Research Article

Isolation And Identification Of Fungi Associated With Date Palm Fruit Rot (Phonex Dactylifera) Disease And Their Impact On Date Quality

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Abstract

This study was undertaken to isolate and identify the fungal species associated with fruit rot of date palm (Phoenix dactylifera L.) at different stages of fruit ripening. The isolation and identification procedures revealed a diverse fungal community associated with diseased and deteriorating date fruits. The predominant isolates included Alternaria alternata, Aspergillus flavus, A. parasiticus, A. niger, Penicillium expansum, Stemphylium spp., Nigrospora sp., Pestalotia sp., Ulocladium sp., Curvularia lunata, Bipolaris sp., and several other fungal taxa.

Among the isolated fungi, Aspergillus spp. exhibited the highest occurrence and frequency, accounting for 73.4% and 37%, respectively. The fungal isolates were identified using conventional morphological characteristics based on established taxonomic keys and further confirmed through molecular identification by amplification and sequencing of the ITS1–ITS4 region of ribosomal DNA. The identified isolates of Aspergillus flavus and Aspergillus parasiticus were deposited in GenBank under accession numbers OP602315.1 and LC752805.1, respectively.

Furthermore, quantitative analysis of aflatoxin contamination using high-performance liquid chromatography (HPLC) revealed that the majority of the examined date fruit samples were contaminated with aflatoxin B_1 (AFB_1), with concentrations ranging from 20 to 90 ppb. These findings highlight the substantial diversity of fungi associated with date fruit rot and, importantly, demonstrate the potential food-safety risk posed by aflatoxin-producing Aspergillus species in date fruits.

Keywords: Dates, fruit rot , fungi, Aflatoxin.

Introduction:-

Date palm trees are extremely important in some Asian countries such as Iraq, Saudi Arabia, the United Arab Emirates and Iran, and in North Africa such as Algeria, Tunisia, Libya and others (AL-Faris & Lee, 2008). Dates are also a basic food source in the producing countries as a low-cost food source, and they have special importance among Muslim peoples, especially during the holy month of Ramadan (Fayyadh & Mohammed ,2022). Date palm fruits are susceptible to several fungi while in the field, after harvest, and during transport and storage, especially in seasons characterized by high humidity (Kader & Hussein, 2006). Fungi cause damage to the fruits, reducing the yield, but their greatest impact lies in their ability to produce toxic compounds known as mycotoxins, which have harmful effects on human and animal health (Sweetry & Dobsom, 1998). The risks of these compounds are increased by consuming fresh dates directly without any additional processing.

Among the most important fungi found on dates and other agricultural products that produce mycotoxins are Aspergillus, Penicillium, Fusarium, Alternaria, and others (Iqbal, et al. 2013). More than 400 types of toxins produced by fungi are now known, the most important of which are aflatoxins (AFs) produced by A. falvus and A. parasticus, and in very small quantities by A. nomius (Iqbal& Jinap, 2013; Hassan & Kassaify, 2014), and ochratoxins produced by A. ochraceus and A. niger.(Ali,2015). Studies on mycotoxin contamination of dates are few compared to studies on contamination of other products. Among the studies that addressed the topic of mycotoxin contamination of date palm fruits is the study by Iqbal et al. (2014), in which they indicated that 39.6% of date samples collected from different regions in Pakistan were contaminated with aflatoxins at levels ranging from 2.9 to 4.96 mg/kg. Eman. (1994) also indicated the contamination of local and imported dates in Egypt with aflatoxins, and a survey conducted in the United Arab Emirates showed that dates contained high levels of aflatoxin B1, reaching 133 parts per billion. In Iraq, Ali (2015) reported that dates of the Zahdi and Khastawi varieties in central Iraq were contaminated with aflatoxins and ochratoxins. The dried date trade ranks second globally among other dried fruit trades, necessitating careful attention to ensure compliance with international standards and the absence of mycotoxins and other contaminants.

The current study aimed to isolate fungi from dates sold in local markets and estimate the aflatoxin levels.

