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Fungi Associated with Post-harvest Spoilage of Date Palm *(Phoenix dactylifera* L.) in Yola, Adamawa State

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ABSTRACT

One thousand (1000) fruits of date palm obtained from four (4) markets (Jimeta Old Market, Jimeta Ultra-Mordern Market, Jimeta Shopping Complex and Yola Town Market) in Yola were assessed for incidence of rot. The highest incidence of rot was 20.4% in Jimeta Shoping Complex while the least was 14.4% in Yola town Market. Fungal isolates from the samples carried out on potato dextrose agar (PDA) in the laboratory gave the following total frequencies: Aspergillus niger (40.42), Fusarium solani (19.39%), Scoulariopsis brevicaulis (17.35%) and Rhizopus stolonifer (22.45%). Pathogenicity tests showed that all isolates were pathogenic on healthy date palm fruits. The pathogens varied (P= 0.05) from the control test as well as among themselves in severity of rot and this was determined by measuring the size of rot lesion on infected fruits. Weight of fruits before and after inoculation with the pathogens varied significantly (p=0.05) with a considerable weight loss for the infected fruits. The proximate composition of infected and non-infected fruits varied with decrease in percentage of fiber, lipid, protein, moisture and ash with a corresponding increase in carbohydrate for the infected.

Keywords: Date palm Fruit Post harvest rot, Fungi incidence

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a palm in the genus *Phoenix*, cultivated for its edible sweet fruit (Wikipedia, 2014). Dates are widely distributed facilitated by the fact that dates lend themselves perfectly to being carried along as a high calorie food, with a long-keeping quality (FAO, 2012). Date fruit also can be made into juice, vinegar, wine, beer, sugar, syrup, honey, chutney, pickle, paste, dip, and food flavoring (Barreveld, 1993 and Glasner *et al.*, 2002). It is an ideal food for all age phases, providing the most important essential nutrients like protein, fiber, carbohydrates, fat and minerals (Al-Farsi *et al*., 2005 and Vyawahare *et al.*, 2009).

The plant is affected by various pests and diseases, but the nature of the problem varies with geographic location (Howard *et al.*, 2001). Atia (2011) observed that the date palm fruits are mostly loaded with mixture of microbes: bacteria, moulds and yeast but people go ahead eating after clearing the pericarp, while some eat it whole irrespective of the state of the pericarp. Species of *Alternaria, Aspergillus, Fusarium* and *Penicillium* have been reported to cause fruit rots of date-palm (Bokhary, 2010).

A report stated that the major agents of date palm spoilage are moulds, followed by bacteria and yeast at all stages of ripening on trees, as well as during storage and processing (Amal *et al.*, 2014). ChihCheng and Robert (2007) also report that the fruit quality is influenced by size, colour, texture, cleanliness, freedom from defects (sunburn, insect damage, sugar migration to surface, fermentation), and the effects of decay-causing pathogens. Some pathogens and potential mycotoxin producing microbes isolated from date fruits include species of *Aspergillus, Penicillium, Alternaria* and *Fusarium* (Kader, 2007; Hamad, 2008; Hayrettin *et al*., 2012; Aido *et al*., 1996, El-Sherbeeny *et al*., 1985 and Abdulsalam *et al*., 1991).

Hawking of date palm fruits is a common sight especially north of Nigeria were Yola is located. The high patronage this merchandise 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.

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Also, Christensen *et al*. (2007) reported that the agricultural industries sustained huge crop losses 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's food crops are significantly contaminated with mycotoxins (WHO 1999). Mycotoxins are highly toxic and cause severe intoxications in human and animals, some of them are carcinogens (Khomutov *et al*., 2011). Amal *et al*. (2014) observed that methods that will curb the microbial infestation of fruits at post-harvest storage are required and should be made mandatory.

The objectives of this work therefore are to isolate, identify and determine the severity of the fungi causing spoilage of date fruits sold in markets in Yola as well as determine nutritional change of fruits.

