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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(4): 3347-3352. **OPEN ACCESS**

Fungi linkage in rotting of date fruits (*Phoenix dactylifera L*) after sharecropping and reduction in its nutritional values

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The date palm (*Phoenix dactylifera*) is multipurpose, an ancient tree typically grown in the arid and semi-arid regions of the world and is globally famous for its nutritional fruit. After harvesting date fruit is readily attacked by microbes such as fungi. Therefore, the aim of this study was to detect the fungi associated with post-sharecropping putrefaction of date fruit of the district Khairpur Mir's and to detect their negative effect on weight and nutritional value. One hundred fruit samples of fresh and dry dates were collected from four regions of district Khairpur Mir's, the date varieties included Aseel, Karbalayin, Fasli and Kupro and the regions included Taluka Kingri (25 samples), Therhi (25 samples), Kot digi market (25 samples), Pir-jo-Goth (25 samples). For isolation and identification, conventional microbiological culturing and microscopic methods were applied. Nutritional contents of spoiled date fruit and control was calculated by using the Association of Analytical Communities (AOAC) method. Three different fungal species namely *Aspergillus fumigatus*, *Fusarium solani*, and *Aspergillus niger* were detected in date fruits. All the fungi isolates were found significant harmful (p=0.0001) to healthy date palm fruit in this study. The decrease in nutritional content including carbohydrates, fiber, lipid, protein, ash and moisture was found in date samples spoiled with fungi. High incidence of postharvest decay found in this study highlights the need of efficient strategies to control these fungi to reduce economic losses.

Keywords: Date palm fruit; Post-harvest rot; Fungi

INTRODUCTION

Date Palm (Phoenix dactylifera L.*)* is an old perennial date fruit tree belonging to family Aceraceae. Date fruit is sweet, delicious and staple food. There are currently more than 100 million date plants cultivated all over the world. The 10 top countries that are famous for dates cultivation, increased production and yield in are Pakistan, Saudi Arabia, Algeria, Iraq, Iran, Egypt, Tunisia, UAE, Libya and Oman (Chi & Pat, 2017). Date fruits are produced in many tropical and subtropical areas around the world. Furthermore, date fruits are now used as a food in different parts of the world, especially Europe (Abbas, 2013; Abd-Alla et al. 1999). It has a great economical, industrial and religious importance. Date fruits contain minerals including selenium, potassium, calcium, manganese, iron dietary fiber,

vitamins, proteins, amino acids, procyanidins, sterols, flavones, anthocyanidins, isoflavones, phytoestrogens phenolic acid, cinnamic acid derivatives, volatile compounds and have strong antioxidant potential. The β-glucan from date fruits have strong anti-tumor, immune-modulating, anti-diabetic, anti-inflammatory and cholesterol lowering potential, encourage the growth of beneficial gut microflora. Date fruit is an inexpensive source of nutrients (Abdulsalam, 1991). Dates are spread and facilitated highly in order to get high calorie food as they are believed. They can be kept and used for a long time without using special preservation procedures. Juices, vinegar, wine, bear, sugar, syrup honey, chutney, pickle, biscuits, confectionaries, and food flavorings agents are among the foods produced. They can be used by people of all ages (Aidoo et al. 1996). They contain necessary nutrients like proteins, fiber, carbohydrates, and minerals. The date palm plants are influenced by pests and different diseases and it depends upon the types of the problems and geographic change of place where trees are fertilized (Al-Farsi et al. 2005). After harvesting date fruits are readily attacked by microorganisms. For example, (Ani et al. 2012) has reported that date fruits were spoiled with bacteria, yeast, and molds. These microorganisms spread from contaminated environment through wind, water, insect vectors and unhygienic handling and processing that can be a cause of fruit spoilage under favorable conditions (Ali et al. 2009; Al-Jasser 2010; Al-Shahib & Marshall, 2003; Al-Shahib & Marshall, 2002). The major causative agent of date fruit decay is mold (Al Hazzani et al. 2014). Dates are prone to contamination in the field; during harvesting, transporting, storage, and marketing and/or by the consumer. Although several fungal species were implicated in causing contamination to date fruits such as *Aspergillus spp*., *Penicillium spp* and *Rhizopus spp*., the most predominant fungal genus is *Aspergillus* species (Ani et al. 2012). The *Aspergillus niger* represent one of the most important source of fungal toxins (mycotoxins) present in dates. People use to eat date fruit after clearing pericarps only and sometimes clearing pericarps. As research showed, that modulus is highly affected upon date palm (Medina et al. 2006; Thiex et al. 2012). The mycotoxins cause great loss of date industries. Mycotoxins are very harmful and cause serious harm to human. The purpose of this study was to detect the fungi associated with post-sharecropping putrefaction of date fruit of the district Khairpur Mir's and to detect their negative effect on weight and nutritional value.

