

Mycoflora Associated with Date Palm (*Phoenix dactylifera*) from Ad Darb, Jizan , Saudi Arabia.

Abstract

Date Palm (*Phoenix dactylifera*) is a very important and commonly growing tree found in Saudi Arabia. The fruit is nutritionally important and adds to the economy of the country. This is the first attempt to isolate and identify the phytopathogenic fungal flora found on the phylloplane and other plant parts of the date palm from Ad Darb region of Jizan province . The media employed for the study was Potato Dextrose Agar on which 0.5cm direct pieces of the diseased parts (phylloplane ,fruit , rachis and trunk) of the date palm. The isolated fungi were analysed qualitatively and quantitatively and the results interpreted in the form of table, bar diagram and photomicrographs. The mycoflora is represented by *Aspergillus*, *Botrytis*, *Ceratocystis*, *Fusarium* , *Graphiola* ,*Penicillium* *Rhizopus* ,*Saprolegnia* and *Syncephalastrum*. One hundred and fifty four fungal isolates were obtained from the nine represented genera . *Aspergillus* was the predominant genera with 74 isolates. Isolation and identification of the phytopathogenic mycoflora is an important tool in the diagnosis of the pathogenic genera which are damaging to the palm under study. This will help in the protection and conservation of this very important plant in the province. The study can also help the plant pathologist in disease forecasting and effective monitoring of the different diseases of the date palm thereby helping in the management and minimization of economic loss .

Key words: Ad Darb; Date palm ; Mycoflora ; Jizan ;Saudi Arabia.

Introduction:

This study is conducted to know the mycoflora associated with different parts of the date palm (*Phoenix dactylifera* L.). The date palm is attacked by many pathogenic mycoflora which are responsible for the deterioration of the edible fruit both qualitatively and quantitatively. It is a very important and commonly growing tree found in Saudi Arabia. The fruit is nutritionally important and adds to the economic value of the country.

Saudi Arabia is considered to be one of the major date producing and exporting country (Al-Showiman and BaOsman, 1992). There are more than 400 cultivars of fruiting date palm of economic value (Fayadh and Al-Showiman, 1990; Date-palm Symposium, 1982). The nutritional values of date palm have been well studied regarding the presence of amino acids, vitamins, carbohydrates, minerals (Al-Whaibi et al., 1985; Al-Showiman, 1990), (H.A.Bokhary ,2010).

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit trees growing in the Arabian world and some neighboring countries and represents a good cash crop for many farmers. Palm diseases are among the major factors that affecting the products. Fungi and Phytoplasma are known as the most causal pathogens on date palm trees (Samir K.Abdullah,

2010). Saudi Arabia is one of the most important country and is considered to be the genetic center for the origin of date palm. Historically this tree has been crucial for the survival of nomadic tribes in Saudi Arabia (Hoop, 2003). Saudi Arabia is considered to be one of the major date producing and exporting country (Al-Showiman and BaOsman, 1992). There are more than 400 cultivars of fruiting date palm of economic value (Fayadh and Al-Showiman, 1990; Date-palm Symposium, 1982). The nutritional values of date palm have been well studied regarding the presence of amino acids, vitamins, carbohydrates, minerals (Al-Whaibi et al., 1985; Al-Showiman, 1990). Bacterial, fungal, nematodal and other diseases of date palm have been well studied in Saudi Arabia (Abdalla, 1985; Lhudaib et al., 2007; El-Hassni et al., 2004; Mansoori and Kord, 2006; FAO, 2004; Al-Rokibah, 1991). There are several fungal diseases of date palm that cause severe damage to plant like Bayoud cause by *Fusarium oxysporum* (El-Hassni et al., 2007). Belaet caused by *Phytophthora sp.* (Howard and Carpenter, 1993), bending head cause by *Ceratocystis paradoxa* and *Lasiodiploda theobromae*, Black leaf spot caused by *Chaetosphaeropsis sp.*, Dipodia disease caused by *Diplodia phoenicum*, while *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* cause Fruit rots of date-palm. Other important fungal diseases that cause significant damage to date-palm are Graphiola leaf spot, Inflorescence rot, Khamedj, Omphalia root rot, Pestalotia leaf spot, Taches brunes (brown leafspot), and Terminal bud rot. Also diseases of unknown cause like Al-Hijm, Berhee disorder, Faround, Internal browning and Rapid decline are the cause of concern for the survival of date-palm, Nematodes are also causing severe diseases of date palm like Root Knot and Root lesion. Lethal yellowing disease of date-palm is cause by mycoplasma like organism (Al-Rokibah, 1991) ,(H.A.Bokhary ,2010).

The major diseases noticed were leaf spots, off-shoot decline, black scorch, leaf basal rot and fruit spots (Eman S.H. Farrag and Kamal A. Abo-Elyousr, 2011).

