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Enumeration of Fungal Isolates and Screening of Mycotoxigenic Species in Palm Sugar Samples by Polyphasic Identification

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ABSTRACT The global demand for alternative sugars is rising due to their lower glycemic index and other health benefits. However, improper manufacture, processing, transport, export, and/or marketing of regional sugar products increases the risk of microbial infection upon consumption. Fungal exposure and contamination of alternative sugars may occur as a result of enhanced hygroscopicity of certain sugar forms such as coconut and palm sugars, which strongly attract water molecules to their surface and thereby fungal spores, a property affected by adulteration practices, e.g. incorporation of cane sugar. The present study highlights risks of unregulated processing of palm sugar in the form of fungal contaminants and their toxigenic potential. Palm sugar was sampled for fungal isolates which were identified as *Aspergillus flavus, A. niger, A. carbonarius, A. terreus*, and *A. fumigatus* using a polyphasic approach, followed by colony enumeration, of which one-sixth of fungal isolates were mold propagules. Subsequent extraction of fungal extracts by thin layer chromatography resulted in detection of mycotoxins, including aflatoxins B_1 and G_1, citrinin, and ochratoxin. The findings confirmed that humid conditions may be optimal for the presence of toxigenic fungi in the palm sugar and production of toxic metabolites, indicating that more stringent regulation is required for palm sugar processing, as the toxins can lead to detrimental health consequences, including acute poisoning, nephropathy, and liver cancer.

INDEX TERMS palm sugar, exports, microbiology, toxicology, public health.

I. INTRODUCTION

Alternative sugar sources, such as palm sugar, are increasing in demand due to their unique nutritional properties. Palm sugar is prepared from the sap of different palm tree species. In the Indian subcontinent, *Borassus flabellifer* (Palmyra palm) is a prevalent palm tree variety, a member of the *Arecaceae* family (Palm family). The tree is characterized by height of upto 30 m, flowers produced as large clusters of long, white, string-like inflorescences, stalks (1-1.2 m) with a lining of hard spines at the edges, and leathery, gray-green leaves folded along the mid-rib in a fan-shape. Tree parts are utilized for ropes, housing material, and other uses. Palm sugar is processed from unfermented Palmyra tree sap known for its earthy taste while the final sugar product is richer and is characterized by a dark color [1]. Palm sugar is prepared through a tedious process known primarily by those operating smaller home-based industries. The sap is collected in earthen pots coated with slaked lime on the inner surface to cease fermentation. Cleared sap obtained after filtration is taken to be boiled in a galvanized iron pan. While boiling, white residue rises to the top of the surface and is removed; castor beans are crushed and frothing is facilitated. Boiling is stopped after five minutes and the lime is allowed to settle. The clear brown syrup is again poured into a boiling pan. Deliming is done to clarify the syrup if the sap is too alkaline, to get a crystalline product, for greater amounts of clear syrup, or for shape. Palm sugar can take the form of either solid blocks or semi-liquid form like jaggery. Quality can be improved by adding citric acid to precipitate the lime. Challenges include the amount of human labor required to scale the palm trees for sap twice a day and the lack of standardization concerning pan material and heat application [1].

Palm sugar is preferred for consumption due its low glycemic index compared to table sugar or honey; less energy is required to digest the sugar in the human body. Thus, it is known to stabilize blood sugar levels and maintain good energy levels. It contains inulin, a dietary fiber that promotes good digestion through regulation of gut bacteria. Nutritional properties distinguish Palmyra palm jaggery from crude cane sugar; the former shows antioxidant properties as well [2]. It contains 1.04% protein, 0.19% fat, 76.86% sucrose, 1.66% glucose, 3.15% total minerals, 0.861 % calcium, 0.052% phosphorus; also 11.01 mg iron per 100 g and 0.767 mg of copper per 100 g. Fresh sap is reported to contain vitamin B complexes [3]. Palm sugar is used to make molasses, wine, vinegar, sweet soy sauce, certain sweet dishes, and as a substitute to sugar in many cases [4]. Palm sugar has a high level of agricultural value in Southeast Asia and an increasing economic value internationally. It is known by names such as palmyra jaggery, palm jaggery, palm sugar, *karupatti* or *panna vellam* (Tamil), *thaati bella* (in parts of Karnataka), and gur, often acquiring names in coastal region due to prevalence of palm tree growth [1].