MATERIALS AND METHODS

2.1 Collection of the samples

Date fruits of different varieties were randomly collected for investigating their nutritional value. Whereas for fungal study one hundred fruit samples of fresh and dry dates exhibiting spoilage were collected from four areas of district Khairpur Mir's. The date variety included Aseel, Karbalayin, Fasli and Kupro and the areas included Taluka Kingri (25 samples), Therhi (25 samples), Kotdiji market (25 samples), Pir-jo goth (25 samples). Date samples were aseptically collected in sterilized bottle and brought to laboratory of the Institute of Microbiology, Shah Abdul Latif University, Khairpur and Gambat institute of medical sciences, where all necessary tests were processed for further analysis.

2.2 Incidence of the development of rot in the date fruit

Using the formula below, the incidence of fungal spoilage from rotten fruit was calculated in percentages.

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

2.3 Isolation of fungi from affected dates

Under aseptic conditions, the 5mm portion of rotted samples of the date fruit was cut with sterilized scalpel. The sections of the dates were immersed into 1% sodium hypochlorite solution with sterile forceps for 30 seconds to disinfect the surface. Afterwards the pieces were rinsed thrice in sterile distilled water. The small sections were dried using sterile filter papers and placed on Potato Dextrose Agar (PDA) plates. The plates were incubated at 25^0C for 7 days. After appearance of the fungal growth, colonies were sub-cultured on fresh PDA plates (Abul-Soad, 2010).

2.4 Identification of fungi

Fungi were identified based on cultural characteristics (front and reverse side of per trip late) and microscopic characteristics as reported earlier (Sharma et al. 2013). For microscopic analysis, a drop of lactophenol cotton blue was place on clean glass slide followed by transferring small portion of fungi with sterilized mycological needle from PDA plates. The cover slip was placed over the specimen and observed under

microscope using 10x and 40x lenses. The cultural and microscopic characteristic noted were compared with previous literature (Kak *et al.* 2019).

2.5 Test for Pathogenicity and infection severity

Collected dates weighed and washed three times in sterile distilled water before being washed for 30 seconds in 1% sodium hypochlorite solution. Healthy and fresh date fruits were punctured and injected/inoculated with fungi by a sterile cork borer, tissues of healthy fruit were removed and placed in punctured area and then vesper jelly was used to seal the hole to avoid contamination. To differentiate with the test (t), a similar set up was used as a control (c) by using distilled water. The samples were incubated at 25^0Cto allow for potential rot growth and the isolates were compared to the previously isolated fungi. The degree of rot (infection severity) was measured with a vernier caliper and re-weighed.

2.6 Nutritional content of dates

The contents of Ash, fiber, protein, oil/lipid, moisture and carbohydrate were calculated using the Association of Analytical Communities (AOAC) method (Thiex *et al.* 2012).

RESULTS AND DISCUSSION

3.1 Incidence Rate of Rot in Date Samples

Rot incidence were recorded as 11.2% to 20.4% in date samples purchased from various markets (Table 1). These vast amounts of rotten fruits are sold for direct consumption in these markets. The injured dates are readily rotten which contaminate healthy dates. These rotten dates are poisonous for human health. Similar to these results previous study has also reported this problem (Abul-Soad, 2010).