Black scorch, also called Medjnoon or Fool's disease, is y *Ceratocystis paradoxa* (Hohn) which is the perfect form of *Thielaviopsis paradoxa*. Black scorch has been observed on date palm in all date growing areas of the world. Symptoms are usually expressed in four distinct forms: black scorch on the leaves, inflorescence blight, heart or trunk rot and bud rot on palms of all ages. Infections are all characterised by partial to complete necrosis of the tissues. Typical lesions are dark brown to black, hard, carbonaceous, and, as a mass, give the petioles, fruit strands and fruit stalks a scorched, charcoal-like appearance(A. Zaid, et. al).

Black scorch disease :The disease has different names according to the type of infection and the developed morphological symptoms. These names include: Theilavopsis bud rot, Bending head, Crazy disease (Medjnoon), Inflorescence blight, Terminal bud rot, Trunk rot, Leaf black scorch, Stem bending, Dry basal rot, Heart rot.

Causal agent : The fungus *Thielaviopsis paradoxa* (perfect stage: *Ceratocystis paradoxa*).

Symptoms on palm of all ages are usually expressed in four distinct forms: black scorch on the leaves, inflorescence blight, heart or trunk rot and bud rot. In all cases, infections are characterized by partial to complete necrosis of the tissues. Typical lesions are dark brown to

78 black in color, hard, carbonaceous, and - as a mass - give the petioles, fruit strands and fruit
 79 stalks a scorched, charcoal-like appearance (Ahmed A.M. Dawabah , 2017).

80 Saprolegnia is an important genus of the family Saprolegniaceae (Oomycetes) with about 22
 81 species. Various species usually grow saprophytically in freshwater or less commonly in soil
 82 (A.L.S.ISMAIL,S. S.RATTA & TAWFIKM MUHSIN , 1979)

83 The causal organism responsible for bayoud is a microscopic fungus which belongs to the
 84 **mycoflora** of the soil and is named *Fusarium oxysporum forma specialis albedinis* (Killian and
 85 Maire, 1930; Malencon, 1934 and 1936),(A. Zaid, et. al).

Although the name "Fusarium wilt" implies that the primary symptom is a wilt, vascular wilts in palms look different from wilt diseases of hardwood trees. Instead of limp leaves or leaflets, they cause leaf desiccation and eventually leaf death symptoms. As with any disease, a progression of symptoms is observed with Fusarium wilt (Monica L. Elliott)

The name bayoud comes from the Arabic word, "abiadh", meaning white which refers to the whitening of the fronds of diseased palms. This disease was first reported in 1870 in Zagora-Morocco. By 1940, it had already affected several date plantations and after one century, the disease has practically affected all Moroccan palm groves, as well as those of the western and central Algerian Sahara (Killian and Maire, 1930; Toutain, 1967). Bayoud disease causes considerable damage that can sometimes take on spectacular proportions when the disease presents its violent epidemic aspect. Bayoud has destroyed in one century more than twelve million palms in Morocco and three million in Algeria. Bayoud destroyed the world's most renowned varieties that are susceptible to the disease and particularly those which produce high quality and quantity fruit (Medjool, Deglet Nour, BouFegouss). It also accelerated the phenomenon of desertification. The result is an influx of farmers who have abandoned their land and moved to large urban centres(A. Zaid, et. al)

Bayoud disease of date palm: early symptom indicated by whitening of some of the pinnae. Other symptoms accompany or follow the whitening and drying of the pinnae on certain leaves. Streaks or longitudinal bands occur on midribs and leafstalks which are at first just a little off-color, changing to light brown, then dark brown, and extending up from near the leaf base fungi(H.S. FAWCETT AND L. J. KLOTZ , 1932).

Date palm infected with several fungi resulting in decline of the growth and production. Several fungal diseases of date palm trees have been reported from many date producing countries. The most common disease of date palm was Bayoud disease caused by *Fusarium oxysporum* f.sp. *albedinis*. This disease is the most serious date palm disease especially in Morocco and Algeria (Elarosi, 1989; El-Hassni et al., 2005; Chakroune et al., 2008). The Inflorescence rot (Khamedj), Black scorch, rooting of aerial offshoots and Leaf spots are considered of date palm serious diseases (Al-Rokibah, 1991; Al-Obeed, 2005)(M.I. Ammar and M.A. El-Naggar, 2011).

BAYOUD (Fusariose),The disease was first reported from Morocco in 1870, and derived its name from the Arabic word "Abiadh" which means white. So, "Bayoud" in Arabic means whitish

referring to the whitening of the diseased palm fronds.

Causal agent: The fungus *Fusarium oxysporum* f. sp. *albidinis*

Morphological Symptoms : External symptoms start to appear on one or two leaves of the middle crown. Leaves takes the grey then white color from bottom to top in a very distinguished pattern. Spines on one side of the frond begin to whitened from bottom to top, then whitening turn to the spines of the other side starting from top to bottom. Brown stains also appear on the dorsal side of the rachis starting from base to the tip of the fronds (Ahmed A.M. Dawabah , 2017)

Fusarium wilt :The fungus attack old palms as well as offshoots and young palms. Spines on one side of the frond begin to whitened from bottom to top, then whitening turn to the spines of the other side starting from top to bottom, with a brown discoloration along the midrib of the leaves. Symptoms extend from the outer leaves to the young ones, then to the apical meristem, leading to the death of the palm tree(Ahmed A.M. Dawabah , 2017).

