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PATHOGENIC FUNGI ASSOCIATED WITH DATE PALM TREES IN TURBAT, BALOCHISTAN

Irshad Ahmed, Muhammad Abid, Faisal Hussain, Syed Qaisar Abbas and Toqeer Ahmed Rao

Dr. A.G. Laboratory of Aerobiology and Plant Pathology, Department of Botany, Federal Urdu University of Arts, Sciences & Technology, Gulshan-e-Iqbal Campus, Karachi-75300 Pakistan

ABSTRACT

District Turbat, Balochistan, Pakistan is famous for its stingy and delicious dates. Recently, date palm trees have been badly affected by different plant pathogens which significantly reducing yield and farmers facing heavy losses annually. During a survey of different date palm cultivation in District Turbat and surrounding areas, fruit, root and soil samples were collected. Five fungi viz., *Aspergillus niger, A. flavus, Penicillium* sp. *Cladosporium* sp. and *Rhizopus* sp. were isolated from fruits. Among these fungi, *A. niger* was dominant and isolated from all the samples. The fungus causes fruit rot disease in different date palm varieties resulting in heavy losses annually. Fungi isolated from root samples included *Fusarium solani, F. oxysporum. Drechslera elisii, D. hawaiiensis, Humicola* sp., *Blastomyces* sp., *Acremonium* sp., *Alternaria* sp., *Phytophthora* sp., *Aspergillus niger, A. flavus* and *Penicillium* sp. *F. solani* was the dominant fungus and causes sudden decline syndrome (wilt disease) in Date palm trees. The level of occurrence of this fungus was significantly high in root samples of Gokna and Kungun varieties as compared to other varieties. *F. solani, A. niger A. flavus,* and *A. terreus* were isolated from soil at higher percentage as compared to some other fungi such as *Drechslera hawaiiensis, Humicola* sp., *F. oxysporum, Macrophomina phaseolina, Acremonium* sp., *Alternaria* sp., *Penicillium* sp. and *Rhizopus* sp.

Keywords: Date palm, Pathogenic fungi, Fruit, root-rot

INTRODUCTION

The areas which have long, dry summer and mild winter are best for date palm cultivation. It can also grow in saline soil (Sanderson, 2001). Date palm covers 3% of the world cultivated area (Dowson, 1982). Egypt is the main date's producer in the world followed by Saudi Arabia, Iran and United Arab Emirates. Pakistan is the 5th largest country in date's production (Chao and Robert, 2007). In Pakistan, Sindh province produces 45.4%, Balochistan 44.8%, Punjab 7.9% and Khyber Pakhtonkhwa 1.9%. Khairpur district of Sindh province produces 85% of the total production of the province (Anon., 2008). In Balochistan high quality date palms are cultivated and total productivity of dates is about 236205 tons/year. Approximately 80% dates are produced in district Kech (Turbat). Out of 80 Date palm varieties, farmers are commonly cultivating seven varieties in Turbat *i.e.* Begham jangi, Mozati, Shakar, Pashpag, Halini, Abdandan, Hussaini however Begham jangi is widely being cultivated due to good taste (Anon., 2000). From two to three decades, date palm is under stress due to biotic stresses. Several pathogens are causing diseases to date palm and it is affecting the yield of date palm in Balochistan particularly in Turbat district. Among other plant pathogens, fungi cause different diseases in date palm.

Date palm trees suffering a serious disease known as sudden decline syndrome (wilt disease) caused by *Fusarium solani, F. proliferatum* and *F. oxysporum* (Abdalla *et al*., 2000; Rashed and El-Hafiz, 2001; Al Yaseri *et al.*, 2006). Infection occurred at any time of the year particularly when plants are in at fruiting stage, pre mature fruits are dropped (Abul-Soad *et al*., 2011). *Phytophthora* sp. was reported by several workers which caused Balaat disease in Date palm. There are several symptoms observed at the crown of palm, young fronds become whiten and die, so the infection leads to downward in trunk result as a heart rot form (Zaid *et al.*, 2002; Abdullah *et al.*, 2010). Fruit rot varies from year to year depends on humidity and rainfall. It was estimated that this disease cause 10 to 50% post harvest losses (Darley and Wilbur, 1955; Calcat, 1959; Djerbi *et al*., 1986). The most common fungi causing fruit spoilage is *A. niger* and side spot decay by *Alternaria* sp. (Zaid *et al*., 2002).