Certain preparation methods utilizing prolonged heating have shown better quality and antioxidant properties [5]. According to the Centre for Promotion of Imports from developing countries, Netherlands Ministry of Foreign Affairs, the total European market for palm sugar ranges 1,500 to 3,000 tons and comprises a sugar market that comes up to over 16 million tons. In Europe, brick and cake forms of palm sugar are not as preferred as granulated sugar, yet palm sugar has become a popular replacement in Germany in the search for alternatives to synthetic sweeteners and white sugar. India provides an export output of 3,000,000 tons of crude sugar every year, of which 10% is palm sugar. However, palm sugar is not formally tracked in the large-scale trades. The worldwide palm sugar market was valued at USD $1684.2 million in 2017 and estimated to reach USD $2205.8 million in 2025. Income from trade is anticipated to gain at a compounded annual growth rate of 3.4% in the prediction time period from 2017–2025 [6].

Hygroscopicity and Fungal Utilization of Sugars

Fungi are ubiquitous organisms that often live and survive as symbionts or parasites, found in unicellular or multicellular forms when they grow saprophytically on substrates, and obtain their nutrients from dead organic matter by utilizing enzymes to digest and absorb the nutrients from food extracellularly. They readily absorb and metabolize numerous soluble carbohydrates and proteins as carbon and nitrogen sources, respectively. Optimal growth conditions consist of broader ranges in pH, temperature, and moisture content values compared to bacteria. Fungal reproduction is either sexual or asexual, where spores play a substantial role in their ecological dispersion.

Fungi grow on a number of substrates composed of either a single sugar or a variety [7]; research on sugar utilization rates by fungi has shown that they rarely encounter a single sugar in the substrate composition, rather encountering a mixture of sugars [8]. Moreover, sugars like sorbose, in combination with other sugars, block the utilization of any other sugar, but the addition of malt extract overcomes this inhibition. Sorbose has the ability to kill hyphal tips of fungi and does so better at a higher incubation temperature than at a lower one. Fungi like *Aspergillus niger* utilize galactose more slowly than glucose [8].

Oligosaccharides yield monosaccharides upon hydrolysis and they contain α and β bonds between the simple sugars. Sucrose is a non-reducing oligosaccharide composed of glucose and fructose that hydrolyzes easily upon acidic conditions. Several fungi with the ability to utilize glucose cannot degrade sucrose and certain fungi utilizing sucrose do not necessarily hydrolyze it. The two prevailing theories are that a) oligosaccharides are hydrolyzed before utilization and b) that they are utilized by using pathways that do not require hydrolysis [9]. Sugars not utilized alone may be utilized rapidly when in combination with another sugar. Depending on composition, structures and properties are compared among either several sugars or structural forms of a single sugar. In case of monosaccharides, D-glucose, D-mannose, and D-fructose appear collectively and share a common enol form; in an alkaline solution, when one sugar is present, the other two sugars also appear. A definite relationship between sucrose, invert sugar, and water content of various sugars in relation to the water content of the surroundings can be established [9]. Controlling the optimal level of relative humidity for sugar maintenance is key to curbing the hygroscopic nature of sugar by which sugar hardens and softens due to increased water content, thus stimulating fungal and bacterial growth. For mixtures of sucrose and 0.1-0.2% invert sugar, the point at which absorption began was lower than that for pure sucrose. Dittmar observed a sharp point at which liquefaction occurred; the crystals were able to hold upto 15% water, after which the crystals passed into solution. If the relative humidity is lowered before this point, the sugar will dry out rather than liquefy. These observations have been said to hold true for brown sugar as well as any product of high sugar content. The production of invert sugars through microbial degradation increases the hygroscopicity of palm sugar, resulting in greater microbial growth [1]. Species of *Aspergillus, Penicillium*, and *Talaromyces* are very productive in the case of secondary metabolites which are defined as products that do not have a known role in the 'internal economy of the producer' and are not required in growth [10]. Frisvad termed secondary metabolites as exometabolites, small molecules produced during morphological and chemical differentiation which are directed in an outward manner, secreted or deposited in or on the cell wall, and accumulated. Exo-metabolites may either transform into endo-metabolites or feed into morphological structures [10].