Table1: Incidences of Rot development percentage at the District Khairpur Mir's.

S.No:	Location	Incidence of Rot (%)
1	Kingri market	14.4
2	Therhi Market	16.4
3	Kot- digimmarket	11.2
4	Pir- jo – Goth market	20.4
	Average	17.6

Rotten dates should be discarded immediately since they destroy quality of healthy fruits and ultimately loss of economy. High temperatures and long storage periods have been linked to the development of ripe rots (Jiang *et al.* 2006).

3.2 Fungal Specied Isolated from date fruits

Three different fungal species were isolated from the dates collected from various markets of district Khairpur (Table 2). These fungal species were identified as *Aspergillus fumigatus*, *Fusarium solani*, and *Aspergillus niger* (Figure 1).

In this study, *Aspergillus fumigatus* was found tobe more frequent cause of spoilage of dates followed by *Aspergillus niger* and *Fusarium solani*. Previously other researchers have also found the same trend (Abass, 2013; Al Hazzani et al. 2014). Some researchers have found these fungal species in other fruits including peanuts, hazelnuts, walnuts, and figs (Wu et al. 2018).

The presence of fungi in dates is cause of concern since they produce mycotoxins that cause serious intoxications in humans and animals. In addition, these fungi are also known to be carcinogenic and cause allergy as well (Zain, 2011).

3.3 Test for Pathogenicity and infection severity

All the three fungi isolated in this study were found pathogenic and showed significant infection in date fruit samples (p=0.0001). Severity of infection was linked with size of lesion produced and weight loss. The lesion size measurement obtained in date fruits after inoculation and incubation with fungal isolates is shown in Table 3. *Aspergillus fumigatus* was found to cause more severe infection followed by *Fusarium solani* and *Aspergillus niger* since it showed high lesion size in date fruits compared to other two isolates. The ability of the isolated fungi to bypass the fruits' natural defense mechanism or to induce resistance in infected fruit could explain the severity differences.

All pathogens caused significant weight loss after inoculation and incubation with fungal isolates (Table 3). The more weight loss was found in date samples inoculated with *Fusarium solani* compared to other two isolates.

Table 2: Location wise frequency of fungal isolates

S.No.	Fungi Isolated	Location wise fungal Isolation (%)			
		Khairpur	Thehri	Pir Jo Goth	Kot Diji
1	*Aspergillus fumigatus*	40.54	45.50	37.46	42.40
2	*Fusarium solani*	30.25	23.25	29.77	24.27
3	*Aspergillus niger*	29.21	31.25	32.77	33.33
Total		100	100	100	100

Table 3: Lesion size measurement after inoculation and incubation with each fungal isolate

S.No.	A Pathogenic Organism	Lesion Measurement (mm) Average ± Standard deviation	Weight loss (mg)			
			Aseel	Karbalayin	Fasli	Kupro
1	*Aspergillus fumigatus*	9.44 ± 0.16	8.17 ± 0.05	9.09 ± 0.09	7.35 ± 0.12	9.19 ± 0.03
2	*Fusarium solani*	8.12 ± 0.02	8.74 ± 0.16	9.13 ± 0.14	8.38 ± 0.16	8.17 ± 0.06
3	*Aspergillus niger*	7.85 ± 0.09	8.28 ± 0.11	8.76 ± 0.18	8.31 ± 0.1	8.11± 0.025