Material And Methods:-

Samples of date palm fruits were collected during different stages of ripening (Kamari, Khalil and rutab) that showed symptoms of rot. These samples were taken from different date palm varieties (Sayir, Halawi, Khadrawi, Zahdi, Barhi, Dairi and Brem) and from different areas of Basra Governorate (Abu Al-Khasib, Al-Qurna, and Shatt-Al Arab). The infected fruits, which were still attached to the bunches, were collected and placed in sterile polyethylene bags and brought to the Mycotoxins Laboratory in the Plant Protection Department, College of Agriculture, University of Basra. The infected palm fruits were washed with running water for 10 minutes, then washed with sterile distilled water and dried on filter paper. The fruits were then cut into small pieces and transferred to 9 cm diameter Petri dishes containing PDA medium sterilized with a steam sterilizer (autoclave) and supplemented with the antibiotic tetracycline at a rate of 100 mg/L, with four pieces per dish. The dishes were incubated at a temperature of 25±2 C for 5-7 days. Occurrence and Frequency were calculated according to following formula:-

$$\text{Occurrance}(\%) = \frac{\text{Nubmer of samples contaning the fungal species}}{\text{Total number of samples}} \text{X } 100$$

$$\text{Frequece } (\%) = \frac{\text{Nubmer of species or genus occure}}{\text{Total number ofspecies}} \text{X } 100$$

Morphological identification: -

The fungal isolates growing from palm fruit pieces were purified by transferring the edge of the fungal hypha of each isolate to plates containing PDA medium. The fungal isolates were incubated at 25°C for 5-7 days and identified to the genus level based on the following taxonomic keys (Ellis, 1976; Domsch, et al, 2007,
Klich ,2002 ; Leslie and Summerell, 2006;; Woudenberg et al, 2013).

Molecular identification:-

Molecular identification techniques were used to identify Aspergillus sp., Alternaria alternata, and Chaetomium globosum fungi due to their high frequency in the samples. Additionally, Aspergillus spp. is one of the most important mycotoxin-producing fungi. The surface of pure colonies of fungal isolates from date palm fruits grown in PDA was scraped, and the mycelium was transferred to test tubes containing liquid nitrogen. DNA was extracted, and its purity was determined using a NanoDrop device with the DNA Extraction Kit from Genaid. The following primers were used to amplify the ITS1-ITS4 gene region.
(ITS1 F: 5'- TCCGTAGGTGAACCTGCGG -3')
(ITS4 R:5' TCCTCCGCTTATTGATATGC-3')
The amplification program included five minutes at 90°C for the activation step, one minute for the denaturation step at 94°C, one minute for the annealing step at 58°C, and two minutes for the extension step at 72°C. The gene amplification steps comprised 35 cycles (Kerenyi et al., 1999; Murray, 1980). The amplified ITS1-ITS4 gene products from each isolate were sent to the Korean company for nucleotide sequence determination of the amplified gene region. These sequences were then compared with those of the fungal isolates in the GenBank database to determine the similarity percentage.

Extraction of aflatoxin B1 toxins from dates of different palm varieties.

Date samples were thoroughly mixed, and 50 grams of each sample were taken. 150 ml of the solvent chloroform: water (10:90) was added, and the mixture was mixed using a Gosonic GSB 825 electric mixer for three to four minutes. The mixture was then filtered using Whatman N0.1 filter paper under a Value vacuum system. Re-extraction was performed using 67 ml of the same solvent. The filtrate was collected in 250 ml glass bottles and evaporated in a rotary evaporator at 55°C until dry. The dried samples were stored in a freezer at -18°C until further testing. (Wilson and King, 1995).

Quantitative assessment of aflatoxins using HPLC technology

The quantitative determination of aflatoxin was performed using HPLC in the laboratories of the Department of Environment and Water/Ministry of Science and Technology, following the method of Liu et al. (2012), using a high-performance liquid chromatography (HPLC) instrument, model SYKAMAN (German origin). The carrier phase was acetonitrile : water (70:30), and a C18-ODS (25cm) 4.6mm separation column was used to separate the phenols. A fluorescence detector (ex=365nm, em =445nm) was used, and the carrier phase flow velocity was 0.7 mm/s.

Results And Discussion :-

Fungi isolated from palm fruits infected with fruit rot disease.

Table 1 shows the isolation of several fungi from date palm fruits infected with fruit rot disease Fig (1) the most important of which are Alternaria spp, Aspergillus spp, Cladosporium spp, Rhizopus spp, Penicillium spp, and others. The results also showed that the that Aspergillus species recorded the highest percentage of occurrence and frequency, at 73.4% and 37.2% respectively, followed by Alternaria spp. at 62.6% and 24.6% respectively. Cladosporium spp. ranked third with an occurrence and frequency of 23.06% and 7.5% respectively. The percentage of occurrence and frequency for the remaining fungi varied between 0.6% and 12.6%, and between 0.1% and 7% respectively.