TABLE 1
Common Mycotoxin Types, Sources, and Health Effects

Mycotoxin Types	Associated Health Effects	Associated Fungi	Common Food Sources
Aflatoxins	[Genotoxic] Liver cancer	*A. flavus,* *A. parasiticus*	Cereals, Oilseeds, Spices, Tree nuts
Ochratoxin A	Renal damage and disorders Fetal growth impairment	*Aspergillus* spp. *Penicillium* spp.	Cereals, Coffee beans, Dry vine fruits, Wine and grape juice, Spices
Fumonisins	Neural tube defects Esophageal cancer	*Fusarium* spp.	Corn
Deoxynivalenol	[Immunotoxic] Gastroenteritis Acute toxicoses	*Fusarium* spp.	Wheat

Fungi are well known for their ability to produce beneficial metabolic products such as pigments, enzymes, and antibiotic compounds of industrial significance, as well as harmful metabolites such as mycotoxins [11], which are 'extrolites' that contaminate human food and animal feed, harm the human food production chain, and cause diseases in vertebrates upon entry into the bodily system through ingestion, skin absorption, or inhalation. Common mycotoxins are tabulated with their health effects. Mycotoxins may be acutely toxic, chronically toxic, or both since some act in a synergistic interaction, increasing their toxicity. Nielsen and Frisvad have found that the number of mycotoxin-producing species is low but the production occurs at high water activity. Harmful fungal metabolites are encountered commonly due to the ease of dispersion and resultant colonization that fungi demonstrate, attributed to their spore production which is a bioaerosol [12]. Spores are produced by toxigenic strains of fungi like *A. flavus* and *A. parasiticus*. Moreover, this has presented great risk to agricultural workers exposed to dust containing increased conidial residue from these fungi due to the presence of aflatoxins B_1 and G_1 [13].

Mycotoxin contamination in food can be associated with lack of regulation or implementation by food safety authorities. Sugar product regulation is mediated by both importers and exporters since fungal contaminants may enter at any point of manufacture, handling, transport, or even at the site of sale. The Government of India has amended the 2018 guideline for 'Sweetening agents including Honey' under the Food Safety and Standards (Food Products Standards and Food Additive) from 'product obtained by pressed out of sugarcane' to 'product obtained by boiling or processing juice pressed out of sugarcane or extracted from palmyra palm, date palm, or coconut palm' by the FSSAI. The Bureau of Indian Standards (BIS) and Prevention of Food Adulteration (PFA) Act still seek information on alternative sugars for regulation, yet these products often lack industrial production; home-based microenterprises use traditional processes that do not ensure a sterile environment for production. A risk assessment has shown that more stringent regulatory limits on the mycotoxin content in exported food products would lead to a 3-fold greater profit loss for industrial nations such as the U.S.A., China, and Argentina [14]. Since sugar promotes microbial growth upon degradation, more food safety guidelines are required to prevent contamination during manufacture and handling, as well as to protect food security [15]. A large concern is local unregulated sale on roadsides, air markets, or in open environments where large numbers of contaminants can be found, including dust and spores that may attach to the particles.

Currently, there are few analyses of fungal contamination in the palm sugar supply chain and during storage, resulting in a lack of focus on its implications in relation to food safety. This work aims to highlight the importance of research in sustainable solutions for palm sugar preservation. The objectives of the present study include characterizing the fungi isolated from palm sugar and analyzing the isolates for production of mycotoxins, thus identifying the toxigenic fungi.

II. MATERIALS AND METHODS

A. SAMPLE COLLECTION AND PRELIMINARY FUNGAL ISOLATION

Solid bar forms of the palm sugar were collected from a regional goods outlet directly sourced from the manufacturer

and stored in sterile plastic containers. The initial fungal isolation was done by swabbing the sample and streaking onto a plate of Sabouraud Dextrose Agar (Himedia) with composition of 4 g dextrose, 1 g peptone, and 2 g agar in 100 mL distilled water sterilized at 121℃ for 15 minutes at 15 lbs per square inch. The plates were then incubated at 28℃ for 2-3 days and colonies were noted.