The aim of this study was to conduct survey of different date palm growing areas of Turbat and collect soil and diseased plant samples to update the status of different fungal diseases and develop awareness in date palm growers of the area regarding date palm diseases.

MATERIALS AND METHODS

Site description

Kech or Turbat district is located from 25˚-24¢ to 26˚-39¢ North latitude and from 61˚–49¢ to 64˚-31¢ East longitudes and located in south west province of Balochistan, Pakistan. The climate depends upon three major

coordinates, temperature, humidity and wind. Rain is the most important factor of environment, rain during the flowering and at harvest season is likely to cause some damage to the fruits (Anon. 2010).

Collection of samples

Roots, fruits and soil samples were collected from two areas namely Turbat and Buleda (two tehsils of district Turbat) during the months of July and August, 2012. More than 80 samples of infected fruit, root and soil were collected and observed under lab condition of Department of Botany, Federal Urdu University of Arts, Science & Technology, Karachi. The soil samples were collected from rhizosphere of infected plants. The samples were brought to the lab and kept at 5-10°C.

Soil dilution technique for the isolation of fungi

One gram of soil was suspended in 9 mL of sterilized distilled water which gave a dilution of 1:10, from which the dilutions of 1:100, 1:1000 and 1:10000 were made. One mL aliquot of each dilution 1:1000 and 1:10000 was poured in sterilized Petri plates containing Potato Dextrose Agar medium (PDA) supplemented with penicillin (100,000 units) and streptomycin sulphate (0.2 g) to inhibit bacterial growth. Three replicates for each dilution were made. The plates were incubated at 28 ± 2°C for one week. Fungal colonies growing on plates were counted and identified. The number of colonies of each fungus was multiplied by the dilution factor which gave a total number of propagules/g of soil (Waksman and Fred, 1922).

Isolation of fungi from roots and fruits (dates)

The collected roots and fruits samples were washed with running tap water and cut into about 1cm long pieces separately. The pieces were surface sterilized with 1% $Ca(OCl)_2$ and placed on Petri plates containing PDA supplemented with Penicillin (100,000 units) and Streptomycin sulphate (0.2 g) to inhibit bacterial growth. The plates were incubated at 28°C for 4 to 5 days. After incubation, infection and colonization % were calculated with the help of following formula:

$$\textbf{Infection } \% = \frac{\text{Number of plants infected by a pathogen}}{\text{Total number of plants}} \times 100$$

$$\textbf{Colonization } \% = \frac{\text{Number of pieces colonized by a pathogen}}{\text{Total number of pieces}} \times 100$$

Identification of isolated fungi

Isolated fungi were identified using standard references (Ellis, 1971; Barnett and Hunter, 1972; Ellis, 1976; Domsch *et al.*, 1980; Sutton, 1980; Nelson *et al.*, 1983; Singh *et al*., 1991).

Analysis of data

Data were analyzed and subjected to Analysis of variance (ANOVA). The follow up of ANOVA included least significant difference (LSD), Duncan's multiple range test was used to compare the treatment means.

RESULTS

a. Fungi Isolated from Dates

Fungi isolated from infected dates (fruit) including *A. niger*, *A. flavus*, *Penicillium* sp., *Cladosporium* sp. and *Rhizopus* sp. These fungi were isolated in different mean value from fruit are shown in Table 1. *A. niger* was dominant fungus with the mean value of 4% among fruits of date palm varieties including Gokna, Rogeni, Begham jangi, Pashpag, Husaini and Abdandan. *A. flavus* was dominant in Halini, Pashpag and Koroch with the mean value of 3.3, 1.6 and 1.3%, respectively. *Rhizopus* sp. and *Penicillium* sp. were also found in some varieties with very low mean value and *Cladosporium* sp. was isolated from one variety (Shakri) with mean value of 2.3%. *A. niger* was predominant abundance in all varieties with high infection mean value and caused fruit rot. This disease is common in date palm of Turbat which reduced the annual production of dates. The results of ANOVA of different fungi were isolated from different varieties of date palm. Three fungi including *Aspergillus niger* ($F=7.35$, $P<0.001$), *A. flavus* ($F=8.69$, $P<0.001$) and *Cladosporium* sp. ($F=12.25$, $P<0.001$) showed highly significant differences whereas *Rhizopus* sp. and *Penicillium* sp. have non- significant differences amongst different varieties.