MATERIALS AND METHODS

The survey of fungi associated with date palm (*Phoenix dactylifera* L.) spoilage was conducted in four major markets in Yola (Jimeta Old Market, Jimeta Ultra-Mordern Market, Jimeta Shopping Complex and Yola Town Market). A sample of 250 fruits were randomly collected from sellers in each market. These were brought to the laboratory of Plant Science Department, Modibbo Adama University of Technology (MAUTECH) Yola, where all determinations and tests were carried out.

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:

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

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 into 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 ± 2^0 C for 7 days. Sub- culturing onto fresh sterilize PDA using the methods of Klick and Pitt (1988) and Robert *et al*. (1996) were carried immediately when new colonies began to grow through hyphal-tip transfer.

Identification of Fungi

Microscopic examination was carried out to observe the structure and characteristics of the fungal isolates in addition to macroscopic cultural observations. A sterile needle was used to pick a little portion of the hyphae containing spores and placed on a sterile glass slide stained with Lactophenol cotton blue and examined under the photographic microscope using the method of Fawole and Oso (1995). Micrograph of the isolates showing (conidia etc.) were taken. The morphological and cultural characteristics observed were compared with structures in the identification guides of Hunter and Barnet (1998).

Pathogenicity Test

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 $33\pm2\ ^0$C. 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.

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 hand lens and re-weighed. Result were analyzed with Statistical Analysis System (SAS) version 7.

Determination of the Nutritional Content

Crude fibre, crude protein, carbohydrate contents were determined using the method of AOAC (2007), percentage of oil/ lipid and moisture were determined using the method of Ani *et al*. (2012), while of ash using methods of Ani *et al*. (2012) and AOAC (2007).

Calculation of parameters

Crude fibre %, crude protein %, ash %, lipid %, moisture % and carbohydrate % was calculated by weight using the following methods;

$$\text{The \% of fibre content} = \frac{\text{Weight of crucible + residue} - \text{Weight of crucible + Ash}}{\text{Initial weight of sample}} \times 100$$

% crude protein = % of Nitrogen by weight (N) = V0.1 HCl x 0.28

% of crude protein = % of Nitrogen (N) x 6.25

Where V0.1 M HCl represent volume of HCl

$$\text{Ash content (\%)} = \frac{\text{W ash}}{\text{W sample}} \times 100$$

Where W ash is the weight of ash

W sample is the weight of sample

$$\text{Percentage of oil/lipid yield)} = \frac{\text{W oil}}{\text{W sample}} \times 100$$

Where W oil is the weight of oil

W sample is the weight of sample

$$\text{\% of moisture content} = \frac{\text{W sample - W dry}}{\text{W sample}} \times 100$$

Where W sample is the weight of sample before drying

W dry is the weight of sample after drying.

% of carbohydrates = 100 – (% moisture + % ash + % protein + % lipid + % fibre)

RESULTS & DISCUSSION

The results of the survey of post-harvest rot of date palm in Yola metropolis is shown in Table 1. Rot incidence ranges from 14.4% in Yola town to 20.4% in Jimeta Shoping Complex with an average rot incidence of 17.6. These rather high numbers of rotted fruits are sold in our markets for direct consumption. Fungal contamination is strongly influenced by the health status of dates. Oustani (2011) reported that the presence of injured dates provide the sources of contamination, encourage the development of contamination and therefore it can only be to the disadvantage of storing dates in long-term. Besides traders are reluctant to dispose rotted fruits which provide in ocula for infection of new supplies.