B. PRIMARY FUNGAL ISOLATION AND ENUMERATION

Primary isolation of fungi for the study was performed by taking the sample of palm sugar and scraping off portions of the bar by using a sterile spatula, then weighing it out to a quantity of 1 gram. This process was carried out quickly due to the hygroscopic nature of palm sugar upon exposure to air. The sample was then serially diluted till the 10^{-7} dilution using sterile distilled water. A quantity of 0.1 mL was taken from each of the 10^{-2}, 10^{-3}, and 10^{-4} dilutions and aseptically inoculated onto the respective plates of SDA medium by spread plate technique. The inoculated plates were then incubated at 28° +/- 3℃ for 2-3 days and observed for growth. The colonies were enumerated and the number of propagule-forming units was calculated for each sample dilution by using the following formula, then tabulated.

Colony-Forming Units/Propagule-Forming Units per mL = (Number of Colonies or Propagules x Dilution Factor)/Volume of Sample

C. IDENTIFICATION OF FUNGI

The fungal species were identified by utilizing a polyphasic approach which included observation of colony morphology and hyphal structure by LCB staining [16]. Identification was done using several criteria to attempt a polyphasic approach. The points of evaluation included:

- Colony Morphology and Pigmentation
- Microscopic Structure by Lactophenol Cotton Blue Staining
- Reverse Coloration of the Colonies

The results were tabulated.

D. SCREENING FOR PRESENCE OF MYCOTOXINS

To determine whether the fungal isolates were mycotoxigenic, they were cultivated as liquid broth fungal cultures and the metabolites were extracted. The cultures were prepared in Sabouraud Dextrose Broth containing 2 g dextrose, 1 g mycological peptone in 100 mL distilled water. After sterilizing and dispensing the medium into tubes, pure liquid sub-cultures of fungal isolates were prepared. The inoculated tubes were then incubated at 28℃ for 3-4 days and observed for growth regularly.

E. EXTRACTION OF MYCOTOXINS

In a microfuge tube, 0.5 mL of liquid culture (including part of the mycelial layer) was taken along with 1.0 mL of pure chloroform used as the extraction solvent. The contents of the tube were shaken for 30 minutes, then the mixture was allowed to evaporate in order to remove the chloroform layer. The contents were filtered through Whatman No. 1 filter paper and 0.1 g of anhydrous sodium sulfate (Na_2SO_4) into 1 mL of pure chloroform [17].

F. THIN-LAYER CHROMATOGRAPHY (TLC)

Silica gel G was used to prepare a slurry in a 1:2 ratio with distilled water, then activated at 60℃ for 2 hours. The mobile phase solvent system was taken to be a 9:1 ratio chloroform-acetone mixture [18]. The silica gel was spotted using a capillary tube at a line 2 cm away from the bottom edge of the plate. The solvent system was taken in a glass tank and after allowing vapors to saturate the closed system, the plates were placed within the tank in such a way that the solvent front remained below the line where the sample was spotted. The solvent was allowed to run till near the top edge of the plate and the plates were then carefully removed from the tank [19].

G. DEVELOPMENT AND VIEWING OF TLC PLATES

The plates were then developed by spraying the silica gel layer with 20% conc. H_2SO_4 using a syringe, then heating the plates at 110℃ in the hot-air oven for 10 minutes [17]. The TLC plates were placed in a UV transilluminator for 15 minutes, then a Labline UV Cabinet was used to expose the plates to visible light and UV light at short and long wavelengths. Visible bands were marked and the R_f value was calculated by measuring the distances that the solvent and solute(s) traveled from the specified point, respectively, and dividing the values. The R_f values were compared to known analyte values and the analytes were determined based on band characteristics as well [18].

R_f Value = Distance Traveled by Solute/Distance Traveled by Solvent

H. IDENTIFICATION OF MYCOTOXINS

The mycotoxins that were isolated were identified by collectively using R_f values, band colorations, and fluorescence patterns under UV light.

