



ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH DATE FRUITS (*PHOENIX DACTYLIFERA*, LINN.) SOLD AT BAYERO UNIVERSITY, KANO, NIGERIA

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ABSTRACT

Mycological investigation on spoilage fungi in 30 apparently infected date fruits (Phoenix dactylifera, Linn.), sampled from both new and old campuses of Bayero University, Kano, Nigeria was carried out between August and September 2008. 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 2 minutes, placed on Potato Dextrose Agar and incubated at room temperature for 5 days. Pure cultures of the resulting fungi were obtained from subcultures 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 Rhizopus sp. (100%) and Mucor sp. (100%), followed by Torula sp. (40%), Penicillium sp. (30%), Aspergillus sp. (16.67%) and Alternaria sp. (13.33%) respectively. Of all the samples, soft dates were the most heavily contaminated probably owing to the artificial increase in moisture content and improper storage facilities associated with it. Aspergillus species appeared to be the most toxigenic fungi recovered from the dates. It was recommended that soft dates should be stored in a cold environment while dry dates should be stored under dry condition so that they will not increase in moisture and avoid constant exposure to air at sales time, hence the use of paper wraps should be encouraged. Similarly, contaminated dates should be sorted and eliminated to avoid re-infection while washing the dates with clean water prior to consumption should be strongly encouraged by appropriate authorities with the view to checking the spread of these fungi.

Key words: *Phoenix dactylifera*, fungi, contamination, spoilage, Kano

INTRODUCTION

Although date fruits (*Phoenix dactylifera*, Linn.) form a vital component of diet in Arabian Peninsula especially Saudi Arabia, they are as well being consumed in many countries of the world, Nigeria inclusive (Redmond, 2009). In Islamic countries dates are among the religious first meals in breaking Ramadan fast and therefore largely consumed by Muslims for religious and traditional purposes. However, like other fruits, they are attacked by various fungal species, thus causing their spoilage at ripening as well as during storage and processing stages (Ahmad, 2003). Thus, although several fungal species have been implicated in causing damages to date fruits, Djerbi (1983) stated that the most common fungi causing date fruits spoilage are *Aspergillus* sp. and *Alternaria* sp.. These fungi growing on field and stored products can cause damages resulting in reduction in quality and quantity of the fruits. In addition, many fungal species are capable of producing mycotoxins (aflatoxins), which are secondary metabolites that are highly toxic to humans and animals alike (Shamsuddeen and Magashi, 2005). Shenasi *et al.* (2002) reported that *Aspergillus flavus* and *A. parasiticus*, both of which produce aflatoxins have been found to invade dates at all stages of maturation as well as date products.

In Bayero University, Kano, date fruits are being sold by local vendors in which case they store both soft and dry ones in polythene bags, natural fibre, 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. The aim and objective of this research were therefore to culture, isolate and identify spoilage fungi associated with the date fruits sold at Bayero University, Kano as some species are potentially toxigenic. The results of this research is also intended to be used in suggesting possible ways of minimizing or avoiding possible health problem associated with these fungi.

MATERIALS AND METHODS

Sampling Site

For the purpose of this research apparently infected date samples were collected from new and old campus of Bayero University, Kano-Nigeria. The university is located in north-western Nigeria and founded in 1966. Based on 2006 statistics, the students' population stood at 30,026 with academic staff population being 575 (www.buk.edu.ng).

At present the university has two campuses commonly known as Old and New Campus. So, the conduction of this research on both campuses becomes important as many staff and students purchase and consume the dates from both campuses and some probably are not aware of the possible health risk that may be associated with that habit.

Sample Collection

Samples were collected based on their ripening stage (which is related to the moisture and sugar content of the dates). Infested dates were identified by physical examination following the method of Jha (1995) while the associated fungi were identified in accordance with Cheesbrough (2000). Thirty (30) samples were collected; fifteen (15) from each campus, made up of five (5) from each variety which include the soft date with high moisture and high sugar content literally known as "Dan madina", and the dry date of low moisture and very high sugar content literally called "Degla", both imported from Niger Republic, and the other sample is the one cultivated in Nigeria which is pale yellow with reduced sugar content, literally known as "siki" or "gaude" mostly grown in Jigawa State.