Table1. *Incidence of Rot of Date Palm in Yola Markets*

Location	**Incidence of Rot (%)**
Jimeta Old Market	18.8
Jimeta Ultra-Modern Market	16.4
Jimeta Shopping Complex	20.4
Yola Town	14.4
Average	17.6

Four (4) fungal rot pathogens of date palm fruit were isolated in Yola. These fungi are *Aspergillus niger*, *Fusarium solani*, *Scoulariopsis brevicaulis* and *Rhizopus stolonifer*. The frequency of these pathogens in Yola markets is shown in Table 2. These also have been reported by Hashem (2009), Abass (2013), Amal *et al*. (2014), Atia *et al*. (2009) and Al-Jasser (2010) who found these fungi on date palm fruit. *Aspergillus niger* had the highest frequency of occurrence in date palm fruit rot with 40.82 %, followed by *Rhizopus* spp with 22.45 %, *Fusarium solani* with 19.39% and *Scoulariopsis brevicaulis* had the lowest frequency of 17.71 % (Table 2). *Aspergillus niger* and *Fusarium solani* showed a wide distribution occurring in all locations. This was also reported by Amal *et al*. (2014) and Atia (2011) who also found *Aspergillus niger* in the spoilage of date palm in every their studied areas. Similar, results were found on other dried fruits and kernels such as peanuts, hazelnut, walnut and figs (Abd-Alla *et al*., 1999). The most abundant genus found in date palm fruits collected from Figuig oasis of Morocco was *A. niger* (Hasnaoui *et al*., 2010). Hashem (2009) isolated 39 species of fungi from local varieties grown in Saudi Arabia including *A. niger* and *Fusarium* spp. Considering that fruits are eaten raw it should be of health concern that these mycotoxin producing moulds as reported by Mohammed *et al*. (2013) also have the widest spread in our markets. Mycotoxins are reported to be highly toxic and cause severe intoxications in human and animals, some of them being carcinogenic (Khomutov *et al*., 2011)

Table2 *Frequency (%) of Fungal Pathogens of Date Palm Fruit Rot in Yola*

Number of isolates per market (%)					
Fungi isolated	**JOM**	**JUM**	**JSC**	**YTM**	**Total**
Aspergillus niger	37.50	41.67	38.46	45.83	40.82
Fusarium solani	29.17	12.50	15.38	20.83	19.39
Scoulariopsis brevicaulis	33.33	20.83	15.38	-	17.35
Rhizopus stolonifer	-	25.00	30.77	33.33	22.45
Total	**100**	**100**	**100**	**100**	**100**

Key:

JOM: Jimeta Old Market

JUM: Jimeta Ultra-Modern Market

JSC: Jimeta Shopping Complex

YTM: Yola Town Market

Nil

The analysis of variance to determine the level of severity at p= 0.0001 showed to be highly significant among all the pathogens. *Aspergillus niger* had the highest severity mean of 10.44, followed by *Scoulariopsis brevicaulis* with 8.26, *Rhizopus* spp (7.94), while *Fusarium solani* had the least mean of 6.12 (Table 3). The differences in severity of the fungi isolated might be due to their ability to overcome the natural defense mechanism of the fruit or their ability to induce resistance in the fruit when infected (Brian and Gwyn, 2008). Some reports have shown that the severity and incidence of fruit rots (storage and ripening) are affected by temperature and the ripeness of the fruit (Dixon *et al*., 2003a; Dixon *et al.*, 2004). High temperatures and long storage periods were reported to favour the development of ripe rots while low temperatures and fast ripening times generally inhibit the growth of ripe rots (Dixon *et al*., 2003a).

Table3. *Severity of Fungal Pathogens on Date Palm Fruit*

Pathogen	Lesion size (mm)
Aspergillus niger	10.44
Fusarium solani	6.12
Scoulariopsis brevicaulis Rhizopus stolonifer LSD (0.001)	8.26 7.94 1.92

The weight of date palm fruit before and after inoculation is shown in Figure 1. All pathogens produced significant weight loss after inoculation. Fruits inoculated with *Fusarium solani*, (4.92g) *Scoulariopsis brevicaulis* (4.43g) and *Rhizopus stolonifer* (4.10g) lost more weight than those of *Aspergillus niger* (4.02g).

