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Mycoflora of *Phoenix Dactylifera* (Date Palm) Fruit Sold in Some Markets in Ilorin

Adedayo, Majekodunmi Racheal
Lecturer, Microbiology unit, Department of Biosciences and Biotechnology,
College of Pure and Applied Sciences, Kwara State University, Malete, Nigeria
Babatunde, Shola Kola
Senior Lecturer, Microbiology unit, Department of Biosciences and Biotechnology,
College of Pure and Applied Sciences, Kwara State University, Malete, Nigeria
Ajiboye, Adeyinka Elizabeth
Lecturer, Microbiology unit, Department of Biosciences and Biotechnology,
College of Pure and Applied Sciences, Kwara State University, Malete, Nigeria
Adetoyi, Eunice Omolara
Student, Microbiology unit, Department of Biosciences and Biotechnology,
College of Pure and Applied Sciences, Kwara State University, Malete, Nigeria

Abstract
Aim: the research was conducted to isolate and characterize spoilage mycoflora present on dried date palm fruit commonly sold in some markets within Ilorin metropolis.
Method: samples were collected from eight different market locations within Ilorin metropolis. The samples were surface sterilized and split opened to isolate fungi from them and the isolates were characterized morphologically on plate and under the microscope. Characteristics observed were compared with literature for the identification of the specimen following standard methods. Physicochemical analysis was also conducted on the samples according to standard techniques.
Result: A total number of six fungi were isolated and identified as Apergillus niger, Neurospora crasa, Aspergillus fumigatus, Aspergillusflavus, Penicillum chrysogenum, and Syncaehalastron racemosus withA. niger having the highest percentage of occurrence. The physicochemical analysis revealed that the samples has high percentage of sugar and mineral contents.
Conclusion:the study revealed that date palm fruit sold within Ilorin metropolis is heavily contaminated with fungi. The fungi responsible for its spoilage are those that have been known to produce toxic substances which have adverse effect on the consumer's health.

Keywords: date palm, mycoflora, fungi, physicochemical, spoilage

1. Introduction

The occurrence of fungi on fruits has often cause wastage to farmers and reduction in market value. Microbial attack and deterioration is a major setback on agricultural produce. This to a large extent has limited the durability of most food crops including fruits and vegetables. Microorganisms essentially fungi are the major cause of spoilage on foods classified as semi-perishable as a result of their ability to thrive in low moisture environments. The Agricultural industries sustained huge crop losses annually as a result of fungal disease of fruits and plants (Christensen *et* al., 2007). The ubiquity of fungi has been regarded a source of contamination of foods leading to spoilage and/or food-borne mycotoxins. Of all the species of fungi that have been described fewer than 400 are of medical importance and less than 50 species cause more than 90% of fungal infections of human and animals (Geo *et al.,* 2007).Fungal spoilage of fruits is a source of potential health hazard to man and animals. This is due to their production of toxic metabolites/mycotoxins (naturally occurring aromatic compounds) which are capable of incducing mycotoxicoses in man following ingestion or inhalation. However they differ in their degree and manner of toxicity (Effiuvwevwere, 2000). Owing to the role played by fungi, whether from economic or public health point of view, mould and yeast counts on food is a standard test for checking general sanitary conditions. More importantly as mould growth on foods that are to be consumed directly can result to direct exposure to mycotoxins.