Table 1. Fungi isolated from fruit of different date palm varieties with Mean and Standard Error (S.E).

Name of Fungi	Mean and Standard Error (±)												
	PES	GOK	KOR	HAL	ROG	BEG	MOZ	SHAK	SHA	PAS	NAZ	HUS	ABD	KUNG	KUN
Aspergillus niger	2±0.5	4±0	2.3±0.3	2.6±0.3	4±0	4±0	3.6±0.3	1.3±0.8	3.6±0.3	4±0	3.6±0.3	4±0	4±0	3.6±0.3	1.3±0.3
Rhizopus sp.	0±0	0.3±0.3	0±0	1±1	0±0	0±0	0.6±0.6	0±0	0±0	1.6±0.8	0.6±0.6	0±0	0±0	0±0	0±0
Aspergillus flavus	0±0	0±0	1.3±0.8	3.3±0.3	0.3±0.3	0±0	0±0	0±0	0±0	1.6±0.3	0±0	0±0	0±0	0.3±0.3	1±0.5
Penicillium sp.	0±0	0±0	0.3±0.3	0±0	0.6±0.6	0±0	0±0	1±0.5	0±0	0±0	0±0	0±0	0±0	1±0.5	1.3±0.6
Cladosporium sp.	0±0	0±0	0±0	0±0	0±0	0±0	0±0	2.3±0.6	0±0	0±0	0±0	0±0	0±0	0±0	0±0

PES= Peshna, **GOK**= Gokna, **KOR**= Koroch, **ROG**= Rogeni, **HAL**= Halini. **BEG**= Beghan jangi. **MOZ**= Mozati, **SHAK**= Shakri, **SHA**= Shakar, **PAS**= Pashpag, **NAZ**= Nazini. **HUS**= Husaini, **ABD**= Abdandan, **KUNG**= Kungun, **KUN**= Kunzanabad

Table 2. Fungi isolated from root of various Date Palm varieties with Mean and Standard Error (S.E).

Name of Fungi	Mean and Standard Error (±)														
	PES	GOK	KOR	HAL	ROG	BEG	MOZ	SHAK	SHA	PAS	NAZ	HUS	ABD	KUNG	KUN
Fusarium solani	0±0	4±0	0±0	0±0	0±0	0.6±0.6	1.3±0.6	2.3±0.6	2±1	1.6±0.8	2±0	1.6±0.8	0±0	4±0	2±0
Drechslera hawaiiensis	0.6±0.3	2±0.5	1±0.5	1±0.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.6±0.6	0.6±0.3	0±0
Fusarium oxysporum	1.3±0.6	2.3±0.3	1±0.5	1.3±0.6	0.6±0.6	0±0	0±0	0±0	0±0	1.3±1.3	0±0	0.6±0.6	0.6±0.3	3.6±0.3	1±0
Drechslera elysii	0±0	0±0	0±0	0±0	1±0.5	0±0	2.3±0.3	0±0	1±0.5	0±0	0.6±0.3	0±0	0±0	0±0	0±0
Penicillium sp	0±0	0±0	0±0	0±0	0.6±0.3	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Aspergillus niger	0±0	0.6±0.3	0.6±0.6	3.6±0.3	1.3±1.3	0.6±0.3	1.3±0.3	1±0	1±0	1.3±0.3	1.6±0.3	3.6±0.3	1±0.5	0±0	0±0
Aspergillus flavus	0±0	1.6±1.2	2.6±1.3	0.6±0.3	0±0	0.3±0.3	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Blastomyces sp.	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	2.6±0.3
Humicola sp.	0±0	1±0.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Acremonium sp.	0±0	0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	1±0.5	0±0	0±0
Phytophthora sp.	0±0	0±0	0.6±0.6	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Alternaria sp.	0±0	3±0.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.6±0.6	2.6±0.3