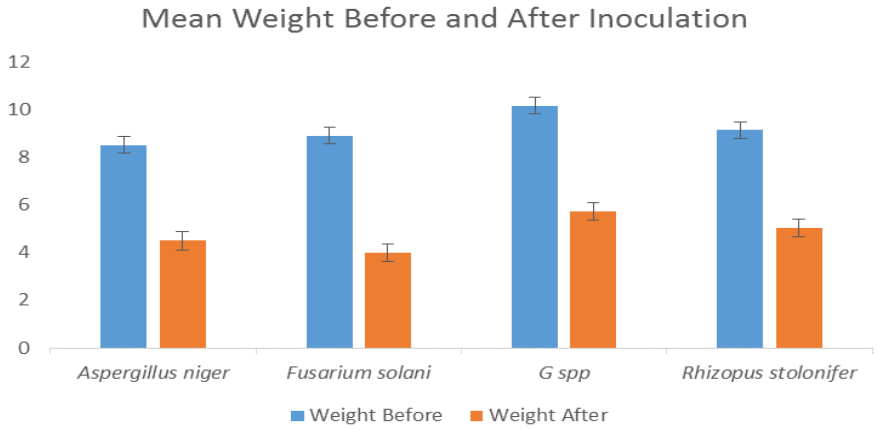

Figure1. *Mean weight of date palm fruit before and after inoculation*

The analysis of proximate composition of date palm fruit flesh and the one infected with fungi (Table 4) indicate that carbohydrate content rose. Variation in the composition of date fruit can be attributed to the pathogens (Nehdi *et al.*, 2010; Saffi *et al.*, 2008; Ali *et al*., 2009 and Hasnaoui *et al*., 2011). Increase in carbohydrate content might lead to subsequent increase in sugars (not desirable for diabetic patients).

Table4. *Proximate Composition of Date Fruit Infected with Isolated Fungi*

Fungi	Fiber %	Lipid %	Protein %	Moisture %	Ash %	Carbohydrate %
A. niger	0.5	0.5	1.23	9	1.5	87.27
F. solani	1	0.5	1.05	15.5	2.5	79.45
S. brevicaulis	0.5	0.5	0.88	6	1	91.12
R. stolonifer	1.6	0.5	0.88	13.5	2	82.12
Control	6.5	4	8.58	23	4.5	53.42

The value of fiber obtained with *Aspergillus niger* had 0.5%, *Fusarium solani* had 1.0%, *Scoulariopsis brevicaulis* had 0.5% and *Rhizopus stolonifer* had 1.6%. All varied with their normal values indicating a loss of fiber for the infected fruits. Similarly there was corresponding decrease in percentages of lipid, protein, moisture and ash content due to infection. The crude fiber value of 6.5% obtained in this work is well within the range reported by Al-Shahib and Marshall (2002). Date fruit can be considered as a good source of dietary fibre such as cellulose, hemicellulose, lignin, pectin, etc. (Biglari, 2009; Habib and Irahim, 2011). Dietary fibre is known to influence digestion and absorption processes in the small intestine (Cherbut *et al.*, 1995; Asp, 1996).

The level of lipids content of date with the fungi is 0.5% each and normal had 4.0%. The result is in accordance with that of Elleuch *et al.* (2008) who found date have a range of 0.5 -3.3% of fat. Also, is less than that of Ebtehal (2014) who reported that the fat concentration of two date samples (Sukkari and Swei) was 3.16% and 2.95% respectively. Low level of fat was also reported by Myhera *et al.* (1999) and Saleh *et al*. (2011) which is not in agreement with the present study (2.90). Omowunmi and Ayoade (2013) reported Nigerian date contain 9.61% lipid. The low level content of fat and that of sugars is desirable however, indicating that the date palm is safe for the heart and high blood pressure patients because it contains a low level of fatty acids and cholesterol (Omowunmi and Ayoade, 2013).