Historically, date–palm (P*hoenix dactylifera*) tree had been crucial for the survival of nomadic tribes in Saudi Arabia. The fruit forms a vital component of diet in many countries of the world, Nigeria not exceptional. In Islamic countries, dates are among the religious first meals in breaking Ramadan fast and therefore largely consumed by Muslims for religious and traditional purpose. Fungal spoilage of date palm fruit has been reported in several part of the world (El-Deeb *et al*., 2007). They are attacked by various fungal species, thus causing their spoilage at ripening, as well during storage processing stages (Ibrahim and Rahma, 2009). Dates contain high sugar content and moderate percentages of minerals and vitamins. On the other hand, dates are relatively low in protein and fat (Yousif *et al*., 1987). Most of dates produced are consumed directly with little or no further processing. Like other fruits, date palms are attacked by various fungal species, thus causing their spoilage at ripening as well as during storage and processing stages. This majorly is attributable to the ability of fungi to tolerate low moisture and near acidic pH associated with the fruit. It has been observed that the date palm fruits are mostly loaded with mixture of microbes including bacteria but more essentially molds and yeast (Atia, 2011) but people eat it after clearing the outer and or inner environment, while some eat it whole irrespective of the state of the internal environment of the fruits (Atia, 2011).Djerbi (1983),stated that the most common fungi causing date fruits spoilage are *Aspergillus* sp., and *Alternaria* sp. This fungus secretes many kinds of enzymes and toxins that causes decay and loss of the nutritional value of the date rendering it unsuitable for consumption. *Aspergillus flavus, A.niger, Fusarium solani, Penicillium digitatum,* and *Rhizopus stolonifer* have been isolated and confirmed to be pathogenic ondate fruits in varying degrees(Berbendi, 2000).
In Kwara State, precisely, Ilorin, date fruits are being sold by local vendors who store both soft and dry ones in polythene bags, wrapped trays or wooden boxes. In addition, personal observation revealed that the fruits (especially dry ones) are normally sold to consumers un-washed and some do consume it also un-washed. Due to the toxins produced and the health implications of consuming these toxins, there is the need to study the spoilage mycoflora present on this dried date palm fruit because it is widely consumed all over the state.

2. Materials and Methods

2.1. Sample Collection and Preparation
A total of 40 date palm fruits were collected into sterile covered plastics from the following locations within Ilorin metropolis: Oja Oba, Oja Ipata, Post office, Surulere road, Kulende market, Tipper garage, Oke odo, Akerebiata from different hawkers. The collected samples were then transported to Kwara State University laboratory, Malete, Kwara State for analysis. With gloved hands, the fruits were cut open by means of sterile pair of forceps and scalpel blades, the outer surface and the inner environment were scrapped aseptically.

2.2. Fungal Isolation from Date Palm Fruits
Samples from each sampling points was cut into pieces. These pieces were put on wet sterilized filter paper and placed on Petri dishes; the plates were incubated at ambient temperature for 168 hours. Visible Fungal colonies over the fruit pieces were taken out with the help of sterilized needle and subcultured on potato dextrose agar (PDA). This was done repeatedly to obtain pure cultures for characterization and identification (Blackburn 2006).

2.3. Characterization and Identification of Fungal Isolates
Colonial and microscopic examinations were carried out on the pure isolates for their vegetative and reproductive structures. Some of the colonial characteristics observed were: colour/shape of spores, rates of growth, colour of hyphae, presence or absence of septa, presence or absence of pigment etc. Identification of fungi was based on the illustrations described by Samson and Von-ReenHoekstra (1988) and designated as F1 to F6.

2.4. Determination of Physicochemical Characteristics of the Samples
The physicochemical parameters of the samples were determined following standard methods. Moisture content (AOAC, 2000), pH (Lenette *et al*, 1985), titratable acidity (Kirk and Sawyer, 1991), total fat, total ash(AOAC, 2000), sugar content (Dubols *et al.*, 1956) using glucose as a standard and crude protein content (Lowry *etal*., 1951).
Results

3. Results

3.1. Description of Isolates
A total number of six fungi were isolated and identified as *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Neurospora crasa, Aspergillus fumigatus, and Syncaephalastron racemosus* (Plates 1-6).

- ISOLATE $F_{1:}$ Colonies on PDA (at room temperature), spreads rapidly attaining a diameter of 4-5cm within 7 days. Colony was initially white and then later turned dark brown to completely black due to formation of conidia as it matures. Conidia heads radiate tending to split into loose columns with age.The fungus identified was as *Apergillus niger.*
- ISOPLATE $F_{2:}$ Colonies on PDA was yellowish at first becoming bright green, spreading over the plate within 4-5 days of growth. Under the microscope, its conidium tends to break up releasing the spores when aged. The spores are green or yellowish green. The fungus was identifies as *Aspergillus flavus*.