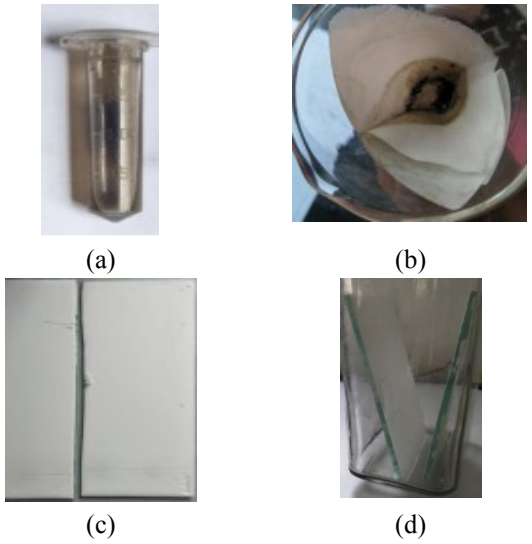


FIGURE 1. a) Extraction of Mycotoxins from Liquid Culture, b) Filtration into Pure Solvent, c) Spotted Silica Gel Plates, d) Running of Solvent in TLC Process.

III. RESULT

A. ISOLATION OF FUNGI

From the initial isolation of fungi, most of the resulting colonies on the SDA plate were found to be members of the genus *Aspergillus*, confirming the presence of fungi in the sample.

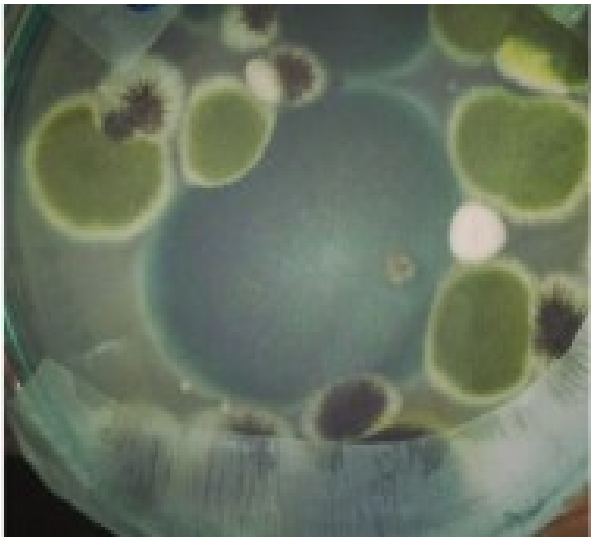


FIGURE 2. (Right) Fungal colonies isolated early on after obtaining the sample.

B. PRIMARY ISOLATION (MAIN ANALYSIS)

The fungi were isolated on plates of Sabouraud Dextrose Agar after incubation for 3-4 days but colonies were only distinguishable after 7 days of growth, as fungal colonies often change color over the course of growth. They are identifiable by colony morphology, growth patterns, and reverse colony coloration in the later stages of growth, although the growth becomes confluent after a certain point. The fungi were isolated twice and both series of plates were documented as below.

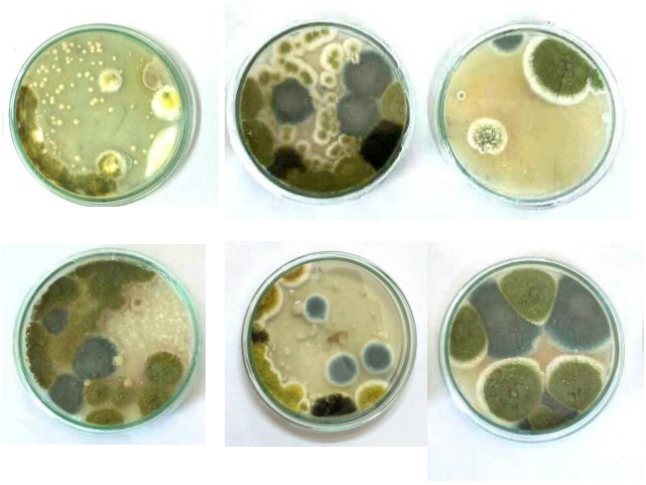


FIGURE 3. Plates of Sabouraud Dextrose Agar spread with 0.1 mL of sample from the 10^{-2}, 10^{-3}, and 10^{-4} dilutions (from left to right) after 5 days of incubation at 28℃. [*Above*: Set 1 and *Below*: Set 2].