The date fruit with *Aspergillus niger* had 1.23% protein, *Fusarium solani* had 1.05%, *Scoulariopsis brevicaulis* and *Rhizopus stolonifer* had 0.88% and normal had 8.58% protein. The value of protein obtained in this report is less than of Omowunmi and Ayoade (2013) who reported high protein content of 17.09%. The drop in protein content is a loss in value of these fruits.

The high loss of moisture especially in fruits infected with *Scoulariopsis brevicaulis* lead to a rather dry and shriveled fruits of low quality not appealing to consumers.

Ash content is an index to the nutritive value of foods (Pearson, 1976). The ash contents obtained in this work were 1.5% with *Aspergillus niger*, 2.5% with *Fusarium solani*, 1.0% with *Scoulariopsis brevicaulis*, 2.0% with *Rhizopus stolonifer* and 4.5% with normal. The value are higher than the values reported by Elleuch (2008) who reported date palm have 2.5% ash and that of Ebtehal (2014) who reported that the ash concentration of two date samples (Sukkari and Swei) was 2.50% and 2.02% respectively. Ash results were not in agreement with Saad *et al*. (1986) who reported that ash content in dates was around 1.88-2.96%, and that of Ogungbenle (2011) who recorded that value was 3.27%. The result is less than that of Omowunmi and Ayoade (2013) who reported Nigerian date have 9.8%. all these differences could be attributed to the area and the factors surround the growth

Date palm inoculated with *Aspergillus niger* had 87.27%, *Fusarium solani* had 79.45%, *Scoulariopsis brevicaulis* had 91.12%, *Rhizopus stolonifer* had 82.12% and normal had 53.42% carbohydrate content. Khan *et al*. (2008) reported that sugars are the most important constituents of dates, making them a rich source of energy for the human system. The most important carbohydrate components in date fruit are glucose, fructose and sucrose, which can reach up to 70–80% (Nehdi *et al*., 2010; Ashraf and Hamidu-Esfahani, 2011; Vayalil, 2002). Borchani *et al*. (2010) analysed the main chemical components of date fruits from 11 Tunisian cultivars and found that they were rich in sugar (799.3–880.2 g kg-1 dry matter). The result is not in accordance with that of Ebtehal (2014), who reported high sugar value in Swei sample of Egyptian date (81.49%). Also, Mikki (1999) reported that Saudi date varieties contain about 70% reducing sugars with an almost equal quantity of glucose and fructose. Similarly, Al-Shahib and Marshal (2003) reported flesh of date palm fruits contained high carbohydrates (73.5%) and might be (88%) in some varieties. Omowunmi and Ayoade (2013) reported Nigerian date contain 65.0% carbohydrate. The result shows that Nigerian date is lower in sugar content than that of Saudi and Egypt. Even though the amount of carbohydrate obtained was high this was useful for getting the energy for metabolic processes.

CONCLUSION

In conclusion from the study, four pathogenic fungi were isolated which are responsible for rot of date palm in Yola. Fungal contamination is a direct relationship with both the physical initial dates and environmental conditions of the premises including the storage temperature and humidity can alter the organoleptic parameters of dates, and consequently decrease the market value. The sensitivity of dates to fungal spoilage is related to poor conservation of places of production and storage. There is need for alternative storage practices. The health status of dates, humidity and temperature during storage are of critical concern if losses are to be minimized. The presence of injured dates can support the development of sources of contamination, encourage the development of source of contamination and therefore it can be to the disadvantage of storing dates in long-term.

There is a health threat arising from the fact that mycotoxin producing moulds are implicated in this spoilage.

Agricultural industries sustained huge crop losses as a result of fungal diseases of fruits and plants. Hence methods that will curb the microbial infestation of fruits at post-harvest storage are equally required and should be made mandatory. There is need for further research to determine the nutritional loss in fruit as a result of the activities of fungi on date palm fruit.

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