- ISOLATE F3: Colonies grows fast on plate with blue green to light green colour and broad white margin on PDA, colony spreads fast, smooth and velvety, dry on the surface and becomes grayish green as days' advanced. The isolate was identified as *Penicillum chrysogenum.*
- ISOLATE $F_{4:}$ Colonies on PDA appeared initially fluffy and fast growing, covering the plate within 2-3 days, the plate was covered with orange powdery spores, turning pinkish in colour after 4 days. The mycelium consists of mass aerial hyphae producing countless spores, making the fungus to be easily recognized. The fungus was identified as *Neurospora crasa*.
- ISOLATEF5: Colonies on PDA appeared cloudy. Growth on plate shows crowded head (grows closely to each other making it difficult to see its root). After two days of inoculation, the whole plate becomes covered with smoky green spores. Microscopically, structure is similar to*A. flavus*, when mounted in lactophenol blue have a very characteristic appearance. The fungus was identified as *Aspergillus fumigatus*.
- ISOLATE F6: Colonies on PDA plate after two days of inoculation shows white mycelium covered with tan to brown colour with whitish margin,colony becomes covered with brown spores as it advanced in age. When viewed under the microscope hyphae that bear sporangium containing brown spores. It was identified as *Syncaehalastron racemosus*(Plates 1-10).

The frequency of occurrence of the identified fungi and percentage of occurrence of each isolate from each sampling point is as shown in Tables 2 and 3. Form the results, *P. chrysogenum* and *Syncephalastron racemosus* was found only in samples collected from Oja-Oba Market while *A. niger* was found on all othersamples from other sources. *Aspergillus niger* has the highest occurrence (41.7%) followed by Neurospora crasa (20.9%), *Aspergillus fumigatus* (15.4%), *A.flavus* (16.5%), *Penicullum chrysogenum* (3.3%) and *Syncaehalastron racemosus* had the least (2.2%).

The physichochemical study showed that the pH and the titratable acid of the date palm fruits sample tends toward acidity, the fat and sugar content, were reasonably high, the moisture content revealed low water contentand ash content revealed that dried date palm is rich in mineral ash (Table 4).

4. Discussion

The isolation and characterization of fungi from date palm samples in this research confirmed that there is heavy contamination of date palm fruit sold in Ilorin. The six isolates were similar to the fungi observed by Nawal and Salalh (1997); who also had earlier reported heavy contamination of date fruit by filamentous fungi. On the other hand, an earlier mycological analysis of semi dry dates showed a remarkable low incidence of diverse fungal contamination of the analyzed samples (El-deeb *etal.,* 2007).*A. niger* had the highest percentage of occurrence among the isolates and was found in all the locations. This finding is also comparable to the record of Hashem (2009), who reported that *Aspergillus* was the predominant genus of spoilage fungi on date palm with ten species followed by *Penicillium* with four species. Other authors with similar reports are Ibraheem and Klaef (2003); El-Deeb *et al*. (2007). Furthermore similar work on date fruit in Maiduguri metropolis in Nigeria revealed parallel result (Colman *et al.,* 2012).

However contrary finding was documented by Ibrahim and Rahma (2009) who reported that *Rhizopus* spp were the dominant isolate also research carried out on spoilage mycoflora of date palm fruits on sample obtained from different points within the two campuses of Bayero University, Kano state, Nigeria showed least occurrence of these organisms (Ibrahim, 2009) while EL-Deeb *et al*. (2007) could not isolate any of the above fungal organisms in his own research. These differences could be attributed to variance in geographical location, handlers and conditions of transportation and storage.

