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## ABSTRACT

Studies was carried out on Isolation and Identification of pathogenic fungi in 180 date fruits (*Phoenix dactylifera*, Linn.), sampled from the three major Markets in Gombe Metropolis, Gombe, Nigeria between April and June 2018. The samples were cut into 3 mm pieces on a clean and sterilized tile with the aid of sterilized razor blade, surface-sterilized in 1% hypochlorite for 5 minutes, placed on blotter paper and incubated at room temperature for 5 days. Pure cultures of the resulting fungi were obtained from subcultures on Potato Dextrose Agar of the primary plates. These were identified morphologically and microscopically in accordance with standard procedures. The investigation showed that the most dominant isolated fungi were *Motierella alpina* (38.8%) and *Mucor* sp. (25%), followed by *Aspergillus niger*. (13, 8%), *Apophysomyces variabilis* (11.1%), *Neosartorya pseudofischer*. (5.5%) and *Rhizopus stolonifer*. (5.5%) respectively. Of all the samples, Dates from Old Market were heavily infested. *Aspergillus niger* appeared to be the most toxigenic fungi recovered from the dates. Also, it has been observed that most of the fungi isolated from dates are known to produce harmful mycotoxins that could pose health risk to consumers. It was concluded that variety (Dan Mali) should be consumed because it had least percentage of fungal infestation as reported in the study. Contaminated dates should be sorted and eliminated to avoid re-infection and washing the dates with clean water prior to consumption should be strongly encouraged by consumers to avoid infection and thus, have a healthy community, while farmers and hawkers should ensure proper handling during or after harvesting, processing, storage or transition. Therefore, in-depth study should be conducted on mycotoxins associated with date.

**Keywords:** fungi; date palm fruits; contamination; mycotoxins; market.

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healthy community, while farmers and hawkers should ensure proper handling during or after harvesting, processing, storage or transition. Therefore, in-depth study should be conducted on mycotoxins associated with dates.

**Keywords:** fungi; date palm fruits; contamination; mycotoxins; market.

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## I. INTRODUCTION

*Phoenix dactylifera* commonly known as date or date palm (it is also referred to as Dabino in Hausa language) (GRIN, 2017). It is a flowering plant species belonging to the family Arecaceae. Date palm is one of the oldest domesticated fruit crops (since 4000 BC in Ancient Egypt), and one of the most important growing trees in desert areas (Ramawat, 2010). Date palm trees are widely spread in arid and semi-arid areas within 10° and 39° in the Northern Hemisphere, the optimal latitude being between 24 and 34°N in the Old World, and between 33 and 35°N in the West Coast of the USA (Zaid *et al.*, 2002). It has also been introduced into other countries such as Pakistan, Australia, Mexico, South America and South Africa and Nigeria because of its nutritive, religious and cultural values (Janick *et al.*, 2008; Chao *et al.*, 2007). Date palm is believed to have been introduced into Nigeria in the early 8th century by Arab traders from North Africa. The Date palm tree (Plate 4) can be grown extensively and commercially in the region of Northern Nigeria from latitude 10°N in the Sudan savannah

