since the mid-1990s, domestic price of crops has become higher than the world price. Peanut (*Arachis hypogaea* Linn.) is one of the crops produced in the country which is also cultivated in the southern portion of the Iloilo Province. It is technically considered as pea which belongs to the family (*Fabaceae*) of bean or legume and is consumed all over the world in a wide variety of forms, most of which are traditional cuisine (Arya et al., 2016). This legume is also important in cycling of nutrients especially nitrogen which is a vital plant nutrient. In Southern Iloilo, it is one of the products which are made as a popular delicacy. Many products are derived from raw peanuts such as peanut brittle, peanut candy, peanut butter, roasted peanuts, salted and coated peanuts, as well as *bandi* (peanut in caramelized sugar)- one of the famous peanut products in Southern Iloilo. Peanut products in not only good as snacks or desserts but is also used as condiment in some of the menus in the Philippines like in *kare-kare* (beef stew in peanut sauce). Peanut is one of the products being consumed with complete dietary source (Arya et al., 2016) since it is rich

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in protein, oil, and fibers (Suchoszek-Lukaniuk et al., 2011). However, it was reported that peanuts are frequently contaminated by the fungal species *Aspergillus flavus*, which can produce aflatoxin (Arya et al., 2016). In addition, peanuts are one of the most susceptible substrates for aflatoxin production and many studies have reported heavy contamination of aflatoxins in peanut samples (Ashiq, 2015): Ethiopia (Chala et al., 2013), Malawi (Monyo et al., 2012), Congo (Kamika & Takoy, 2011), and Kenya (Mutegi et al., 2009). Aflatoxin is reported to be highly toxic and carcinogenic secondary metabolites of concern in food safety (Achar et al., 2009).

Peanut contamination is generally brought up by microorganisms such as microfungi. Microbial contaminations vary from every region because of different environmental conditions based on their geographical locations. Fungal contamination shows evidence of a higher degree of diversity in tropical than temperate regions (Piepenbring et al., 2011), and some are contaminated during transportation or storage of peanut meals (Arya et al., 2016). According to Bennett and Klich (2003), specific fungal strains produce mycotoxins which are capable of causing diseases to humans and animals. The following are some mycotoxins produced by different fungal species as stressed out by Bennett and Klich (2003) which include (1) Aflatoxin derived from *Aspergillus spp.*; (2) Zearalenone, a *Fusarium* metabolite with potent estrogenic activity; (3) Ochratoxin recognized by the United States as potent nephrotoxin derived from metabolites of many species of *Aspergillus* (*A. alliaceus*, *A. auricomus*, *A. niger*, *A. carbonarius*, *A. glaucus*, and *A. melleus*); (4) Citrinin isolated from *Penicillium citrinum* and identified in species of *Penicillium* and *Aspergillus*; (5) Fumonisin produced by *Fusarium* species; and (6) Deoxynivalenol produced by *F. graminearum* found in cereals and cereal products (Ashiq, 2015; Bennett and Klich, 2003). Large factor that affects the crops in the Philippines is maybe brought by parasitic microbial fungi. Mycotoxin contamination may reduce the returns to farmers by deductions made by peanut processors, which therefore gives no security to future supply of a locally-grown crop (Ganzer, 1999). Furthermore, this phenomenon threatens the health of the general public who consumes this crop and other products derived from it. In the country, it is hard to detect the level of aflatoxin contamination in peanuts due to lack of resources and facility that evaluates such fungal pathogens. However, other countries used a rapid chemical test called the mini-column method to detect the

presence and level of aflatoxin contamination in farmer's stock peanuts, this method is used by the Peanut Company of Australia (Hansen and Norman, 1999). In the Philippines, there is no strict regulation in fungal contamination that may produce harmful mycotoxins especially in the local markets.