Graphiola leaf spot is caused by *Graphiola phoenicis* (Moug) Poit., which is a smut fungus. It develops sub-epidermal, in small spots on both sides of the pinnae leaves, on the rachis and on the leaf base (Figure 1). The numerous fruiting structures emerge as small-yellow/brown to black sori, 1 to 3 mm in diameter, with two layers. These sori are abundant on three year-old leaves, conspicuous on two year-old, but absent or infrequent on one year-old leaves. This is because of the 10 - 11 month incubation cycle for this pathogen. On a leaf, sori are abundant on apical pinnae, less abundant on the middle section becoming even less on the basal section. *Graphiola* leaf spot disease is most common in Egypt (Delta region and Fayum) but absent in the less humid oases. In Saudi Arabia, it is abundant in Kattif, Demam and Jeddah, but absent in Iraq (A. Zaid, et. al).

Graphiola leaf spot, also called false smut, is due to a fungus, *Graphiola phaenicis* Poit., related to the smut fungi. It attacks the leaves, producing numerous pustules. It appears to be world-wide in distribution. The disease occurs on the pinnae of leaves in the form of small, cylindrical pustules with a dark brown or black envelope from which yellow spore dust escapes. These pustules are about % to 1 mm (% to % 5 in.) in diameter and protrude from the surface of the leaf. The leaf tissue immediately surrounding the pustule is often yellowish in color, and parts of the pinnae may dry out when the pustules are numerous (H.S. FAWCETT AND L. J. KLOTZ , 1932).

Untimely rains and moist weather just preceding and during the harvest are very disastrous because of the accompanying decay and spotting of the fruit. The injurious effects of rain on dates in Tunisia have been fully pointed out by Kearney. (14) Rains that occasionally occur in the Coachella Valley during the ripening period of the fruit are very destructive, causing cracks in the dates an exposing the inner saccharine flesh to fungi. Soft rot of date fruit due to: A, *Aspergillus niger*; B, *Citromyces ramosus* (artificial inoculations). Note that immature fruit on left resisted the attack of the fungi(H.S. FAWCETT AND L. J. KLOTZ , 1932)

This type of decay is promoted by the cracks that develop in low- hanging fruit due to high humidities and to condensation water that collects on the fruit, exposing the rich inner tissue. The

effects of high humidities and water on date fruit was discussed by Fawcett and Klotz (1932).

Syncephalstrum is the causal organism for fruit rot in date palm (www.redpalmweevil.educations.net/2007)

Initial symptoms on infected plants were small, chlorotic spots on blades and edges of leaflets. As spots enlarged, yellow or reddish-brown margins and brown or gray, wrinkled, sunken centers developed. Sometimes, spots or lesions coalesced to cause blight of young expanding leaves or death of apical buds. *Botrytis cinerea* Pers.:Fr was recovered consistently from sections of infected tissues (disinfected for 1 min in 1% NaOCl and rinsed in sterile water) plated on malt extract agar. On potato dextrose agar (PDA), colonies of *B. cinerea* were first hyaline, then turned white, and later turned dark gray when spores differentiated. On potato dextrose agar (PDA), colonies of *B. cinerea* were first hyaline, then turned white, and later turned dark gray when spores differentiated. Six- to eight-day-old-cultures developed white sclerotia that turned black after three more days. Although the disease only occasionally caused death of plants, evidence indicated that *B. cinerea* reduced commercial value of infected pygmy date palms (G. Polizzi and A. Vitale ,2003).

86 **Materials and Methods :**

87 **Sample collection from the Sampling site :**

88 The samples were collected in sterile polythene packs using sterile instruments like
89 scissor , needle and knife. The diseased parts of the date palm which were collected are
90 symptomatic rachis, fronds , trunk , phylloplane and fruits from Ad Darb , Jizan. The
91 major diseases noticed were leaf spots, black scorch, leaf basal rot and fruit spots , wilt
92 and rust . The samples were stored in the laboratory refrigerator .Nine different fungal
93 genera were isolated from date palm which are the causal organisms of the different
94 diseases during this study.

95 The samples were collected from symptomatic rachis, fronds and fruits in four
96 localities (El-Minia, Assiut, Sohag and Qena Governorates). The major diseases
97 noticed were leaf spots, off-shoot decline, black scorch, leaf basal rot and fruit spots.
98 Seven different fungal species were isolated from date palm exhibited different
99 symptoms (M.I. Ammar and M.A. El-Naggar, 2011) .