and the Sahel regions. This includes Kaduna, Katsina, Kano, Sokoto, Kebbi, Zamfara, Jigawa, Yobe, Borno, Gombe and Bauchi States. Date fruits are a highly valued delicacy among many communities in Northern part of Nigeria especially during ceremonies and festivals. There is an increasing rate of dates fruit consumption as reported in 2009 which is estimated at 8,958 metric tons which ranked the country among the world top 10 consumers of date (Sani *et al.*, 2009). The fruit is affected by various pests and also infested with disease causing microbes, but the nature of the problem varies with geographic location (Howard *et al.*, 2001). Atia (2011) observed that the date palm fruits are mostly infested with mixture of microbes: bacteria, moulds and yeast but it is seldom consumed after clearing the pericarp with bear hands, while some eat it whole irrespective of the state of the pericarp. Studies have shown that species of *Alternaria spp*, *Aspergillus niger*, *Fusarium spp* and *Penicillium spp* are the causal agents of fruit rots of date-palm (Bokhary, 2010). Hawking of uncovered dates fruit is a common business especially in Northern part of Nigeria where Gombe is located. The high patronage of this valued fruit attracts (both fresh and dried fruits) is a cause for pathological concern in light of reports indicating that some potential mycotoxin producing moulds are implicated (Anjili *et al.*, 2016). Also Christensen *et al.*, (2007) reported that Agricultural Industries sustained huge crop loss as a result of fungal diseases of fruits and plants. The food and Agricultural Organization of the United Nations (FAO) has estimated that up to 25% of the world food crops are significantly contaminated with mycotoxins (WHO, 1999). Mycotoxins are highly toxic and cause severe intoxications in humans and animals, some of them are carcinogens (Khomutov *et al.*, 2011). Studies have been conducted on the Isolation and Identification of Pathogenic fungal species from Date palms in other parts of Nigeria, but so far, there is no available report on the isolation and identification of Pathogenic fungal species from date palm fruits around Gombe metropolis, Nigeria. Therefore, it becomes imperative to

investigate both the inner and outer surface of the fruits, as regard the presence of mycotic agents, as well as its suitability for human and animal consumption. In Gombe Metropolis, Gombe, date fruits are being sold by local vendors and they store both soft and dry ones in synthetic sacks or wooden boxes. Moreover, personal observation revealed that the fruits (especially dry ones) are normally sold to consumers un-washed and some do consume it also un-washed. For this study, three varieties were considered as follows:

## II. MORPHOLOGICAL DESCRIPTIONS OF VARIETIES OF DATE PALMS UNDER STUDY.

A. Dan Mali-It is bigger in size, fleshy, soft, and is more expensive because it is less available than the other two varieties. It is sweeter in taste and the skin is more or less twisted. It is probably imported from Mali.



*Plate: 1.* Dan Mali

B. Dan Agadaz-It is brownish in colour, very dry and cheaper than Dan Mali because it is more available probably from Niger republic



*Plate: 2.* Dan Agadez

C. Farin Dabino-It is brighter brown in colour, a little bit softer than Dan Agadaz and is also cheap because it is indigenous to Nigeria.



*Plate: 3.* Farin dabino



*Plate :* Date Palm Tree

The aim of this research is therefore to culture, isolate and identify fungi associated with the date fruits sold at Gombe Metropolis, The results of this research is also intended to be used in suggesting possible ways of minimizing or avoiding possible health problems associated with these fungi.

### III. MATERIALS AND METHODS

#### *3.1 Sample collection*

The survey of fungi associated with date palm (*Phoenix dactylifera* L.) was conducted in three major markets in Gombe: Gombe Main market, Pantami market and Old market in Gombe metropolis. A sample of 180 fruits consisting of three varieties of date palm fruit namely: Dan Agadas (60), Dan Mali (60) and Farin Dibino (60) were randomly obtained from sellers in each market. These were brought to the laboratory of Department of Biological Sciences Department, Gombe State University Gombe, Nigeria where all determinations and tests were carried out. The samples were surface-sterilized with 1% Sodium Hypochlorite for 5 minutes in order to remove the surface contaminant from the samples collected.

### 3.2 Incidence of Rot of Date Palm Fruits in Storage

The samples purchased from each of the markets were sampled out, taking the number of date palm fruits having rot, out of the total number of date palm fruits purchased from the market. The incidence of fungal spoilage/rot was expressed in percentage using the formula:

$$\frac{\text{Number of date palm fruits with rot}}{\text{Total number of date palm fruits}} \times 100$$

### 3.3 Culture Media

Potato Dextrose Agar (PDA) as general culture medium was prepared aseptically according to the manufacturer's instructions to culture fungal isolates.