Culture Media

Two media were used, Potato Dextrose Agar (PDA) as general culture medium and Sabouraud Dextrose Agar (SDA). They were prepared according to the manufacturer's instructions.

Isolation of Fungi

The infected samples of dates were cut into 3 mm pieces with sterile razor blade, surface-sterilized in 1% hypochlorite for 2 minutes, then placed on Potato Dextrose Agar (PDA) and incubated at room temperature for 5 days. After incubation, colonies of different shape and colours were observed on the plates. A pure culture of each colony type on each plate was obtained and maintained. The maintenance was done by sub-culturing each of the different colonies onto the SDA plates and incubated at room temperature again for 5 days (Jha, 1995).

Identification of Isolated Fungi

The technique of James and Natalie (2001) was adopted for identification of the unknown isolated fungi using cotton blue in lactophenol stain. The identification was 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 then mounted and observed with x10 and x40 objective lenses respectively. The species encountered were identified in accordance with Cheesbrough (2000).

RESULTS

Table 1 shows the fungi isolated from the three varieties of dates based on their differences in the

moisture content sold at Bayero University Kano campuses. Six species of fungi were identified from the samples, namely; *Rhizopus* sp., *Mucor* sp., *Penicillium* sp., *Torula* sp. (yeast), *Aspergillus* sp. and *Alternaria* sp.. The six species identified were all found isolated from the semi dry date ("siki") and soft date ("Dan madina") due to their high moisture content. On the other hand, only three species were isolated from the dry date ("Degla"). Plates I – IV show some of the microphotographs of the fungi isolated from the spoiled dates on the campus.

Table 1: Fungi isolated from three varieties of dates sold at Bayero University, Kano.

S/No.	Variety of dates based on moisture content	No. of Samples	Campus	<i>Aspergillus</i> sp.	<i>Alternaria</i> sp.	<i>Rhizopus</i> sp.	<i>Torula</i> sp.	<i>Penicillium</i> sp.	<i>Mucor</i> sp.
1.	Semi dry date (siki or gaude)	10	OC	1 (10)	1 (10)	5 (50)	2 (20)	2 (20)	5 (50)
			NC	1 (10)	1 (10)	5 (50)	1 (10)	2 (20)	5 (50)
2.	Soft date (Dan madina)	10	OC	2 (20)	1 (10)	5 (50)	2 (20)	3 (30)	5 (50)
			NC	1 (10)	1 (10)	5 (50)	3 (30)	2 (20)	5 (50)
3.	Dry date (Degla)	10	OC	0 (00)	0 (00)	5 (50)	2 (20)	0 (00)	5 (50)
			NC	0 (00)	0 (00)	5 (50)	2 (20)	0 (00)	5 (50)
Total		30		5 (16.67)	4 (13.33)	30 (100)	12 (40)	9 (30)	30 (100)