With the advent of climate change, microorganisms like microfungi also adapt to sudden change in the environment and may bring disease that may suddenly strike and ruin an entire season's production. It is very essential to study plant pathogens so that we can ensure the quality of our agricultural products since we are not only getting most of our food supply and nutrients in these products but it also boosts our economy. These agricultural products (rice, corn, peanuts, etc.) meet the needs of our country not to fall into hunger and as well as help rise the status of our economy. With these in mind, it is very essential to determine and assess the fungal species found in raw peanuts. Peanut like rice and corn, is one of the staple foods in the country. These peanut products are sold everywhere in markets of Iloilo, especially in the Southern portion. Peanuts may it be raw or processed in several products is not only sold in the local market of the country but also exported in countries abroad. Therefore, it is crucial in identifying and assessing fungal species found in raw peanuts since some of this fungal species releases toxin that can affect the health of the consumers. This will ensure the quality of the peanut-based products sold in the local market or international market.

## METHODOLOGY

### Sample Site

Five stalls in public market of Southern Iloilo were randomly chosen. About 250 grams of raw peanuts were bought in five stalls selling raw peanuts in Miagao Public Market. There were three replicates of each sample collected, labeled with the name of the stall and the date obtained, and placed in a sterile zip-lock bag to avoid contamination. The sampling was done in three weeks in July 2017.

### Preparation of Potato Dextrose Agar Plates.

About 6.9 grams of Potato Dextrose Agar and 300ml of distilled water were mixed in a 500ml Erlenmeyer flask. It was heated to boiling to dissolve the medium completely. It was sterilized by autoclaving at 15lbs pressure (121°C) for 30

minutes. About 1 ml of Ciprofloxacin was added in each prepared medium to prevent the growth of bacteria. It was mixed well before dispensing. The medium was suspended to 50 Petri dishes covering the total area of the base. It was allowed to solidify and stored for further use.

#### *Preparation and Serial Dilution of Peanut Samples.*

About 10g of raw peanut sample in each stall sample was homogenized in 90ml normal saline solution (NSS). One ml of the homogenized sample was transferred into the  $10^{-2}$  test tube containing 9ml NSS using a 1000 $\mu$ L micropipette. The solution was homogenously mixed with the aid of a vortex mixer. Serial dilution was repeated until the  $10^{-6}$  dilution.

#### *Plating of Samples.*

About 100 $\mu$ L of aliquot from tubes  $10^{-2}$ ,  $10^{-4}$ , and  $10^{-6}$  tubes of serially diluted samples were placed at the center of agar surface and spread entirely over the surface of the agar using a sterile L-shaped spreader. After a few minutes, all plates were inverted and were incubated for one week at 35° Celsius in an incubator.

#### *Isolation and Purification of Fungal Cultures.*

After one week incubation, plates were examined to verify the growth of filamentous fungi. Individual distinct colony that grows in agar plates was cut in a small block and aseptically transferred in a new agar plate. Purification was repeated once for three weeks until one colony grows in a new plate. Pure fungal colonies obtained by successive purification steps were photographed, morphologically described, and subjected to microscopic analysis. Fungal isolates were preserved in microbiological tubes containing agar slants, maintained at 2-4°C, periodically transferred for three months. Media used for preservation are the same used for strains isolation and growth.

#### *Characterization of Fungal Colonial Morphology.*

Each individual purified fungal colony was characterized based on their characteristics in a top and reverse views. Description of each colony includes appearance, colony pigmentation, agar pigmentation, shape, and texture.

#### *Slide Culture Preparation*

Aseptic technique was employed to avoid contamination during the preparation. With a pair of forceps, a sheet of sterile filter paper was placed in a Petri dish. U-shaped glass rod was also placed on the filter paper. Enough sterile water (about

4ml) was poured on filter paper to completely moisten it. With forceps, a glass slide was placed on the U-shaped rod. About 5 mm square block of the medium was cut from the plate of potato dextrose agar. The block of agar was picked up by inserting the scalpel and carefully transferred this block to the centre of the slide. The four sides of the agar square was inoculated with spores or mycelial fragments of the fungus examined. A cover glass was placed on the upper surface of the agar cube. The Petri dish was covered and incubated at room temperature for 48 hours or until such the entire agar block was consumed for maximum fungal growth. A new clean glass slide was used to hold the cover slip from slide culture and was examined under high power objective and oil immersion objective.