100 **Inoculation and Slide Preparation:**

101 Potato Dextrose media was employed for studying the mycoflora associated with the
102 different parts of the date palm . 10 ml of the media was poured in the petri plates
103 under sterile conditions which were then inoculated with 0.5 cm square leaf pieces
104 from the infected phylloplane collected from the area. The petri plates were also

inoculated with the pieces of the other different parts of the date palm like the symptomatic **rachis** , fronds ,trunk and infected fruits. A qualitative and quantitative assessment of mycoflora was carried out by the fungal cultures on the petri plates and the slides were identified by microscopic and macroscopic characteristics. **(fullstop)**

Lactophenol and cotton blue in lactophenol were used as mounting and staining media for preparing semi permanent slides which were sealed with DPX mountant.

Microscopic and Macroscopic observation:

Research microscope with adequate high power has been used through out the study.

Identification was carried out by using standard manuals and keys to the identification. Colony characters on media. Morphological characters include like nature of mycelium, its colour ,sexual and asexual structures and their characters , conidiophore nature and spore nature were used in identification . Pictures of the petriplates and photomicrographs were taken during the study.

Figure 1. Pictures of the Different Parts of Date Palm (age**) infected by Mycoflora from the Sampling Site.**



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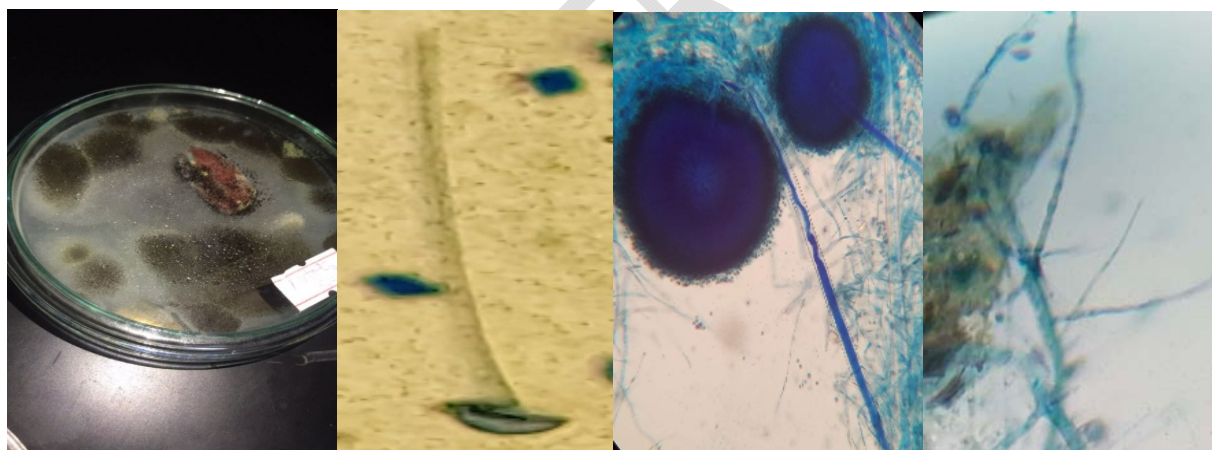
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Figure 2. Photographs of the Mycoflora from Date Palm



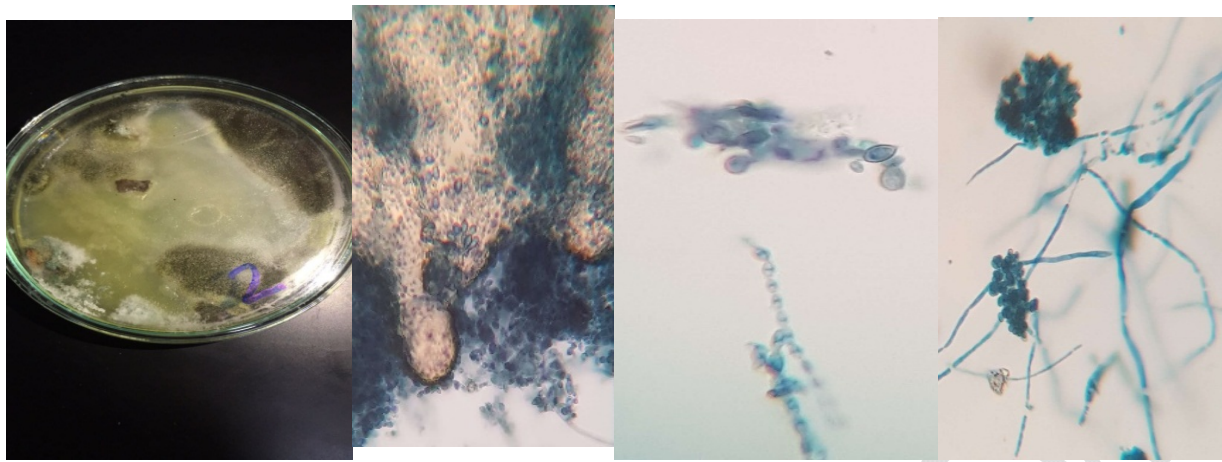
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128 a).Petri Plate with isolates from Date fruit b).Rhizopus c).Aspergillus d) Ceratocystis