Most of the fungi isolated are known to produce harmful chemicals including mycotoxins that could pose health risk to consumers. Results of this study and earlier works have shown that date palm fruits are much more prone to contamination by *Aspergillus* spp than other fungal species. Dates can encounter fungal infestation by influences from the surrounding environment, such as insect's infestation, wound and presence of foreign matter such as sand, dust and debris thus some of the identified fungal species could have come from any of these sources (Djerbi, 1983). From the public health point of view, the contamination of date palm fruits by moulds is significant because of the probability of producing mycotoxins which can cause severe poisoning, emesis, nausea, vomiting and diarrhea, cancer and even death when consumed(Effiuvwevwere, 2000; Abdulla, 2008). Date fruits are sold by vendors without been protected in any way from dust or atmospheric contamination, and are often consumed without any form of cleaning or washing. Similarly, the constant exposure of the dates to the outside environment at the time of sales could aid the deposition of fungal spores on them. These spores germinate when temperature and humidity become favourable. Damage by insects has also been known to provide entry points for fungal infection and aid in their rapid spread. On consumption of such fruits unsuspected consumers are predispose to imminent mycotic infection (Colman *et al*., 2012).

Neurospora craca (the second most occurring organism) commonly referred to as bakery mold because it frequently infects bakery products and causes considerable damage, spreads fast leading to heavy contamination of the atmosphere with its spores. This could hence increase its adaptability and potentiality in causing disease in both man and animals. This organism is widely distributed in nature growing on organic matter and food remnants particularly on maize cob.

Aspergillus fumigatus spores could lead to temporary acute trachea-bronchitis in patients with pre-existing lung damage when inhaled. Aspergillosis, an infection caused by *Aspergillus* spp is of three type: allergic Aspergillosis (where *A. fumigatus* colonizes the bronchial tree of an asthmatic patient), saprophytic aspergillosis (where *A. fumigatus* spores colonizes some areas of the lungs tissue that had been previously damaged by diseases such as tuberculosis; and disseminating aspergillosis (which occurs in individuals that been infected with diseases such as leukemia (Lenette *et al*., 1985).

Mycotoxins produced by *Penicillum* spp (especially citrinin produced by *Penicillium citrinum*)are toxic to human health and also aflatoxins produced by Aspergillus flavus is also believed to be carcinogenic and causes extreme liver toxicity to human (Magan, 2004). Inhaling the spores of *S. racemosus*,a principal member of the genus *Syncephalastrum* and a potential human pathogen, could

lead to primary rihnocerebral, pulmonary and disseminated disease including gastrointestinal, cutaneous/subcutaneous, allergic diseases and even asymptomatic colonization (Domsch *et al*., 1980; Ellis, 1997).

The physicochemical analysis (Table 4) revealed that dates fruit has high sugar content and moderate percentages of minerals ash which enhance the growth of fungal isolate. Similarly, the pH of the sample (tending towards acidity), low moisture content of the dried fruit, and seemingly high sugar content provides a good environment for fungal growth. This report was similar to earlier report by Yousif *et al*. (1987).

5. Recommendation and Conclusion

Evidence from this study on the spoilage mycoflora of date palm fruit, revealed that date fruit sold within Ilorin metropolis is heavily contaminated with fungi. Most of the fungi responsible for its spoilage are those that have been known to produce toxic substances which have adverse effect on the consumer's health. Hence, people should try as much as possible to wash these fruit before eating them with plenty of clean water. One drop of vinegar might also be dropped in the water to enhance cleansing. Washing helps to reduce microbial load and unnecessary consumption of these fungi and their toxins. Conclusively, vendors should be oriented on the importance of hawking these fruits in clean packages to prevent exposure to spoilage agent and also contaminants that could pose great danger to the health of the consumers.