#### *Staining of Fungi.*

A drop of lactophenol cotton blue stain was placed on a clean microscope slide. The cover glass from the slide culture was removed and disposed properly. The fungi on the slide were examined under low power objective, high power objective and oil immersion objective.

#### *Microscopic Characterization of Fungal Isolates.*

Each fungal isolate was characterized microscopically. The hypha was identified as to its type; the stipe and phialides were characterized, measured with ocular micrometer according to length and width and later identified. The diameter of the spore was measured and described as to its arrangement as viewed under the microscope. The color of each vegetative part was also noted. The fungal isolates were identified utilizing a set of dichotomous keys and a set of picture keys by Ainsworth, Sparrow, and Sussman (1973), Arx (1981) and Crous et al. (2009).

## RESULTS AND DISCUSSION

#### *Fungal Isolates Present in Raw Peanut Kernels.*

There were three fungal isolates found in raw peanut kernels. Table 1 shows the frequency and percentage of fungal isolates from five stalls in Southern Iloilo Public Market. Isolate 1 (*Rhizopus sp.*) has the highest occurrence among the isolates. Isolate 3 (*Aspergillus sp.*) has the lowest occurrence and present in one stall only. These fungal isolates from raw peanuts sold in public market of Southern Iloilo corresponds with the findings of Gachomo et al. (2004) which shows the species of *Rhizopus* and *Aspergillus* in different market samples. Furthermore,

**Table 1.** Frequency of fungal isolates present in raw peanut kernels

Isolates	Frequency (f)	Percentage (%)
Isolate 1 ( <i>Rhizopus spp.</i> )	5	50
Isolate 2 ( <i>Aspergillus spp.</i> )	4	40
Isolate 3 ( <i>Aspergillus spp.</i> )	1	10

their study suggests that raw peanuts are very susceptible in fungal infection especially if the storage condition is favorable for fungal growth such as moisture and temperature. Spoilage and production of harmful metabolites called mycotoxins is brought up by infection of toxigenic fungi during handling, storage, and distribution of agricultural commodities (Ashiq, 2015). Moreover, it was highlighted by Ashiq (2015) that *Aspergillus* was the principally dominant species which were associated with peanuts during curing, picking, and storage. However, *Rhizopus*, *Sclerotium bataticola*, and *Fusarium* were also frequently found (Diener 1973).

#### Macroscopic Colony Characteristics of Fungal Isolates in Raw Peanuts.

The top view colony characteristics and reverse view colony characteristics are presented in Table 2. It shows the colony characteristics of fungal isolates in raw peanut kernel samples. In terms of top view colony characteristics, all the fungal isolates' appearance is teleomorph. These fungi have both sexual states (Wingfield et al., 2012). All fungal isolates has a velvety texture except for the third isolate (*Aspergillus sp.*) which has a dry texture. All fungal isolates has a transparent agar pigmentation and entire edge colony shape except for the third isolate (*Aspergillus sp.*) which has an undulate shape. All fungal isolates have different pigmentation.

Fungal isolate 1 (*Rhizopus sp.*) is present in all five stalls, while isolate 2 (*Aspergillus sp.*) is present in four stalls, namely, stall B, C, D, and E. Isolate 3 (*Aspergillus sp.*) is present in stall A only. The present findings correspond with the findings of Gachomo et al. (2004), where they found out that *Rhizopus stolonifer* and *Fusarium sp.* had the highest occurrence rate in both surface-sterilized and non-sterilized peanuts however, the occurrence of these species was higher in the non-sterilized samples. Furthermore, the species with higher occurrence in surface-sterilized samples were *Aspergillus spp.* (*A. flavus*, *A.*

*parasiticus* and other *Aspergilli*) (Gachomo et al., 20014). On the other hand, isolate 3 (*Aspergillus sp.*) is present only in stall A, it may be inferred that there are some factors that are favorable for the growth of isolate 3 (*Aspergillus sp.*) in stall A such as environmental, chemical, or biological. According to Fernández-Cruz et al. (2010), high water activity, high temperature ranging from 27 to 38 °C, and high humidity are conducive to *Aspergillus* growth. It may be derived from the above-mentioned statement that the way raw peanuts were stored in Stall A has a favorable environment or there is no other biological competitor for isolate 3 (*Aspergillus sp.*) to grow.