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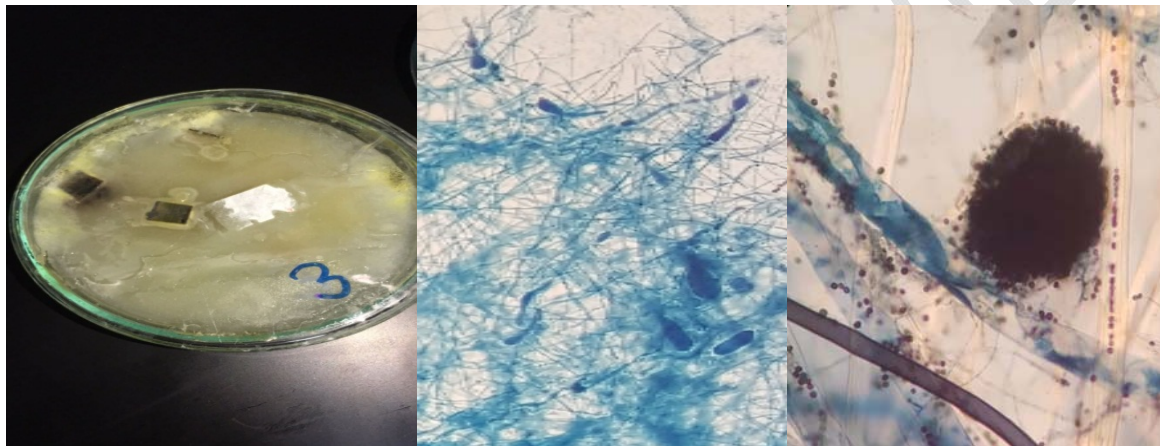
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d).Petri Plate with isolates from Rachis

e). Graphiola

f). Ceratocystis

g) Botrytis



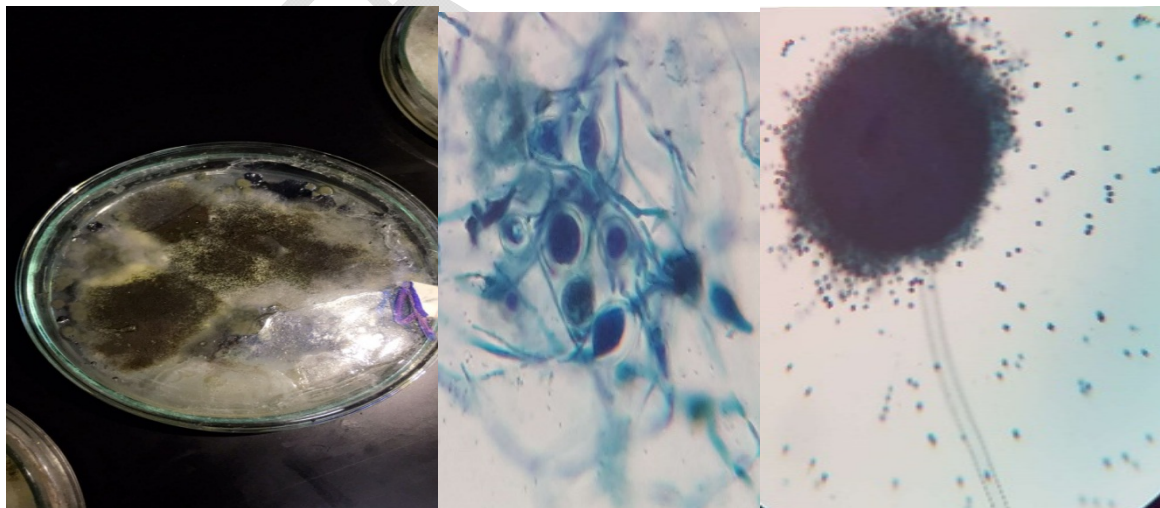
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h).Petri Plate with isolates from Phylloplane

i). Fusarium

j). Aspergillus



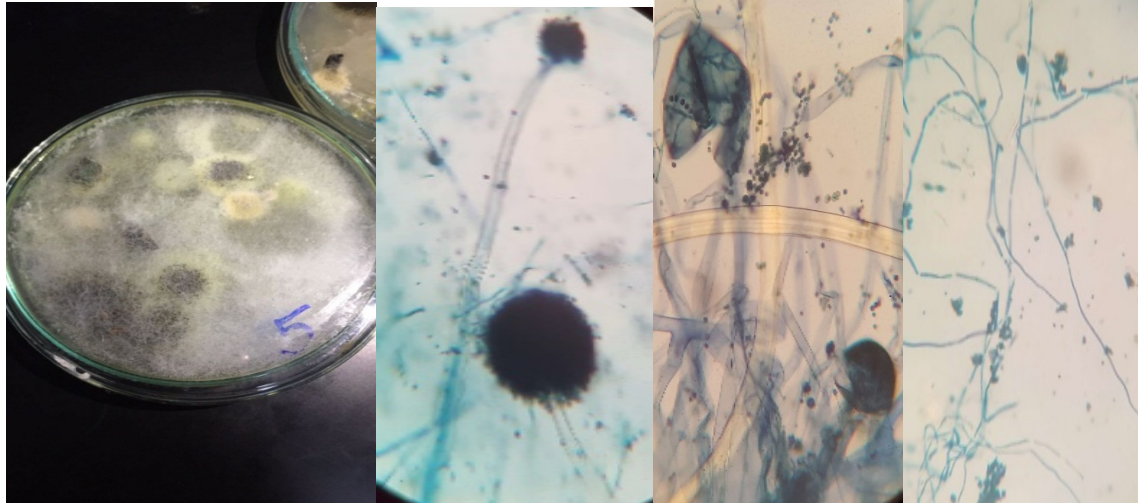
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k).Petri Plate with isolates from Phylloplane