These findings are consistent with several previous studies that identified various fungi in date palms infected with fruit rot disease. Sarhan (2001) reported the isolation of A. flavus, A. niger, A. ochraceus, Alernaria alternata, and Rhizopus stolonifera from the Barhi, Barim, Halawi, Khadrawi, Khastawi, and Zahdi varieties. Abass (2016) also reported the isolation of several fungi associated with fruit rot disease, most notably Aspergillus, Alternaria, and Curvularia. Iqbal, et al (2014) also pointed out the isolation of several fungi from date palm fruits infected with rot disease, the most important of which are Aspergillus, Alternaria, Cladosporium, Stemphylum and Nigrospora. Date palm fruits are infected with several fungi while in the field, after harvesting and transport, or in storage, especially in seasons characterized by high humidity, causing significant losses in most date palm growing regions. Losses resulting from fungal infection of dates during storage in Pakistan were estimated at around 41.3% (Suhail, et al, 2020).

Tab (1) Percentage of occurrence and frequency of fungi isolated from date palm fruits infected with fruit rot disease during different ripening stages

Occurrence &Frequence / Fungi	% Occurrence	% frequency
Alternaria spp	62.6	24.6
Aspergillus spp	73.4	37.2
Chaetomium sp	16.9	6.8
Cladosporium sp	22.6	7.5
Curvularia spp	8.7	3.2
Drechslera sp	0.6	0.1
Fusarium moniliforme	5.3	1.3
Mucor sp	8.8	3.8
Nigrospora sp	2.6	1.8
Penicillium sp	2	1.3
Pestalotia sp	3.5	0.8
Rhizopus sp	10.3	6.3
Stemphylium sp	8.2	3
Ulocladium	2.8	2.9
L.S.D	7.9	4.6

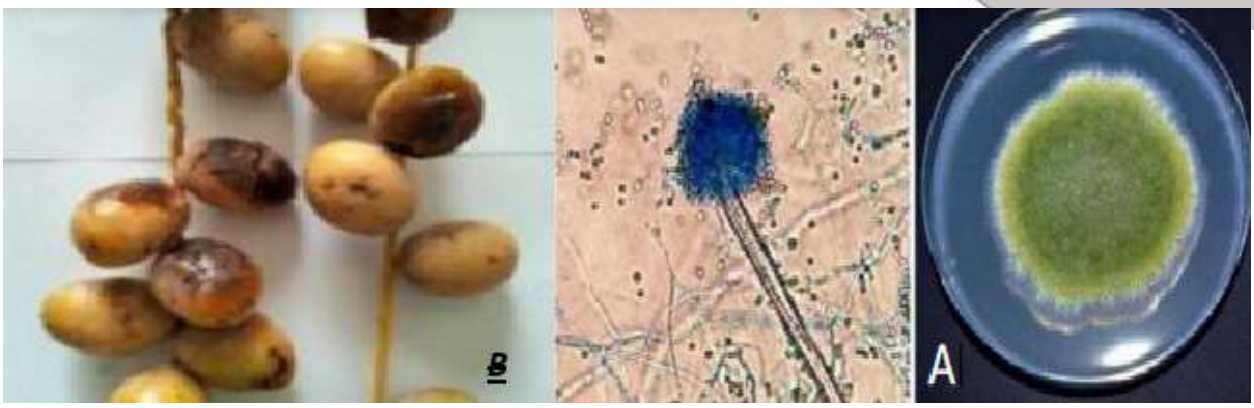

Fig (1) Symptoms of date rot and A. flavus isolated from it .

Morphological and molecular diagnosis of some fungi causing date palm fruit rot disease:

a- Phenotypic diagnosis

Fungi isolated from date palm fruits infected with fruit rot disease were identified based on their morphological characteristics, such as colony color, conidia shape, and the color, shape, and size of asexual spores, according to established taxonomic keys (Figure 1).

Aspergillus colonies appeared on Czapek Yeast Extract Agar (CYA 25) at -25°C, exhibiting various colors such as green, olive, or black, surrounded by a white halo, depending on the species. A. flavus colonies were characterized by dark green to olive-green coloration, sometimes interspersed with greenish-yellow. The phialides were either uniseriate or biseriate. The colonies were colorless or orange on the back of the plate. The conidia and conidia were smooth, while they were rough in A. parasticus. These characteristics are consistent with Raper & Fennell (1965).

b- Molecular identification.