Based on reverse view colony characteristics, all isolates have transparent agar pigmentation. All fungal isolates have different pigmentation, shape, and texture. All fungal isolates have the presence of spore accumulation.

#### Microscopic Characteristics of Fungal Isolates from Raw Peanut.

Microscopic characteristics of fungal isolates are summarized in Table 3. All fungal isolates have coenocytic hypha this would allow nutrients to move quickly throughout the filament because the cytoplasm is continuous, without any dividers to slow transport (Becker, 2017). Moreover, all fungal isolates found in raw peanuts have smooth stipe. Phialide is present only isolates 2 and 3 in which both has a biseriata seriation, in addition, isolate 2 has a pear shape phialide while isolate 3 has a spatulate shape phialide. Conidia is also present in isolates 2 and 3 (*Aspergillus spp.*) in which both has a smooth texture and hyaline color, in addition, isolate 2 has globose and in chain conidia shape while isolate 3 has a oblong head and globose shaped conidia. In terms of vesicle shape, isolate 1 (*Rhizopus sp.*) has globose shape, isolate 2 (*Aspergillus sp.*) has spatulate shape, and isolate 3 (*Aspergillus sp.*) has pyriform shape. By means of spore characteristics, it was found out that isolate 1 (*Rhizopus sp.*) has an elliptical spore shape, isolates 2 and 3 (*Aspergillus spp.*) has a globose

**Table 2.** Macroscopic colony characteristics of fungal isolates in raw peanuts

Fungal Isolate	Stall	Appearance	Top View Colony Characteristics				Reverse View Colony Characteristics			
			Pigmentation	Agar Pigmentation	Shape	Texture	Pigmentation	Agar Pigmentation	Shape	Texture
Isolate 1 ( <i>Rhizopus spp.</i> )	A, B, C, D, E	Teleomorph	Light Brown	Transparent	Entire edge	Velvety	Dirty white/pale brown	Transparent	Entire edge	Velvety
Isolate 2 ( <i>Aspergillus spp.</i> )	B, C, D, E	Teleomorph	Black	Center yellow with transparent center	Entire edge	Velvety	Dark brown with black margin	Transparent	Lobate	Velvety
Isolate 3 ( <i>Aspergillus spp.</i> )	A	Teleomorph	Grayish greenish	Transparent	Undulate	Dry	Light bluegreen	Transparent	Undulate	Dry

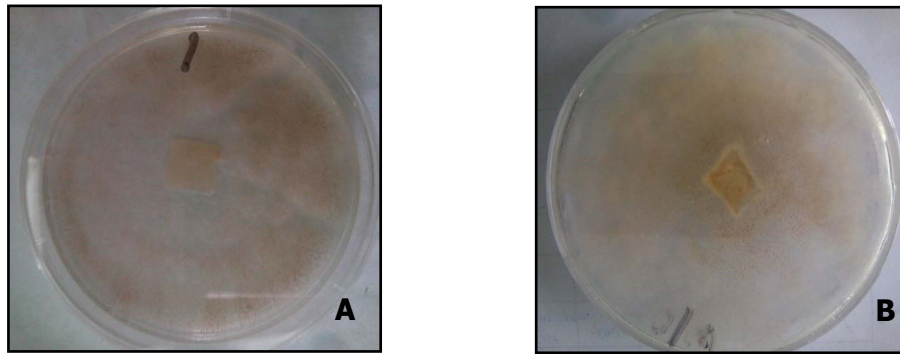
spore shape. Furthermore, the spores of three isolates have no attachment and appendages, the spores' wall is thin and smooth, and their color is hyaline except for isolate 3 (*Aspergillus sp.*) which is pigmented yellow.

