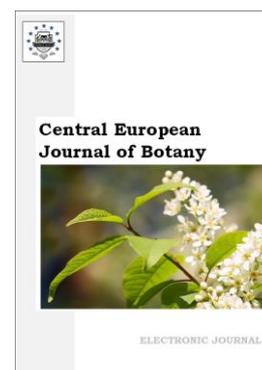


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Articles

Isolation and Identification of Fungi Associated with the Contamination of Different Varieties of Date Palm Fruit

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Abstract

The date palm tree (*Phoenix dactylifera*) is one of the most popular fruit trees of the world. It has religious values as well as cultural importance. Fungi are a source of fruit rot, produce mycotoxins and reduce the economic values of date fruit. The study aimed to isolate and identify the fungi associated with the contamination of different varieties of date palm fruit sold in Bauchi metropolis Nigeria, to isolate and identify the fungi associated with the date's contamination and to microscopically characterize the isolated fungi. Results showed that sample A had 11 organisms, 13 were found from B, 5 from C, 6 from D and 7 were isolated E. Results also showed the fungal count for sample A was 2.36×10^3 , that of sample B was 1.23×10^3 , C had 1.75×10^3 ; D, 1.12×10^3 while E had a count of 1.32×10^3 . Number and percentages of occurrence of *R. stolonifer*, *A. niger*, *P* spp, *M* spp and *C. candida* were 12 and 28.57; 08 and 19.05; 07 and 16.67; 05 and 11.90; 10 and 23.81 respectively.

Keywords: fungi, dates, mycotoxins, contamination, hazardous and toxic.

1. Introduction

Dates popularly known as Dabino among the Hausas and botanically known as *Phoenix dactylifera*, is a fruit obtained from the date palm tree (Anjili et al., 2015; Ramaswamy, 2015). They are extensively produced and consumed in the Middle East, West Africa and also in North Africa over the millennia (Bjornlund et al., 2020). The date palm tree (*Phoenix dactylifera*) is one of the most popular fruit trees of the world. It has religious values as well as cultural importance (Samakov, Berkes, 2017; González-Alcaide et al., 2020). Dates have been used in several traditional medicines for a long time to cure or as prevention against cold, sore throat, fever, abdominal troubles and traditional system (Wang et al., 2014; Satpathy, 2010). Date fruit can be used as juice, vinegar, wine beer, syrup, honey, pickle, paste and food flavorings (Fernández-Cruz et al., 2010; Adeyeye, 2016).

Fungi are a source of fruits rot, produce mycotoxins and reduce the economic values of date fruit. The most identified fungi were *Aspergillus* spp, *Cladosporium* spp, *Penicillium* spp,

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Fusarium spp, *Mucor* spp and *Chalaropsis* (Al-Alawi et al., 2017). The date palm fruits are mostly loaded with a mixture of microbes: bacteria moulds and yeasts but some people continue eating after clearing the pericarp (Gonçalves et al., 2018). Species *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* have been reported to cause fruit rots of date palm (Dojjode, 2020).

Fruits and vegetables are exposed to contamination by microorganisms through direct contact with soil, dust, water, by handling at harvest or during post-harvest processing (Al-Muaini et al., 2019; Odebode et al., 2020). Fruits are reported to be contaminated with toxic and health hazardous chemicals. Contamination of fruits with mycotoxins producing fungi could lead to accumulation of these toxins in the fruit tissues.

2. Materials and methods

Sample collection

Five different varieties of date fruit were collected from different marketers in Bauchi metropolis and the samples were labelled A₁, A₂, A₃, A₄ and A₅. The labelled samples were transferred to the Biological Science Laboratory Abubakar Tatari Ali Polytechnic (ATAP).

Four different test tubes containing 9 ml of sterile distilled water (each) were arranged on a test-tube rack labeled accordingly. One gram of the macerated sample was weighed using a weighing balance. Each sample was homogenized by aspiration using a new syringe in different test-tubes containing 9 mls of sterile distilled water. One milliliter of the suspension was transferred from one original bottle to the first test-tube containing 9 ml of sterile water and was shaken properly to obtain 1:10 dilution. One milliliter was then being transferred from first test-tube to the second test-tube and also be shaken properly 1:100 dilutions up to the fourth test-tube where the dilution factor of 1:10000 (10^{-4}) was obtained.