PES= Peshna, **GOK**= Gokna, **KOR**= Koroch, **HAL**= Halini, **ROG**= Rogeni, **BEG**= Beghan jangi, **MOZ**= Mozati, **SHAK**= Shakri, **SHA**= Shakar, **PAS**= Pashpag, **NAZ**= Nazini, **HUS**= Husaini, **ABD**= Abdandan, **KUNG**= Kungun, **KUN**= Kunzanabad

Table 3. Fungi isolated from soil (at 10^{-3}) in rhizosphere soil from various Date Palm varieties with Mean and Standard Error.

Name of Fungi	PES	GOK	KOR	HAL	ROG	BEG	MOZ	SHAK	SHA	PAS	NAZ	HUS	ABD	KUNG	KUN
Aspergillus niger	2.3±1.2	3.6±0.6	2.6±1.4	1±0.5	1.6±0.8	4.3±2.9	3.6±1.6	0±0	6.6±1.7	2±1.5	8.3±1.6	2±1.1	3.3±0.8	1.3±0.6	3.6±2
Aspergillus flavus	6.6±6.6	2±1.5	5±2.8	0±0	0±0	2.3±0.8	5±2.8	2.6±0.6	2.3±0.6	1±0.5	3.3±1.2	3.6±1.2	3.6±3.1	0.6±0.3	8.3±6
Drechslera hawaiiensis	0±0	1.6±0.3	0±0	0±0	2.6±0.3	1±0.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Fusarium solani	0±0	0±0	0±0	0±0	0±0	0.6±0.6	0.6±0.3	2±1	0±0	0±0	0±0	4.6±2.7	0±0	0±0	0±0
Humicola sp	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.6±0.3	1.3±0.6	1±1	1.3±0.6	0±0	1.3±0.6	0±0
penicillium sp.	3.6±3.6	0.6±0.3	0±0	0±0	2.3±0.5	0±0	0±0	3.3±1.6	2.3±1.2	1.3±0.6	2.6±0.6	1.3±0.6	1.6±1.6	0±0	4.6±2.9
Rhizopus sp.	0±0	0±0	1.6±1.6	0±0	0±0	0±0	0±0	0±0	0±0	0±0	1.3±1.3	0±0	0.6±0.6	0±0	0.6±0.6
Aspergillus terreus	0±0	0±0	0±0	1±0.5	2±0.5	3.3±3.3	1.6±1.6	1±0.5	0.6±0.3	0±0	3.6±1.6	4±3	0±0	3.3±1.6	1.6±1.6
Fusarium oxysporum	0±0	0±0	0±0	0±0	2±0.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Macrophomina sp.	0±0	0.6±0.6	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Acremonium sp.	0±0	0.3±0.3	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Alternaria sp.	0±0	0±0	0±0	1.6±0.3	0±0	0±0	1.3±0.8	0±0	0.6±0.3	0±0	0.3±0.3	0±0	0±0	0±0	0±0

PES= Peshna, **GOK**= Gokna, **KOR**= Koroch, **HAL**= Halini, **ROG**= Rogeni **BEG**= Begham jangi, **MOZ**= Mozati, **SHAK**= Shakri, **SHA**= Shakar, **PAS**= Pashpag, **NAZ**= Nazini, **HUS**= Husaini, **ABD**= Abdandan, **KUNG**= Kungun, **KUN**= Kunzanabad

Table 4. Fungi isolated from soil (at 10^{-4} dilution) from rhizosphere of different Date Palm varieties with Mean and Standard Error (S.E).