The three fungal isolates that are present in raw peanut kernels were probably due to storage conditions (moisture and temperature) and storage time of the different stalls. It was observed that when gathering the samples from the market, most of the stalls store their raw peanuts in an open container and left unattended. This kind of storage condition is very susceptible from contamination of fungal spores present in the air. Furthermore, the climatic condition of the province is favorable for fungal growth. According to the National Water Resources Board (2013), the Iloilo Province has a Type I climate based on Modified Corona's

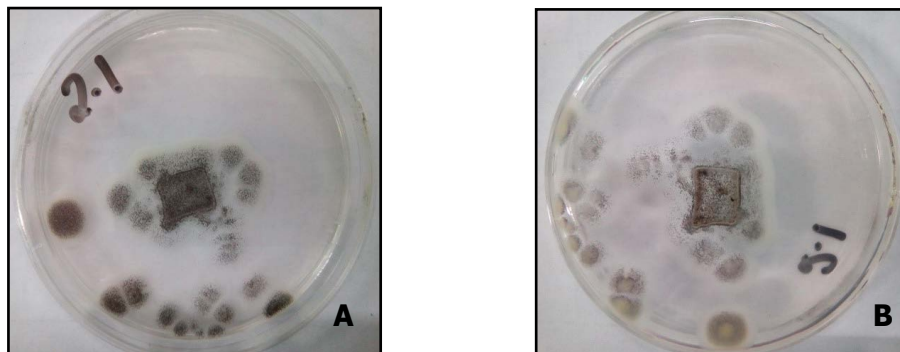
Classification, which is characterized by distinct wet and dry seasons; in addition, rainy period is usually between the months of June to October in which within these months that the samples were collected; and the mean annual temperature in the area is about 27.8°C, while relative humidity ranges from 73 to 84% and averages 81%. These current findings also correspond to the study of Xing et al. (2016) which pointed out that there was a significant variation in fungal isolates per-sample based on storage conditions and storage time. Furthermore, Xing et al. (2016) stressed out that across storage time, *Aspergillus*, *Eurotium*, *Penicillium*, *Rhizopus*, and *Wallemia* were the most predominant genera. In addition, the relative abundance of *Rhizopus* is higher than *Aspergillus* in peanut kernels because they were xerophilic and grew well on substrate with low water activity. However, during growth, they released metabolic

**Table 3.** Microscopic characteristics of fungal isolates from raw peanuts

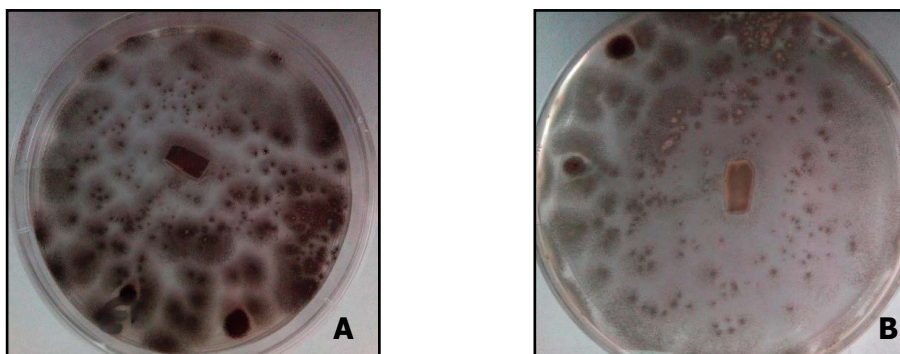
Fungal Isolate	Stall	Microscopic Characteristics
Isolate 1 ( <i>Rhizopus spp.</i> )	A, B, C, D, E	Type of hypha: Coenocytic; Stipe texture: Smooth; Stipe size: 200µm x 5µm; Phialide seriation: None; Conidia: None; Vesicle shape: Globose; Vesicle size: 11µm x 8µm; spore shape: Elliptical; No. of spore cells: in cluster; Spore attachment: None; Spore Appendages: None; Spore wall thickness: Thin; Spore wall characteristic: Smooth; Spore color: Hyaline; Spore size: 2µm
Isolate 2 ( <i>Aspergillus spp.</i> )	B, C, D, E	Type of hypha: Coenocytic; Stipe texture: Smooth; Stipe size: 120µm x 8µm; Phialide seriation: Biseriate; Phialide shape: Pear; Conidia color: Hyaline; Conidia texture: Smooth; Conidia shape: Globose and in chain; Conidia size: 15µm x 15µm; Vesicle shape: Spatulate; Vesicle size: 20µm x 15µm; Spore shape: Globose; No. of spore cells: 1; Spore attachment: None; Spore Appendages: None; Spore wall thickness: Thin; Spore wall characteristic: Smooth; Spore color: Hyaline; Spore size: 6µm
Isolate 3 ( <i>Aspergillus spp.</i> )	A only	Type of hypha: Coenocytic; Stipe texture: Smooth; Stipe size: 90µm x 3µm; Phialide seriation: Biseriate; Phialide shape: Spatulate; Conidia color: Hyaline; Conidia texture: Smooth; Conidia Head: Oblong; Conidia Shape: Globose; Conidia size: 9µm x 3µm; Vesicle shape: Pyriform; Vesicle size: 13µm x 10µm; Spore shape: Globose; No. of spore cells: Clusters; Spore attachment: None; Spore Appendages: None; Spore wall thickness: Thin; Spore wall characteristic: Smooth; Spore color: Pigmented/Yellow; Spore size: 3µm