6. References

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Annexure

Sampling Point	*Aspergillus niger*	*Aspergillus fumigatus*	*Aspergillus flavus*	*Neurospora crasa*	*Penicillum chrysogenum*	*Syncaehalastron racemosus*
Akerebiata	5	_	_	3	_	_
Oja Oba	5	_	5	_	3	2
Oja Ipata	6	_	2	_	_	_
Oke Odo	6	4	-	4	-	-
Post Office	7	-	8		-	-
Tipper garage	5	4	-	2	-	-
Surulere	3	-	-	7	-	-
Kulende	1	6	-	3	-	-
Total	38 (41.7%)	14 (15.4%)	15 (16.5%)	19 (20.9%)	3(3.3%)	2 (2.2%)

Table 1: Fungal isolates and their frequency of occurrence

Sampling Point	*Aspergillus niger*	*Aspergillus Fumigatus*	*Aspergillus flavus*	*Neurospora crasa*	*Penicillum chrysogenum*	*Syncaehalastron racemosus*	**Total (%)**
Akerebiata	5(62.5%)	_	_	3(37.5%)	_	_	100
Oja Oba	5(33.3%)	_	5(33.3%)	_	3(20%)	2(13.4%)	100
Oja Ipata	6(75%)	_	2(25%)	_	_	_	100
Oke Odo	6(42.8%)	4(28.6%)	-	4(28.6%)	-	-	100
Post Office	7(46.7%)	-	8(53.3%)		-	-	100
Tipper garage	5(45.5%)	4(36.4%)	-	2(18.2%)	-	-	100
Surulere	3(30 %	-	-	7(70%)	-	-	100
Kulende	1(10%)	6(60%)	-	3(30%)	-	-	100

Table 2: Distribution of fungal isolates and their percentage of occurrence.

Samples	**pH (at 25^{o}C**	**Fat Content (mol/ml)**	**Titratable Acid(cm^{3})**	**Moisture Content (%)**	**Ash Content (%)**	**Sugar Content (mg/g)**	**Protein Content (%)**
Post office	5.93	9.2	2.31	32	28.0	2.3	20
Oja Oba	6.15	7.8	0.10	20	13.0	2.5	16.5
Oja Ipata	5.61	8.5	0.09	32	26.0	1.8	19.4
Surulere	5.73	9.9	0.10	40	35.0	2.0	18.4
Kulende	6.06	8.4	0.90	21	15.0	2.7	18.5
Akerebiata	5.20	9.2	0.09	42	38.5	2.7	19.8
Oke Odo	5.12	9.2	0.09	30	26.3	1.7	17.9
Tipper Garage	6.00	8.4	0.10	32	28.0	1.5	16.7

Table 3: Physiochemical Properties of the Date Palm Fruit Samples.