The results of molecular diagnosis based on amplification of the ITS1-ITS4 gene region and subsequent nucleotide sequence analysis showed that the Aspergillus isolates belonged to the species A. flavus, A. parasticus, A. niger, A. carbonius, and A. fumigatus. The results also indicated that the Alternaria isolate belonged to the species A. alternata and the Chaetomium isolate belonged to the species C. globosum. The isolates were registered in the NCBI GenBank and assigned accession numbers, as shown in Table 3.

The ITS1-ITS4 gene region has been used as a marker for the identification of several plant pathogenic fungi. Henry et al. (2000) used it to identify Aspergillus species, Ahmed (2021) used it to identify fungal species isolated from date palm tissues, including Aspergillus species, and Mohammed and Eidan (2016) used it to identify Aspergillus species.

Tab (3) Molecular identification of some fungi isolated from date infected with fruit rot.

Scientific name of the fungi	Accession number of isolate	The identical isolate	Percent identity	Query cover %
Aspergillus flavus	OP602315.1	OM319570.1	100	99
A. niger	LC752801.1	MZ331798.1	100	100
A. parasticus	LC752805.1	MT079321.1	99.76	100
A. carbonius	LC752804.1	MZ373755.1	100	99
A. terreus	OP620979.1	MT558939.1	100	99
A. fumigates	OP620980.1	OR905990.1	100	100
Chaetomium .globosum	LC754203.1	MT341778.1	99.17	93.3
Curvularia lunata	OP595609.1	KY40478.1	98.26	93
C. lycopersici	OP621042.1	MT590310.1	100	99
Cladosporium cladosporides	OP620977.1	MH244422.1	96.19	95

Alternaria alternata	LC752802.1	MT644140.1	99.24	99

Quantitative assessment of aflatoxins in date samples:

The quantitative assessment of aflatoxin B1 using HPLC technology showed that 13 out of 18 samples were contaminated with aflatoxins, i.e., 72.2%. The table results showed that the highest concentration of aflatoxins was recorded in a sample of Halawi date variety at a concentration of 0.4415 PPM, and the lowest concentration was recorded in Zahdi date variety at 0.2014 PPM. The results of this study are consistent with several previous studies that indicated the contamination of dates with aflatoxins in date samples in india and Pakistan. Iqbal et al. (2014) indicated that 39.6% of date samples were contaminated with aflatoxins B1. In Egypt, local and imported dates were found to be contaminated with aflatoxins (Eman et al., 1994). Ali (2015) mentioned that some Zahdi and Khastawi date samples collected from central Iraq were contaminated with aflatoxins.

Tab (4) Concentration of AFB1 on date Samples collected from different locations of Basra

Location / Varieties	Concentration of AFB1 PPM		
	Abu Al-Khasib	Al-Qurna	Shatt-Al Arab
Barhi	0.4418	0.3145	0.4025
Brem	0.3955	0.3011	0.3458
Halawi	0.3895	Nil	0.4415
Khadrawi	0.2598	0.4415	Nil
Sayir	Nil	0.1624	0.2014
Zahdi	Nil	0.1148	Nil

Conclusions:

The present study demonstrated the occurrence of a diverse range of fungi associated with fruit rot of date palm fruits. Among the isolated fungi, Aspergillus flavus and Aspergillus parasiticus are of particular concern because of their well-established ability to produce aflatoxins, especially aflatoxin B_1, a potent mycotoxin associated with significant acute and chronic health risks. The detection of these potentially aflatoxigenic fungi in date fruits highlights an important food-safety concern, particularly because dates are commonly consumed fresh and may be subjected to minimal processing before consumption. Therefore, the presence of aflatoxin-producing fungi in date fruits warrants greater attention to fungal disease management, post-harvest handling, and food-safety monitoring.

Recommendations:

In view of the potential risk of aflatoxin contamination and the important role of Aspergillus species in date fruit deterioration, appropriate preventive and management strategies should be implemented throughout the cultivation, harvesting, handling, and storage stages. The following measures are recommended:

Protect date bunches during early fruit development by using suitable protective bags to reduce exposure to fungal spores. Reduce excessive fruit density within bunches where appropriate to improve air circulation and reduce moisture accumulation, and promptly remove infected, damaged, or decaying fruits to minimize the spread of fungal contamination to healthy fruits. Improve harvesting and post-harvest handling practices and Maintain strict hygiene during sorting, packaging, and storag**e**, using clean, dry, and suitable food-grade containers and packaging materials to minimize secondary contamination.

References :-

1- Abass, M. H. (2016). Identification of Different Fungal Fruit Rot Pathogens of Date Palm (Phoenix Dactylifera L.) Using ITS and RAPD Markers. Basra J. Date Palm Res, 15(1-2):1-19.