**Figure 1.** Fungal colony of *Rhizopus sp.* in Potato Dextrose agar, A. top view and B. reverse view

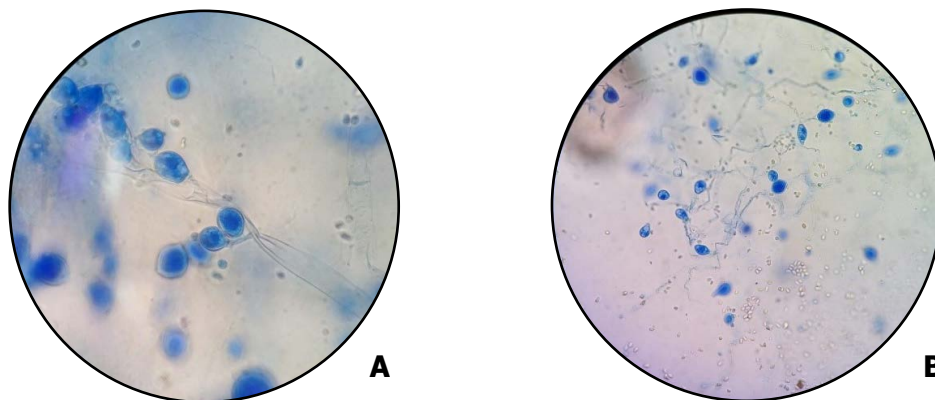


**Figure 2.** Fungal colony of *Aspergillus sp.* in Potato Dextrose agar, A. top view and B. reverse view

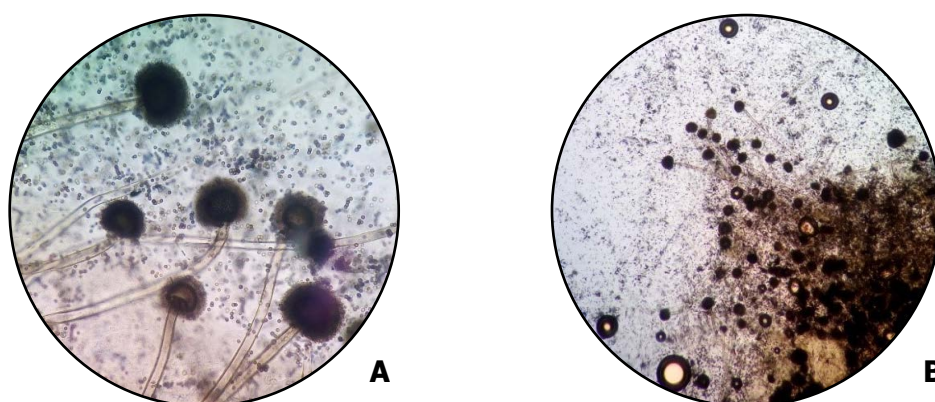


**Figure 3.** Fungal colony of *Aspergillus sp.* in Potato Dextrose agar, A. top view and B. reverse view

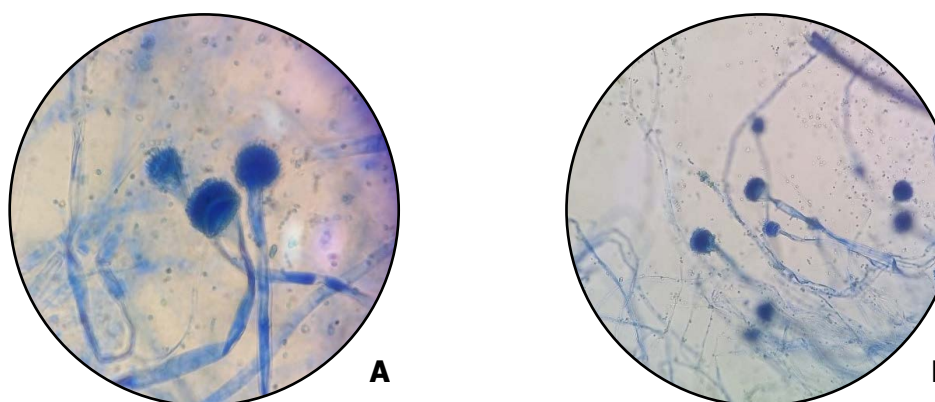
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**Figure 4.** Photomicrograph of *Rhizopus* sp. under A. HPO (400x) and B. LPO (40X)



**Figure 5.** Photomicrograph of *Aspergillus* sp. under A. HPO (400x) and B. LPO (40x)



**Figure 6.** Photomicrograph of *Aspergillus* sp. under A. HPO (400x) and B. LPO (40X)

water, thereby favoring the growth of *Aspergillus* (Xing et al., 2016).

## CONCLUSION

Three fungal species were present in raw peanut kernels in public market of Southern Iloilo namely *Rhizopus sp.* and two *Aspergillus spp.* The presence of these fungal species may imply the mishandling and improper storage of the raw peanuts which is favourable for the growth of fungi. The two species of *Aspergillus* is an indicative of species variation based on their macroscopic and microscopic characteristics and imply significant roles for their presence in peanut sample contamination. All fungal species found in raw peanut samples in five stalls could be associated in producing harmful fungal metabolites such as mycotoxin. Among the three fungal species found in raw peanuts sold in public market of Southern Iloilo, *Aspergillus spp.* releases mycotoxins, specifically aflatoxin. On the other hand, *Rhizopus sp.* also promotes potential harm to humans since they are also considered as human pathogen and parasite to plants especially peanuts. The presence of fungal contamination may affect the market value and the quality of peanut-based products in the future which could be attributed to the proper storage as well as the physical environmental conditions like moisture content and temperature.