l).Saprolegnia

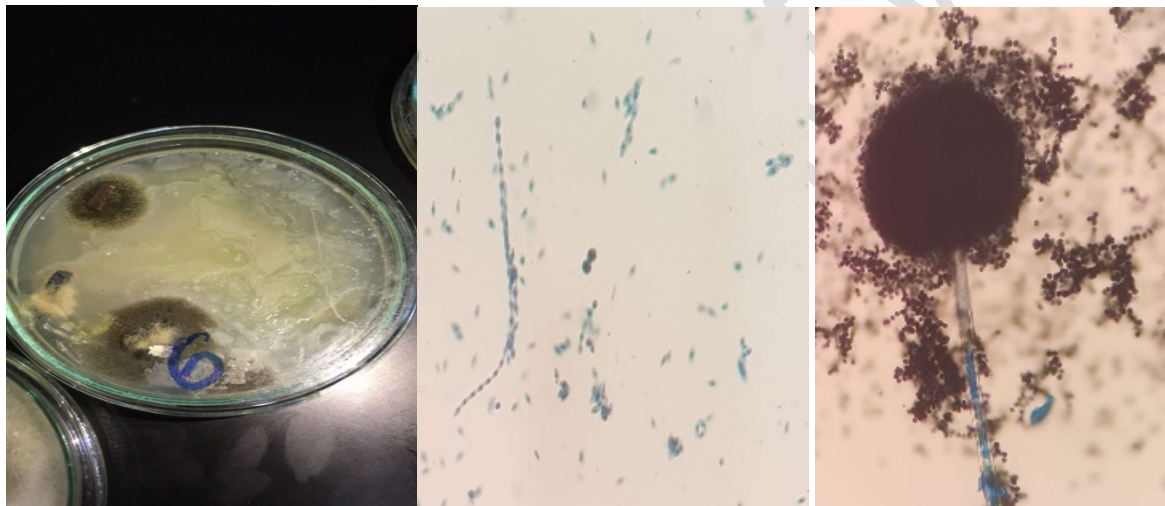
m). Aspergillus



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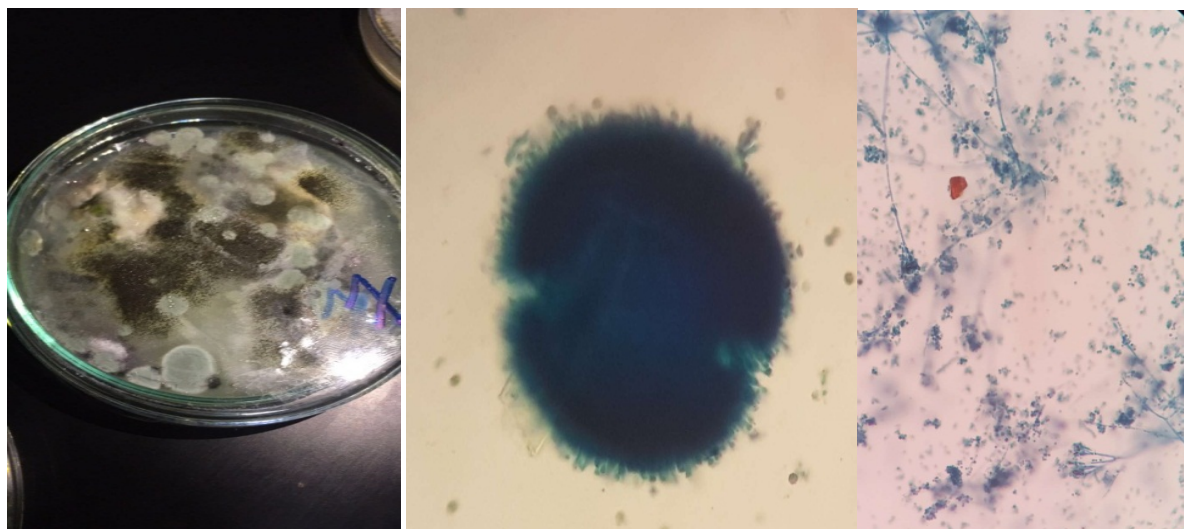
m).Petri Plate with isolates from trunk n). Aspergillus o).Rhizopus p).Botrytis