C. QUANTITATIVE ANALYSIS: ENUMERATION OF FUNGAL PROPAGULES

The number of propagule-forming units is affected by different factors, such as uniformity of sample spreading and sizes of respective fungal spores which affect the number of units on the plate. The results were tabulated.

TABLE 2
Enumeration of Fungal Colonies or Propagules in Two Series of SDA Plates After Inoculation of 0.1 mL of Sample & Incubation at 28℃ for >3 Days

S. No.	Dilution (10^{-x})	Number of Propagule-Forming Units (PFU/mL)	
		Set 1	Set 2
1	10^{-2}	87×10^3	132×10^3
2	10^{-3}	51×10^4	55×10^4
3	10^{-4}	5×10^5	8×10^5

TABLE 4
Morphological Characterization of Fungal Isolates from Palm Sugar by Polyphasic Approach

S. No.	Colony Morphology, Coloration, Reverse Coloration	Microscopic Structure (Staining)	Identified Organism
1	- Edgy, cinnamon brown colonies - White to brown reverse coloration	- Compactly columnar structure - Short, smooth conidia	*Aspergillus terreus*
2	- Wooly, brown colonies - Light reverse coloration	- Entire vesicle surrounded by phialides (forms radial head) - Long, smooth conidia	*Aspergillus niger*
3	- Dark brown to black colonies	- Roughened walls, packed metulae and phialides, spherical conidia - Dark brown to black conidia	*Aspergillus carbonarius*
4	- Velvety colonies - Colonies turn green from yellow - Gold to red-brown reverse coloration	- Entire vesicle is covered - Phialides point in all directions - Varied length of conidia	*Aspergillus flavus*
5	- Powdery colonies - White colonies turn grey with a thin border - White to tan reverse coloration	- Upper vesicle covered - Short, smooth conidia	*Aspergillus fumigatus*

TABLE 3
Comparative Enumeration of Yeast and Mold Colonies on SDA Plate with 10^{-2} Sample Dilution After 3 Days of Incubation

Set No.	Total Number of Colonies	Number of Yeast Colonies	Number of Mold Colonies	Percentage of Mold Propagules from Total Fungal Population (%)
1	87	71	16	18.4%
2	132	115	17	12.9%

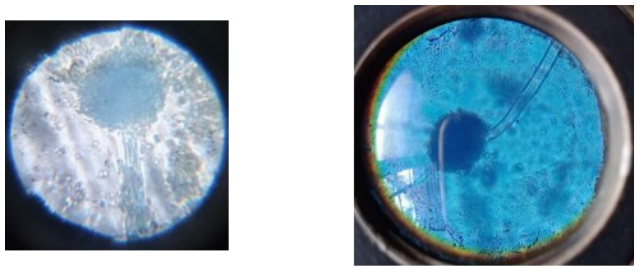
*Significant yeast populations were only found on the SDA plate with 10^{-2} sample dilution

From this data, it is evident that the mold propagules or propagule-forming units actually comprise less than one-fifth of the total number of colonies. The least diluted plate was the most diverse in terms of demonstrating different fungal populations in both sets.

D. QUALITATIVE ANALYSIS:

1. IDENTIFICATION OF FUNGI

Using the criteria of the polyphasic approach, several of the fungal members were identifiable to the genus and species levels and tabulated as below.



(a) (b)

Figure 3: *(a)* Microscopic image of *Aspergillus flavus* and *(b)* Microscopic image of *Aspergillus carbonarius*

Yeasts were also identified. These included *Saccharomyces cerevisiae*, *Cryptococcus magnus*, *Candida albicans*, and *Candida krusei*. They were identified based on their colony morphology and Gram staining/simple staining with crystal violet.