N.B.: Values in parenthesis are percentages, OC = Old Campus, NC = New Campus



Plate I: *Alternaria* sp. isolated from date fruits

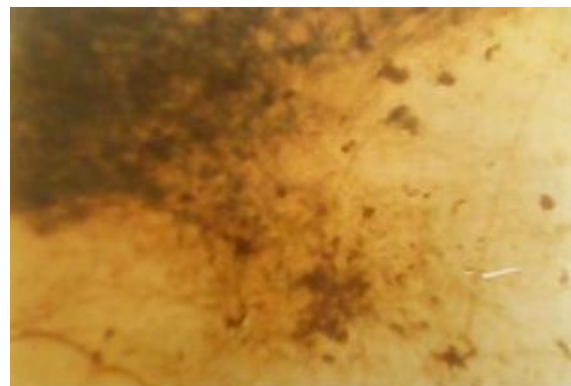


Plate II: *Rhizopus* sp. isolated from date fruits

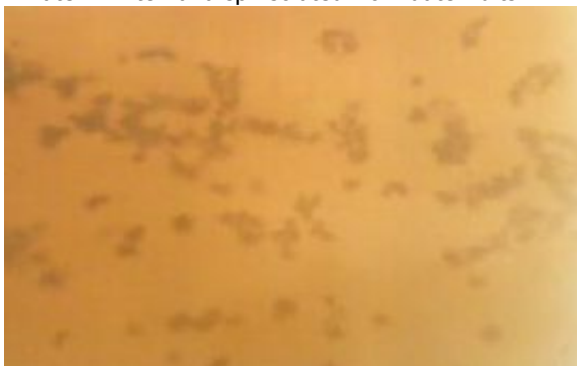


Plate III: *Torula* sp. isolated from date fruits

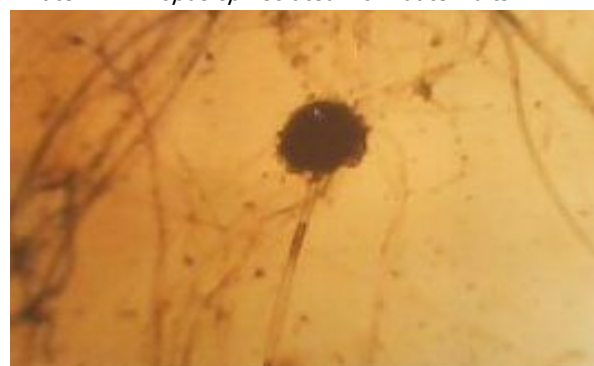


Plate IV: *Penicillium* sp. isolated from date fruits

DISCUSSION

Moisture content appeared to be one of the major factors that support fungal growth in dates (Hill and Waller, 1999) as both the semi dry and soft types had all the six species identified from them. Therefore, storage facilities such as sacks, polythene bags and natural fibre, which are air-tight being used by the traders on both campuses(Personal observation) for storage of all the varieties might have encouraged the fungal growth on the two varieties above. This leads to continuous increase in humidity and temperature of the dates, which consequently favours fungal growth as reported by Ahmad (2003). Indeed, although Djerbi (1983) stated that the most common fungi causing date fruits spoilage are *Aspergillus* sp. and *Alternaria* sp., which were both identified from the two campuses, they however showed the least occurrence in this research with percentage infection of 16.67% and 13.33% respectively and none was associated with dry date (Table 1).

Moreover, dates can encounter fungal infestation by influences from outside environment, such as insect's infestation, wound and presence of foreign matter such as sand, dust and debris among others (Djerbi, 1983), thus some of the identified fungal species could have come from any of these sources. Similarly, the constant exposure of the dates to the out side environment at the time of sales could have aided in deposition of the fungal spores on them. Therefore, spores can germinate on the dates

when temperature and humidity triggers the growth processes. Damage by insects has also been known to provide entry points for fungal infection (Dennis, 2002) and aid in their rapid spread. Hence, presence of insects may under certain critical circumstances be quite essential for establishment of infection.

While several fungal species cause spoilage of dates worldwide, however the presence of *Aspergillus* sp.(Table 1) recovered from semi dry (Gaude) and soft date (Dan Madina) in this research from both campuses of the University shows that there is the fear of consumption of aflatoxins that have a serious health implication, as they are highly toxic and carcinogenic (AOAC, 2002; Shenasi *et al.*, 2002), thus rendering the fruits unfit for human and animal consumption. Consequently, the following recommendations are given:-

1. Soft dates should be stored in a cold environment while dry dates should be stored under dry condition so that they will not increase in moisture and avoid constant exposure to air at sales time, hence the use of paper wraps should be encouraged.
2. Contaminated dates should be sorted and eliminated to avoid re-infection while washing the dates with clean water prior to consumption should be strongly encouraged by appropriate authorities to avoid infection and thus, have a healthy community.

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