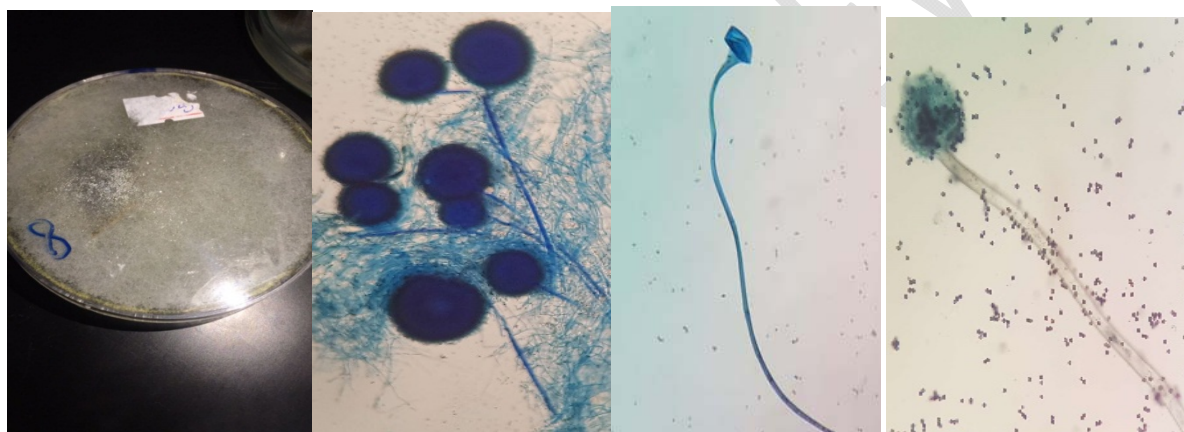
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q).Petri Plate with isolates from Rachis r). Ceratocystis s). Aspergillus



t).Petri Plate with isolates from Rachis u). Syncephalastrum v). Penicillium



w).Petri Plate with isolates from Phylloplane x). Aspergillus y). Rhizopus z). Aspergillus

Table 1. Different Fungal Genera Isolated from the different parts of the Date Palm.

Sr. No	Sample	Zygomycotina		Oomycotina	Ascomycotina					
		Rhizopus	Syncephalastrum	Saprolegnia	Aspergillus	Botrytis	Ceratocystis	Fusarium	Penicillium	Graphiola
1	Fruit	+(16)	-	-	+(22)	-	+(1)	-	-	-
2	Rachis	-	-	-	-	+(3)	+(2)	-	-	+(3)
3	Phylloplane	-	-	-	+(12)	-	-	+(6)	-	-
4	Phylloplane	-	-	+(2)	+(6)	-	-	-	-	-
5	Trunk	+(2)	-	+(6)	+(3)	+(7)	+(1)	-	-	-
6	Rachis	-	-	-	+(3)	-	+(3)	-	-	-

7	Phylloplane	-	+(3)	-	+(11)	-	-	-	+(21)	-
8	Phylloplane	+(1)	-	-	+(1)	-	-	-	-	-
9	Trunk	+(3)	-	-	+(17)	-	-	-	-	-
Total number of isolates		22	3	8	74	10	7	6	21	3

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Total fungal genera = 9

(+) Present

150 **Symptoms of Different Fungal genera on the infected plant parts of the Date palm:**

151 **Aspergillus** :It causes brown , black and powdery rots of infected fruits and brown or black
152 leaf spot on the phylloplane. The fruit surface is paler , discoloured and turns brown, black or
153 powdery. The fruit turns soft and decays thus its taste and quality deteriorates.

154 **Botrytis** : It is called the gray mold and shows gray moldy growth along with light
155 brown lesions on the rachis and trunk of date palm due to infection.

156 **Ceratocystis** : It causes brown necrotic lesions on all parts of the date palm.It causes the fruit rot
157 and the rachis shows bleeding rot leading to partial necrosis . The lesions are dark brown to
158 black carbonaceous and sooty in nature.

159 **Fusarium** : It is a dangerous and commonly found pathogen of date palm . It causes wilting of
160 the leaves and gradually yellowing with streaks and death of leaves .

161 **Graphiola** : The disease is also called false smut on date palm. The symptoms are found as
162 black spots due to rough sori developing on the rachis containing spores.

163 **Penicillium** : The surface of the infected leaves shows blue moldy growth along with
164 rotting and necrosis of the tissue

165 **Rhizopus** : It causes soft rot of date fruit which is characterized by a soft and watery
166 rot that quickly deteriorates and damages the entire fruit. The infected phylloplane and
167 trunk shows brown or black rot symptoms which reduces the quality of the leaves and
168 other infected parts.The surface of the infected plant part shows hairy gray mycelial
169 growth along with black sporangial mass.

170 **Saprolegnia** : It causes white fibrous mycelial patches with yellow lesions on the
171 surface of the infected plant parts

172 **Syncephalastrum** :Phylloplane of the infected palms have brown or black water-
173 soaked spots on the surface which coalesce and enlarge leading to the yellowing
174 , withering and death of the entire leaf . Different font

Results and Discussion :

Total number of nine fungal genera were isolated from the nine samples collected from the different parts of the Date palm from Ad Darb , Jizan. The mycoflora is represented by *Aspergillus*, *Botrytis*, *Ceratocystis*, *Fusarium* , *Graphiola* ,*Penicillium* *Rhizopus*,*Saprolegnia* and *Syncephalastrum*. One hundred and fifty four fungal isolates were obtained from the nine represented genera . (Table -1)(Fig.2 a-z).*Aspergillus* was the predominant genera with 74 isolates..