2. SCREENING FOR MYCOTOXINS

The main colonies of fungi were inoculated into separate tubes of Sabouraud Dextrose Broth medium and observed for growth. The growth was observed and continued for 10 days. After the solvent ran till near the top edge of the TLC plates, the plates were removed from the chamber and they were placed under UV light to observe for band production. However, bands were not visible initially. The plates were sprayed with 20% sulfuric acid in a syringe and heated in a hot-air oven at 110℃ for 10 minutes. They were exposed to long wave UV light and bands began to appear after 15 minutes. The plates were then removed and the distance that the spots traveled from the origin was measured. The TLC plates were then placed into a TLC Plate Viewer and observed for colored bands. The bands were noted and documented under visible light and UV light as below.

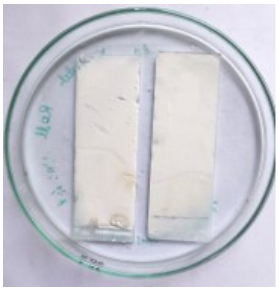


FIGURE 4. TLC Plates Before Development

FIGURE 5. TLC Plates After Development

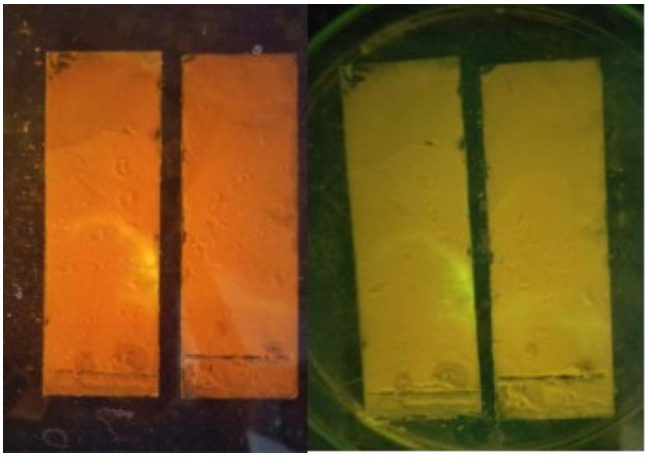


FIGURE 6. Appearance of bands on TLC plates after UV exposure in the UV transilluminator.

3. IDENTIFICATION OF MYCOTOXINS

The R_f values were calculated for each fluorescent pattern by using the formula and corroborated with the recorded R_f values for mycotoxins by considering a) the color of the band in visible light and under UV light, b) solvent system used, and c) the presence of a tail/streaking appearance.

Using the experimental work of Steyn (1981) as a reference, the R_f values obtained were compared to those determined using a chloroform-acetone in a 9:1 ratio based on IUPAC approval. Bands with R_f values of 0.27 and 0.34 were determined to be Aflatoxin B_1 and Ochratoxin A (OTA) as they have blue coloration over several wavelengths of UV light [20]. The band with an R_f value of 0.25 was determined to be Aflatoxin G_1.

FIGURE 7. (Left) TLC plates under visible light.

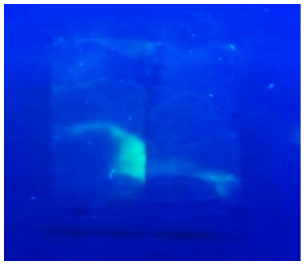


FIGURE 8. (Left) Fluorescent patterns on TLC plates upon exposure to UV light in the TLC plate viewer.

The band that appeared yellow under certain wavelengths of UV light and yellowish-green under other ranges was determined to be citrinin, which was recorded to have a varied range of R_f values due to streaking tendency. However, as the substance coincided on both plates, the R_f value could be proximate based on the coinciding of the distances traveled.