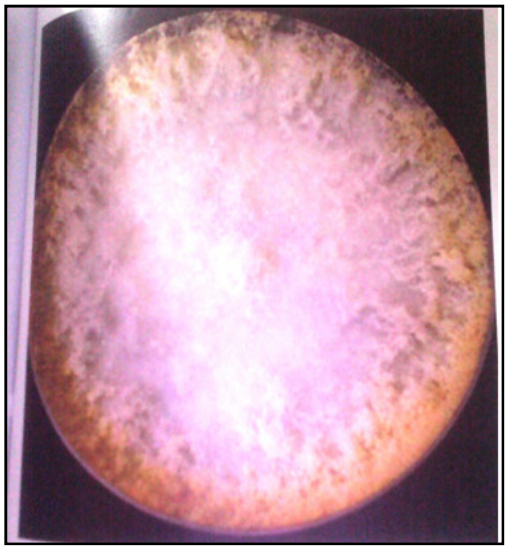


Figure 1: Neurospora Crasa growth on PDA plate

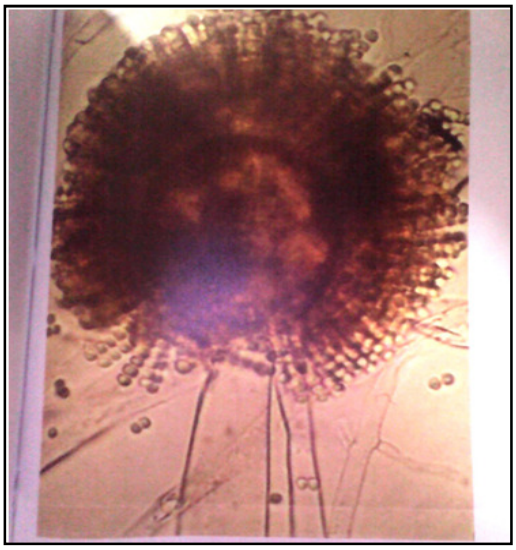


Figure 2: Microscopic view of Sycachalastron racemous

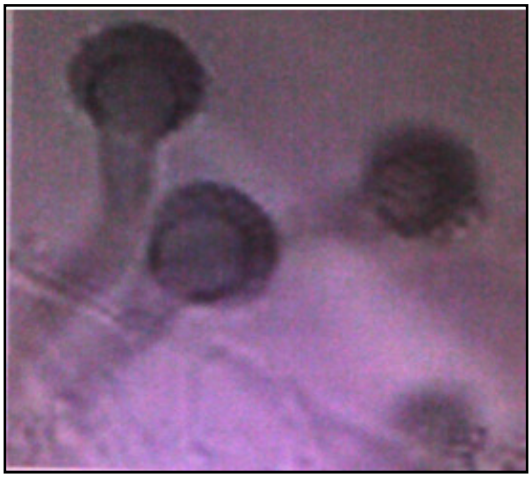


Figure 3: Microscopic view of Aspergilus famigatus

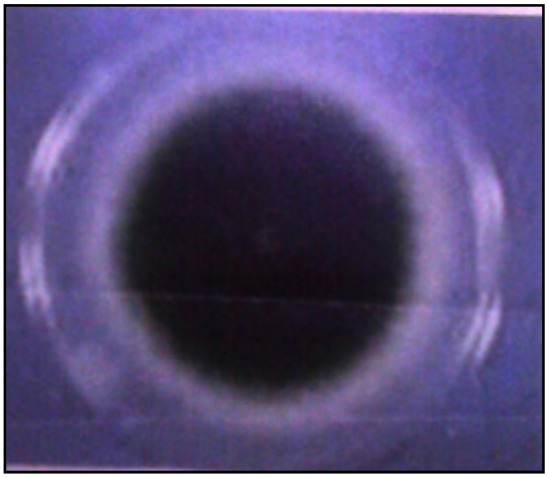


Figure 4: Growth of Aspergilus famigatus on PDA plate

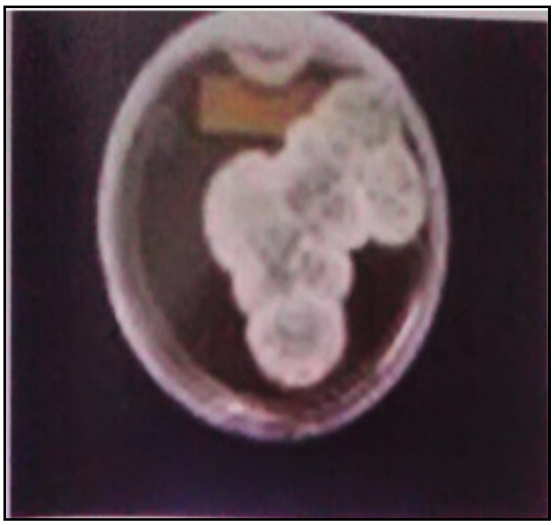