## RECOMMENDATIONS

Based on the conclusions and findings of the recent study, the following are recommended: (1) Peanut samples should be assessed for exact aflatoxin or other mycotoxins using proper laboratory procedure such as high-performance liquid chromatography (HPLC), mass spectroscopy, enzyme-linked immune-sorbent assay (ELISA). (2) The study was limited to raw peanut kernels; thus, it is advised to assess also the peanut pods for the presence of fungi. (3) Assessment and monitoring of peanuts during pre- and post-harvest time should be done to identify the highest possible contamination of aflatoxin-producing fungi. Also, it is recommended to consider the dry season in the sampling of the fungi in peanut samples since physical factors greatly contribute to the contamination of fungi. (4) Stall owners as well as farmers should ensure the proper handling of their products especially peanuts. (5) Raw peanut products must be stored in a cool and dry place to prevent the growth of fungal spores. When processing peanut products

or cooking peanuts at home, consumers should check or monitor their peanuts and other processed peanut-based products must be cooked properly and thoroughly in order to destroy or kill fungi spores present in raw peanuts. (6) Since peanuts are used as feeds for some livestock, feed producers should strictly monitor that their feeds are free of fungal contamination by quality control and laboratory examinations. (7) Quality control of peanut products should also be employed by producers and stall owners to ensure that their products are safe for consumption. (8) And the Local government Units (LGUs) specifically the Department of Agriculture in cooperation with Non-Government Organizations (NGOs) should have a strong program to educate the farmers and other concerned persons in proper handling, storing, and cultivation of these products.

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