IV. DISCUSSION

Fungal species were isolated and identified by polyphasic criteria, as shown in Table 4. The R_f values were calculated as recorded in Table 5 and the presence of four mycotoxins was deduced. The deviation caused by the streaking effect or tail formation is not easily overcome and previous studies have noted this detail. However, on a whole, determining mycotoxins based on the band coloration and the R_f values was done based on the recorded colorations and R_f values for a 9:1 ratio of chloroform-acetone solvent as the mobile phase [20]. Further study could be done through quantification of mycotoxin by using standards. The production of mycotoxins is undoubtedly an important field of study within public health and epidemiology. The risk of intoxication concerns both consumers of contaminated food products and labor workers in crop fields infected with fungal spores, as a consumption risk and an occupational hazard [13]. Fungi found in sugar sourced from sugarcane has already been studied to show aflatoxin production [21]. The unregulated

TABLE 5
R_f Values of Solutes Separated by Thin-Layer Chromatography using a Mobile Phase Solvent System of Chloroform-Acetone in a 9:1 Ratio

S. No.	Color Under UV Light	Presence of Tail/Streaking	Distance Traveled by Solvent from Origin (cm)	Distance Traveled by Solute from Origin (cm)	R_f Value
1*	a) Light Green (Left Plate)	+, Streaking	7.5	2.2	0.29
	b) Yellow-Green (Right Plate)	+, Minimal	7.5	1.5	0.20
2	Light Blue	+, Tailing	7.5	1.6	0.21
3	Light Blue	-	7.5	2.5	0.34
4	Bluish-Green	+ , Minimal	7.5	1.9	0.25

* Values differ due to the streaking effect.
+ Indicates presence, - Indicates absence

sale of palm sugar on roadsides, as well as lack of restriction in handling and manufacture, give way to product contamination of palm sugar which, due to the hygroscopic nature of its components [22], enhances the growth of select fungi through increased water activity, for which a dry form of sugar should be produced for long term storage and use. The adulteration of palm sugar is another factor [23] that alters the composition and affects palm sugar shelf life [24] due to changes in pH and water activity [25], requiring greater food safety implementation since the major component is sucrose, which is known for its functional water-absorbing properties as an humectant [26]. The low glucose concentration may promote initial fungal spore germination and the growth is often sustained through the presence of sucrose, the optimally utilized disaccharide by fungi, namely *A. niger* [27], adding to the findings in Table 3 showing that osmophilic yeasts also grew in the high sugar concentrations, at relatively greater rates than molds. Table 2 indicates the large diversity of fungal species found at a lower sample dilution due to which various metabolites may be produced and cau a number of symptoms. Large loads of palm sugar can be ultrafiltered and spray drying is a sugar processing step [4] frequently used for cane sugar and another alternative is the incorporation of adsorption agents in storage for control of atmospheric water content, such as starch copolymers or cellulose-based solutions [28]. Another solution is the development and use of suitable containers for palm sugar storage, as aluminum vessels have shown to maintain more stable pH values compared to bamboo wrapping, a common biomaterial for palm products [29]. Airtight storage in an environment with proper temperature control is a significant way to control microbial growth but based on the isolation of fungi, the colonization of contaminants indicates that more changes may be required in storage. Preventing the entry of contaminants during handling and processing is more essential. Furthermore, meeting the rising demand for palm sugar exports requires a tightened mode of regulation that has not yet been applied to palm sugar as it is still considered an unorganized rural industry throughout much of Southeast Asia with unregulated raw material costs [26]. Proper training concerning the use of sterile conditions is a necessary step in the future for the palm sugar industry. Although the effects of the mycotoxin upon consumption may not be evident, long term consumption of palm sugar, due to either a preference for a natural sweetener alternative or lack of access to white sugar in a particular area, can lead to detrimental effects that are chronic or even teratogenic, as both aflatoxin B_1 and ochratoxin A are teratogenic toxins. Better regulation of the manufacture and sale should be exercised on a whole.

V. CONCLUSION

Upon isolation and identification of fungi from the palm sugar, there appears to be an affirmation that the adsorption of atmospheric moisture onto the surface of the palm sugar occurs due to its greater hygroscopicity compared to cane

sugar, resulting in greater fungal growth. The hygroscopic nature may be attributed to a greater sucrose concentration in its composition. The toxin production indicates that contamination has occurred in the palm sugar sample at some point during manufacture, transport, sale, or storage and that it is unsafe for consumption due to the potential of acute or chronic effects being caused by the mycotoxins. Regular microbial testing during the food supply chain may be a way to check the fungal contamination and different storage conditions and preservation methods, especially for export, may prove efficient in countering potential intoxication of consumers.

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