Figure 5: Growth of Penicillum chrysogenum on PDA plate



Figure 6: Microscopic view of P. chrysogenum

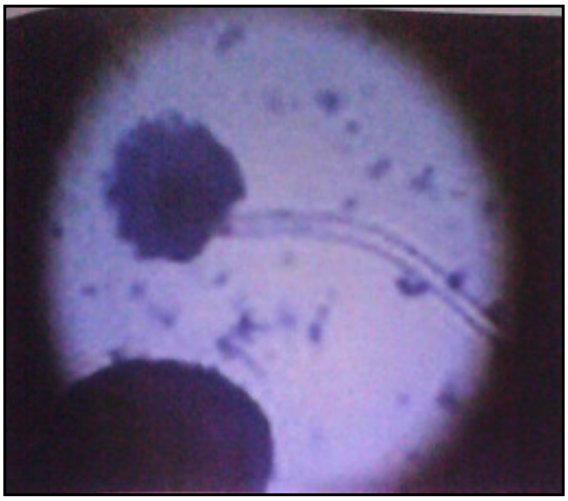


Figure 7: Microscopic view of Aspergilus niger

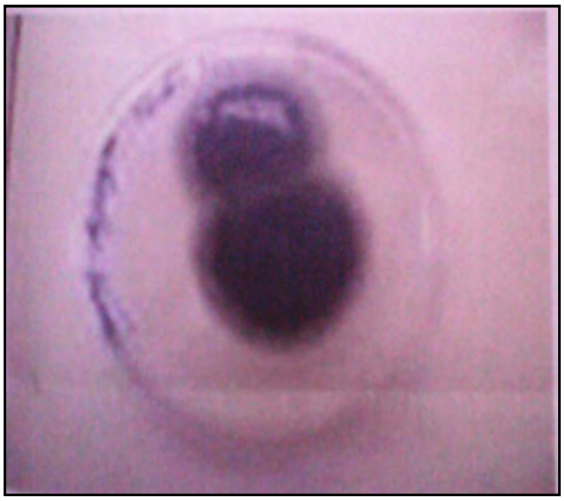


Figure 8: Growth of Aspergilus niger on PDA plate

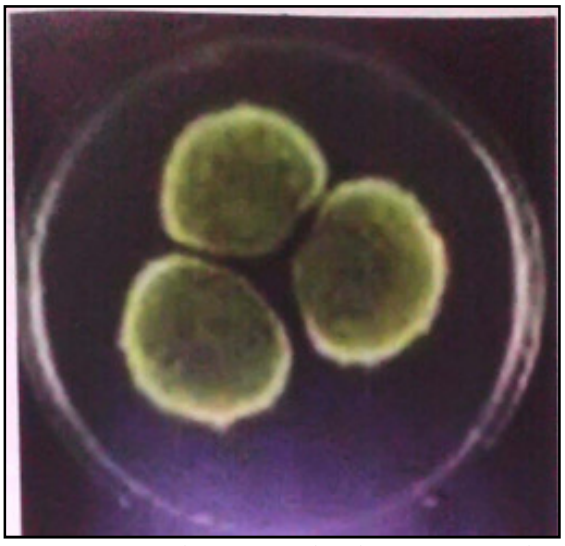


Figure 9: Growth of Aspergilus flavus on PDA plate

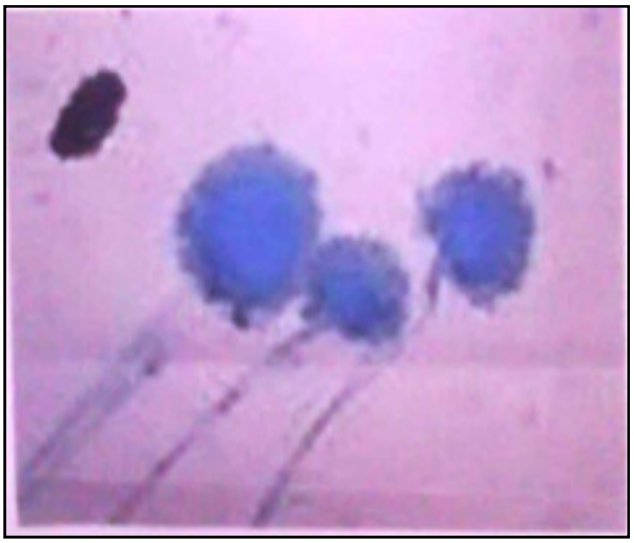


Figure 10: Microscopic view of Aspergilus flavus