### 3.4 Isolation of Fungi from Date Palm Fruits

Under aseptic conditions the diseased sample from date palm fruit showing rot was sectioned into approximately 5 mm square with a heat-sterilized scalpel. The pieces were immersed in 1% sodium hypochlorite contained in a sterile 9 cm diameter Petri- dish for surface sterilization for 30 seconds using sterile forceps. The sterilized pieces were rinsed in three changes of sterile distilled water and then dried between sterile filter papers. With a flamed and cooled pair of forceps, a sterilized piece of date palm was blotted dry between sterile filter papers, then plated aseptically on 9cm diameter Petri-dish containing sterile solidified Potato Dextrose Agar (PDA) and incubated at room temperature of  $33 \pm 20$  C for 5-7 days. After incubation, colonies of different shapes and colours were observed on the plates. A pure culture of each colony type was obtained and maintained. The maintenance was done by sub-culturing each of the different colonies on PDA plates and incubated at room temperature again for 5 days (Ibrahim, 2009)

### 3.5 Identification of the fungal isolates

The fungal isolates were identified using standard methods described by Fawole and Oso (2007).

This was done by matching the microscopic and macroscopic features of each fungal isolate with those of known species described in relevant monographs, standard textbooks and journals. The technique of James and Natalie (2001) were adopted for identification of the unknown isolated fungi using cotton blue in lactophenol stain. The identification were achieved by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the mycelium from the fungal cultures was removed and placed in a drop of Lactophenol. The mycelium was spread very well on the slide with the aid of the needle. A cover slip was gently applied with little pressure to eliminate air bubbles. The slide was mounted and observed under x10 and x40 objective lenses respectively. The species encountered were identified in accordance with Cheesebrough (2000).

Healthy date fruits (semi ripe) were obtained and surfaced- sterilized with 1% sodium hypochlorite for 30 seconds and rinsed in three (3) changes of sterile distilled water according to the method of Chukwuka *et al.*, (2010). A sterile cork borer (2 mm in diameter) was used to puncture and inject healthy date fruits with spores' suspension of each isolated fungus in three replicates. Removed tissue was replaced and vasper jelly was smeared to completely seal each hole to avoid contamination. It was kept at room temperature of  $33 \pm 2$  oC. A similar set up was placed as control using distilled water to complement the fungal inocula. The set up was arranged in a completely randomized design. It was incubated for seven (7) days to allow for possible rot development and the isolates were re-isolated from the new host and compared to the originally isolated pathogen.

### 3.6 Determination of Severity of Rot Caused by Isolated Organisms

Twelve (12) date fruits were randomly selected, weighed and surface- sterilized with 1% sodium hypochlorite then rinsed in three (3) changes of sterile distilled water. A sterile cork borer (2 mm in diameter) was used to puncture and inject healthy date fruits with spores' suspension of

isolated fungi in three replicates. Similarly removed tissue from fruit was replaced and sealed with sterile vasper jelly. This was then incubated for 7 days after which each fruit was collected and the extent of rot (severity of infection) was measured with caliper and rule, with the aid of a hand lens and re-weighed. Results were analyzed with Statistical Analysis System (SAS) version 7.

#### IV. RESULTS

##### 4.1 Frequency of occurrence of fungal isolates

Distribution of the date fruits according to sampling point (Main market, Old market, and

Pantami market) shows that. Sixty (60) dates were sampled from each location (Main market, Old market, and Pantami market) and a total of 6 fungal species were isolated, namely; *Mucor spp*, *Aspergillus niger*, *Neosartokya pseudo fischeri*, *Rhizopus stolonifer*, *Apophysomyces variabilis* and *Mortierella alpina*. Of these six (6) species, *Mortierella alpina* (38.8%) had the highest percentage of occurrence and with the least percentage of occurrences seen in *Rhizopus stolonifer*, (5.5%) and *Neosartorya pseudofischeri* (5.5%) respectively (Table 1).