2- Ali, S, M. (2015). Isolation and identification of the most important fungi contaminating the fruits of the Zahdi and Khastawi date varieties, and detection and control of aflatoxin (B1) and ochratoxin (A) using extracts of sweet cumin and sesame seeds. PhD dissertation. Department of Plant Protection – College of Agriculture , University of Kufa.

3-Al-Farsi, M. A. and Lee, C.Y. (2008). Nutritional and Functional Properties of Dates: A Review Critical R Reviews In Food Sci. In Food Sci. and Nutrition .48:877-887. doi: 10.1080/10408390701724264.
4- Domsch , K.H., Gams, W. and Anderson T. H. (2007). Compendium of soil fungi. 2nd ed. Munchen: IHW Verlag, 672. DOI:10.1111/j.1365-2389.2008.01052.x
5--Ellis, M. B. (1971). Dematiaceous hyphomycetes. Commonwealth Mycological. Kew, Surrey, England. 608p.
6-EMAN (European Mycotoxin Awareness Network). (2011). Fact Shee2.Decontamination of Mycotoxin contaminated Raw materials. www. Imman.jrc.ec.europan.eu.
7- Hassan, H. F., and Kassaify, Z. (2014). The risks associated with aflatoxins M1 occurrence in Lebanese dairy products. Food Control, 37, 68:72.
8-Henry, T., Iwen , P. C., and Hinrichs, S. H. (2000). Identification of Aspergillus species using internal transcribed spacer regions 1 and 2 . Journal of clinical microbiology, 38(4), 1510-1515. DOI: 10.1128/JCM.38.4.1510-1515.2000.
9-Fayyadh, M. A., Moammed ,Z. Khalaf and Mohammed Al-Hamadany (2022) Date Palm Pest . Publications of the Research, Studies and Publishing Center , KUT University College .ISBN : 978-9922-612-49-2 , Deposit number at the National Library and Archives in Baghdad (888).
10- Iqbal, S. Z., Asi, M. R ., and Jinap, S. (2014).Aflatoxin in dates and dates product ,food control ,vol .43,pp.163-166.
11-Iqbal, S. Z., Mehmood, Z.,. Asi, M,R., Shahid, M., Sehar,M., and Malik, N.(2018). Co-occurrence of aflatoxins and Ochratoxin a in nuts, dry fruits, and nuty products, J. of Food Safety, vol. 38, no. 4, Article ID e12462. DOI:10.1111/jfs.12462
12- Leslie, J. F. and Summerell, B. A. (2006). The Fusarium laboratory manual. Blackwell Publishing. 388p.
13- Kader, A. A., and Hussein, A. M. (2009). Harvesting and postharvest handling of dates. Aleppo, Syria: ICARDA. Aleppo**,** DOI**:** 10.12691/jfnr-5-7-8
14- Klich, M. A. (2002). Identification of common Aspergillus species. CBS.
15- Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. (1983) Fusarium Species. In: An Illustrated Manual for Identification, Pennsylvania State University Press, University Park, 193.
16-Murphy, P. A., Hendrich, S., Landgren, C. and Bryant, C. M. (2006). Food mycotoxins: an update. J. of Food Sci., 71(5): 51-65.
16- Rapper, K. B., Fennel, D. I. (1977). The genus Aspergillus Publishing Company Malabar, Florida.
17- Sarhan, A.R.T. (2001). A study on the fungi causing decline of date palm trees in middle of Iraq 2nd Int. Conf. Date Palm, 25-27 March, Al Ain, UAE pp 424430.
18- Suhail, M.,Durrani,Y.Hashami, M.S.(2020) .Postharvest Losses of dhakki dates during supply chain in Pakistan ,Fresenius Environmental Bullentin,vol.29,pp.299-309.
19- Sweeney, M. J. and Dobson, A. D. W. (1998). Production of Mycotoxin Penicillium Species. A Review. Syst. Appl. Microbiology. 10:42-46. DOI: 10.1016/s0168-1605(98)00112-3.
20-Wilson, D. M. and King, J. K. (1995). Production of aflatoxins B1, B2 G1 and G2 in pure mixed cultures of Aspergillus parasiticus and Aspergillus flavus. Food Additives and contamination. 12(3):521-525 . doi: 10.1080/02652039509374339.
21- Woudenberg, J. H. C., Groenewald, J. Z., Binder, M., & Crous, P. W. (2013). Alternaria redefined. Studies in mycology, 75, 171-212. doi.org/10.3114/sim0015