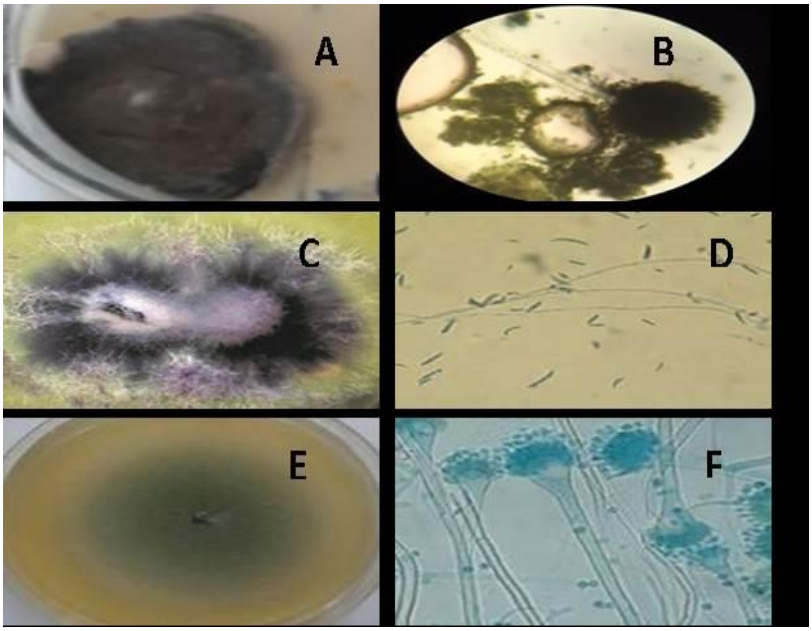

Figure 1: Fungi isolated from dates: A) *Aspergillus niger* on PDA B) Microscopic view of *Aspergillus niger* C) *Fusarium solani*on PDA D) Microscopic view of *Fusarium solani* E) A*spergillus fumigatus* on PDA plate F) Microscopic view of A*spergillus fumigatus*

Table 4: Nutritional value of date fruit spoiled with various fungi and control

Fungi	Fiber (%)	Lipid (%)	Protein (%)	Moisture (%)	Ash (%)	Carbohydrate %
Aspergillus fumigatus	0.55 ± 0.05	0.52 ± 0.72	1.21 ± 0.015	8.95 ± 0.055	1.18 ± 0.02	45.91 ± 0.63
xFusarium solani	0.47 ± 0.025	0.46 ± 0.04	0.84 ± 0.036	5.94 ± 0.19	0.97 ± 0.02	48.84 ± 0.39
Aspergillus niger	1.6 ± 0.15	0.44 ± 0.05	0.84 ± 0.03	13.44 ± 0.04	1.18 ± 0.02	48.29 ± 0.42
Control	6.3 ± 0.15	4.01 ± 0.08	8.39 ± 0.18	22.65 ± 0.37	4.42 ± 0.07	53.17 ± 0.22

3.4 Nutritional value of dates spoiled with fungi

The proximate chemical composition of date palm fruit spoiled with fungi and control is shown in Table 4. Fungal pathogens are to blame for variations in chemical composition of date fruit. In this study decrease in nutritional content including carbohydrates, fiber, lipid, protein, ash and moisture was found in date samples spoiled with fungi.

The lost in fiber content after spoilage with fungi observed in this study is matter of concern since dates are a good source of dietary fibers including cellulose, hemicellulose, lignin, and pectin, among others and have been shown to affect the small intestine's digestion and absorption processes.

CONCLUSION

High incidence of postharvest decay was found in this study that may result in important crop losses. Three pathogenic fungi, *Aspergillus fumigatus*, *Fusarium solani*, and *Aspergillus niger* were found to be major causative agent of postharvest putrefaction of date fruits. Therefore, in Pakistan, the date palm industries should employ efficient strategies to control these fungi to reduce economic losses. Ecofriendly antifungal treatments may be applied to inhibit disease development in date fruits.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

Authors are thankful to the authority of Shah Abdul Latif University, Khairpur and Gambat Institute of Medical Sciences, for providing all the facilities to perform this research work.

AUTHOR CONTRIBUTIONS

ZB and SZI designed and performed the experimental work. AIS, SL, SF and AK collected, assembled and interpreted the data. AH, HF, GAS and MBK analyzed the data statistically, draft and critically revised the manuscript. All authors read and approved the final version.

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