**Table 1:** Fungal isolates and their frequency of occurrence

Sampling Point	<i>Mucor spp</i>	<i>Motierella aipina</i>	<i>Aspergillus niger</i>	<i>Neosartorya pseudofischeri</i>	<i>Rhizopus stolonifer</i>	<i>Apophysomyces variabilis</i>
1	5	25	-	-	-	20
2	15	5	25	10	5	-
3	25	40	-	-	5	-
4	45	70	25			
Total	25%)	(38.8%)	(13.8%)	(5.5%)	(5.5%)	(11.1%)

1: Main market, 2: Old Market, 3: Pantami Market

##### 4.1 Fungal species isolated from the three varieties of Date Fruits

The study revealed that, among the three varieties sampled, the highest number of isolates were

recorded on Farin Dabino (37 %), followed by Dan Agadaz which had 32.% and 29.7 % goes for Dan Mali (Table 2).

**Table 2:** Distribution of fungal species isolated on three varieties of dates

Date varieties	Main market	Old Market	Pantami Market	Total
1	10	20	25	55 (29.7%)
2	20	20	20	60 (32.4%)
3	25	20	25	70 (37.8%)

##### 4.2 Distribution of fungal species across three (3) Markets

The result shows that, the highest percentage of fungi isolated was recorded in Old market with five numbers of species followed by Main market (3) while Pantami market had the least occurrence (2) (Table 3). The fungus with high relative

frequency was *Motierella alpina* (38.8%). This is followed by *Mucor spp* (25%) and while *Neosartorya pseudofischeri* and *Rhizopus stolonifer* are the least with 5.5 % during the survey as shown in Table below. *Apophysomyces variabilis* (11.1 %) was recorded in Main market only (Table 3

isolated fungi in three replicates. Similarly removed tissue from fruit was replaced and sealed with sterile vasper jelly. This was then incubated for 7 days after which each fruit was collected and the extent of rot (severity of infection) was measured with caliper and rule, with the aid of a hand lens and re-weighed. Results were analyzed with Statistical Analysis System (SAS) version 7.

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3	25	40	-	-	5	-
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(13.8%), *Aspergillus niger* causes black mould disease on fruits and vegetables; it is also a common contaminant of food (Sharma, 2012). *Aspergillus* spores are common components of aerosols where they drift on air currents, dispersing themselves both short and long distances depending on environmental conditions. When the spores come in contact with a solid or liquid surface, they are deposited and if conditions of moisture are right, they germinate. The ability to disperse globally in air currents and to grow almost anywhere when appropriate food and water are available means that 'ubiquitous' is among the most common adjectives used to describe these moulds. It also causes a variety of diseases known as aspergillosis, which includes occupational mycoses, allergies, localized mycoses, and invasive mycoses are all assembled together under the same name (Nickle, and Marr, 2005). *Rhizopus stolonifer* (5.5%), *Mucor* spp (25%), and *Apophysomyces variabilis* (11.1%) are agents of human zygomycosis. zygomycosis (mucormycosis) is a rare but often fatal infection caused by fungi in the class Zygomycetes. It usually affects persons with underlying factors such as diabetes mellitus, metabolic acidosis or immunodeficiency states (Ribes, 2000; Linder *et al.*, 1998), Zygomycetes are saprophytic fungi that are ubiquitous in soil or decaying organic material. The clinical manifestations of zygomycosis mainly are rhinocerebral, pulmonary, cutaneous, gastrointestinal, cardiac and cerebral disease (Ribes, 2000). The most common agent of zygomycosis is *Rhizopus* species, but other species in the order Mucorales such as *Absida*, *Mucor*, *Rhizo mucor* are also frequently seen, whereas *Saksenaea vasiformis* and *Apophysomyces elegans* are considered rare pathogens (Linder *et al.*, 1998). Many infections caused by *Apophysomyces variabilis* have been reported including fatal necrotizing fasciitis in immunocompetent patients (Ruiz, *et al.*, 2004; Weddle, *et al.*, 2012). *Apophysomyces variabilis* is considered an emerging species which is able to inflict significant diseases even among immunocompetent patients (Dela Cruz, *et al.*, 2012; Guarro, *et al.*, 2011) and can be fatal. This