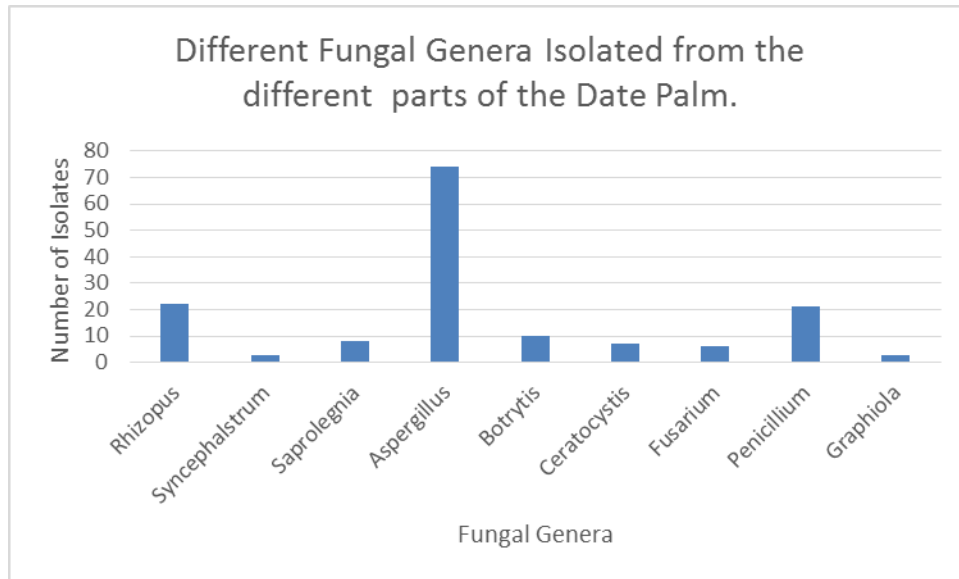
Eleven species belonging to nine different genera of fungi were isolated. The genera isolated were *Alternaria*, *Eurotium*,and *Fusarium* (two species), *Aspergillus*, *Drechslera*, *Penicillium*, *Rhizopus*, and *Curvularia* (one species each). This is the first record of seed-borne fungi from *Phoenix dactyliera* L. in Saudi Arabia (H.A.Bokhary ,2010)

The recording of *C. acutatum*, *Sordaria fimicola*, *F.ventricosum* and *F. torulosum* as true pathogen on date palm was the first time in Saudi Arabia (M.I. Ammar and M.A. El-Naggar, 2011)

Many fungal species have been isolated from affected date palm trees showing DBF (Date Bunch Fading Disorder) (DBF)disorder. These include *Alternaria* sp., *Aspergillus flavus*, *A. niger*, *Penicillium* sp., *Fusarium* sp., *Trichoderma* sp., and *Thielaviopsis paradoxa*(Samir K.Abdullah, 2010).

Fruit rot is caused by *Aspergillus* and *Rhizopus* in this study . It causes heavy damage to the crop. Fruit rot damage varies from one year to another depending on humidity and rain and also on the time of these factors from the Khalal stage until fruit maturation (A. Zaid, et. al).

Figure 3. Bar Diagram to represent the different fungal genera and the number of isolates



One hundred and fifty four fungal isolates were obtained from the nine represented genera. *Aspergillus* was the predominant genera with 74 isolates, followed by *Rhizopus* with 21 isolates and *Penicillium* with 21 isolates. *Graphiola* and *Syncephalstrum* showed 3 isolates each (Fig.3).

Conclusion:

The present study shows that a number of harmful and pathogenic mycoflora are associated with the date palm in Ad Darb region. The quality of the date fruit is deteriorated both qualitatively and quantitatively due to these **mycopathogens**. The list of diverse phytopathogenic mycoflora isolated during this study is associated with a number of date palm diseases of the region which cause a lot of enormous economic loss along with reduced yield to the date producers. This study helps the plant pathologist of the date palm to take up the necessary **phytosanitary**, therapeutic and other control measures in order to protect the date palm and improve the quality and yield of the nutritious date fruit. The field environments must be kept clean always in

order to check these pathogens in the area. The study can lead to an understanding of the occurrence and their activities in relation to their ecological niche and thereby can help in disease forecasting and pathogenesis of the phytopathogens.

The phytopathogenic fungi cause various diseases like leaf spots, wilts, black scorch, smuts etc. on the date palm and hence must be controlled in order to protect the plants thereby helping in better health, good yield and resistance to the crops grown in the region of study. This study can help in controlling the diseases, damage and economic losses to the crop.

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Conflict of interest : The authors declare that there is no conflict of interest.

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