Preparation of media

Potato dextrose agar (PDA) was prepared according to the manufacturer's instruction.

Isolation of Fungi

The aliquot amount of the serial diluents normally 1ml was poured aseptically using a sterile syringe into the agar plate containing solidified Potato dextrose agar medium. The plates were incubated for a maximum of 5-7 days at 28°C. The plates were then observed after two to three days' interval for growth. A sub culture was made by preparing new plates of PDA, a needle was passed through a Bunsen flame until red hot which was used to pick a little from a colony, mostly from the center of the mixed culture. The colony was picked and inoculated at the middle of the fresh agar (PDA) plate and left for another 5-7 days at the same temperature 28°C.

Identification of Fungi

Microscopic examination of fungi was carried out to observe the structure and characteristics of the fungal isolates in addition to microscopic cultural observations. A sample of mycelia growth was picked with the aid of a sterile needle and was placed on a drop of lacto phenol cotton blue on a slide covered with a cover slip. The slide was then mounted and observed with $\times 10$ and $\times 40$ objective lenses of a microscope respectively. The isolates were identified by comparing characteristics under the microscope with diagrams in text book of mycological atlas. The identification was based on colonial appearance, pigment production and micro morphology of the spore produced.

3. Results

Table 1. Fungal count of isolated fungi from date palm fruit

Sample Code	means of colony count (cfu/ ml)
A.	2.36×10^3
B.	1.23×10^3
C.	1.75×10^3
D.	1.12×10^3
E.	1.32×10^3

Table 2. Microscopic characteristics of isolated fungi

Microscopic Identification	Microscopic Appearance	Organisms
Write cottony growth which turns black as the media ages	Presence of stolon and rhizoid with sporangia above rhizoid	<i>Rhizopus stolonifer</i>
Blue-black mould which turns complete powdery black	Conidiophores which terminate in a but-like stricture	<i>Aspergillus niger.</i>
Pale-blue growth that is smooth in texture	non-septate branched hyphal enlarge at the apex to form conidiophores	<i>Penicillium spp</i>
Creamy fluffy white colonics almost covering the whole media surface	Sporangium came directly from hyphal without stolon or rhizoid columella	<i>Mucor spp</i>
Yellowish-whitish colony with smooth and dry texture	Conidial masses which hyphal formed a grape like duster	<i>Candida albicans</i>

Table 3. Numbers and percentage occurrence of isolated fungi

Fungus Isolated	No. of occurrences	% Occurrences
R. stolonifera	12	28.57
A.niger	08	19.05
Penicillium spp	07	16.67
Mucor spp	05	11.90
C. albicans	10	23.81
TOTAL	42	100

Table 4. Frequency of Fungal Isolate from five varieties of date palm fruit

Sample Code	Numbers of Organisms	Frequency (%)
A	11	26
B	13	31
C	05	12
D	06	15
E	07	17

4. Discussion

Five organisms were isolated from dates in the current study. *Rizophus stolonifer*, *Aspergillus niger*, *Penicillium spp*, *Mucor spp* and *Candida albicans*. *R. stolonifer* had the highest no of colonies and percentage of occurrence with 10 and 28.57 % respectively; *A. niger* came second, while *P spp*, *Mucor spp* and *C. albicans* came in third, fourth and fifth respectively, this is not surprising as similar findings were observed by Anjili et al. (2015) and Doijode (2020), they discovered that the fungi *Aspergillus spp.*, *Penicillium spp.*, *Fusarium spp.*, and *Alternaria spp.* are the major fungal species found in stored grains. Sample A had the highest fungal count followed by C, E, B and D that came with second, third, fourth and fifth fungal counts respectively. More than

25 % of fruits and grains global have been stated to be polluted with mycotoxins produced by these fungal species, and over 300 fungal metabolites have been reported to have toxicity on humans and animals as expounded by Gonçalves et al. (2018) and those of Odebode et al. (2020).

5. Conclusion

Date fruit is regularly consumed in several countries in the Middle East and North Africa and is used for innumerable ethnomedical purposes. Date fruits are a virtuous source of ordinary antioxidants and may be used as a purposeful food in the management of oxidative anxiety-correlated and infectious diseases. Date fruit are easily perishable, so they need handling, preservation and supervised environments.

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