study revealed that *Aspergillus niger* (13.8%) occurred only once which is contrary to the findings of (Hashem, 2009; Adebayo *et al.*, 2012; Colman *et al.*, 2015; Angili *et al.*, 2015; Orole *et al.*, 2017; Olan *et al.*, 2017) who reported that *A. niger* had the highest percentage of occurrence among the isolates they studied. This study was also contrary to the findings of Ibrahim and Rahma (2009) who reported that *Rhizopus stolonifer* were the dominant isolates. These differences could be attributed to variance in geographical location, handlers and conditions of transportation and storage. However similarity exists of the general presence of *Aspergillus niger* between the researchers conducted on date palm within all the locations studied. Also, the presence of *Mucor* spp was common to Ibrahim and Rahma (2009) and Colman *et al.*, 2015. Therefore, it is very possible that there could be some active principles in the fruits which could favour colonization by these organisms. (Abdulla, 2008). From the public health point of view, the contamination of date palm fruits by moulds is significant because of the presence of mycotoxins which can cause severe poisoning, emesis, diarrhoea, and even death (Abdulla, 2008). It was observed that the date fruits being hawked around, sold in shops or market places were neither covered nor protected in any way from dust or atmospheric contamination, and when consumed without any form of clearing or washing could predispose such unsuspected consumers to imminent mycotic infection (Shamsuddeen and Magashi, 2005). This study showed that among the different locations where the date palm samples were collected from, Old market had the highest fungal load in the fruits sampled, probably because of the manner in which they handled the fruits, leaving it exposed to all sorts of microorganisms to land on it, followed closely by Main market which might not be unconnected with the dirty and dusty environment of the markets. In the course of isolation, it was observed that some fungi, especially *Mucor* spp grew faster at 37°C, which is the total optimal human body temperature; this could hence increase its adaptability and potential

in causing disease to both humans and animals. (Shamsuddeen and Magashi, 2005).

The investigation concluded that the most dominant isolated fungi were *Motierella alpina* (38.8%) and *Mucor sp.* (45%), followed by *Aspergillus niger.* (13.8%), *Apophysomyces variabilis.* (11.1%), *Rhizopus stolonifer.* (5.5%) and *Neosartorya pseudofischeri* (5.5%) respectively. Of all the samples, dates from Old Market were the most heavily contaminated probably owing to the non-hygienic condition of the market and improper storage facilities associated with it. *Aspergillus* species appeared to be the most toxigenic fungi recovered from the dates. While several fungal species cause spoilage of dates worldwide, however the presence of *Aspergillus* spp. (Table 4) recovered from (Dan Mali) and (Farin Dabino) both in the Old Market in this research shows that there is the fear of consumption of aflatoxins that have a serious health implications, as they are highly toxic and carcinogenic (Ibrahim and Rahma, 2009) thus rendering the fruits unfit for human and animal consumption.

## VI. RECOMMENDATION

Date fruits are sold by vendors without being 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 favorable. 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 predisposed to imminent mycotic infection.

*The following recommendations are given:-*

It's recommended that variety (Dan Mali) should be consumed because it has the least percentage of fungi as reported in the study. Contaminated dates should be sorted and eliminated to avoid

reinfection and washing the dates with clean water prior to consumption should be strongly encouraged by consumers to avoid infection and thus, have a healthy community.

Farmers and hawkers should ensure proper handling during or after harvesting, processing, storage or transition. Most of the fungi isolated from dates are known to produce harmful chemicals including mycotoxins that could pose a health risk to consumers. Therefore, in-depth study should be conducted on mycotoxins associated with dates.

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