Name of Fungi	PES	GOK	KOR	HAL	ROG	BEG	MOZ	SHAK	SHA	PAS	NAZ	HUS	ABD	KUNG	KUN
Aspergillus niger	1±1	2±1.5	0.6±0.3	0±0	0.6±0.3	1.3±0.6	1±0.5	0±0	2.3±0.6	0.3±0.3	3.6±1.3	0.6±0.3	1±1	0.3±0.3	1±1
Aspergillus flavus	3±2	0±0	2.3±0.8	0±0	0±0	0±0	0±0	0.3±0.3	1.3±0.8	0.3±0.3	1±0.5	2.6±1.3	0±0	0.3±0.3	3.6±1.3
Drechslera hawaiiensis	0±0	0.6±0.3	0±0	0±0	1±0.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Penicillium sp.	0±0	0.3±0.3	0±0	0.6±0.3	0±0	0±0	0±0	0±0	0±0	0±0	0.3±0.3	0±0	0±0	0±0	0±0
Aspergillus terreus	0±0	0±0	0±0	0±0	0.3±0.3	0±0	0±0	0±0	0±0	0±0	1.3±0.8	0±0	0±0	0±0	0±0
Alternaria sp.	0±0	0±0	0±0	0.3±0.3	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0

PES= Peshna, **GOK**= Gokna, **KOR**= Koroch, **HAL**= Halini, **ROG**=Rogeni **BEG**= Begham jangi, **MOZ**= Mozati, **SHAK**= Shakri, **SHA**= Shakar, **PAS**=Pashpag, **NAZ**= Nazini, **HUS**= Husaini, **ABD**= Abdandan, **KUNG**= Kungun, **KUN**= Kunzanabad

b. Fungi isolated from roots

Twelve fungi were isolated namely *F. solani, F. oxysporum, Drechslera hawaiiensis, D. elisii, Humicola sp., Blastomyces* sp., *Alternaria* sp., *Phytophthora* sp., *Acremonium* sp., *A. niger, A. flavus* and *Penicillium* sp. from the roots of different date palm varieties (Table 2.) *F. solani* was most abundant fungus with 4% mean value was recorded in Gokna and Kungun varieties, and also observed in Shakri and Pashpag with the mean value of 2.3 and 1.6% followed by *F. oxysporum* and *A. niger* with the 3.6% mean value in Kungun, Halini and Husaini varieties. *Alternaria* sp. was isolated in Gokna variety with 3% mean value. *Blastomyces* sp. was with the mean value of 2.6% in Kunzanabad variety. Thus *F. solani* causes sudden decline syndrome in date palm (wilt disease). *Blastomyces* sp., *Humicola* sp, and *Acremonium* sp. were first time recorded in Date palm.

The ANOVA results of fungi were isolated from date palms root. *F. solani* ($F=7.14$, $P<0.001$), *D. elisii* ($F=7.5$, $P<0.001$), *A. niger* ($F=5.18$, $P<0.001$), *Blastomyces* sp. ($F=64$, $P<0.001$), *Phytophthora* sp. ($F=1$, $P<0.001$) and *Alternaria* sp. ($F=16.73$, $P<0.001$) have highly significant differences among different varieties whereas *D. hawaiiensis, F. oxysporum, A. flavus, Humicola* sp. and *Acremonium* sp. have significant difference in some varieties. *Penicillium* sp. showed no significant differences in all varieties.

c. Fungi isolated from soil

Soil samples were collected from rhizosphere of different localities. Twelve fungi were isolated including *Fusarium solani, F. oxysporum, Drechslera hawaiiensis, Humicola* sp, *Aspergillus niger, A. flavus, A. terreus, Macrophomina phaseolina, Acremonium* sp., *Alternaria* sp., *Penicillium* sp. and *Rhizopus* sp. from rhizosphere soil. These fungi isolated at different mean values in different date palm varieties (Table 3). It has observed that different isolated fungi were recorded with various frequencies but the *A. niger* and *A. flavus* were predominant fungi with 8.3% mean value in the dilution of 10^{-3} in different varieties such as Nazini, Kunzanabad and Shakar followed by *F. solani* with 4.6% mean value and *A. terreus* was also found in abundant with 3.6% of mean value. While the mean value of *D. hawaiiensis, F. oxysporum* and *Alternaria* sp. were 2.6, 2 and 1.3%, respectively observed in date palm varieties (Table 3).

Fungi isolated from soil with various frequencies but the *A. niger* and *A. flavus* were dominant fungi with the mean value of 3.6% in 10^{-4} dilution in different varieties such as Nazini, Kunzanabad and Shakar followed by *A. terreus with* the mean value 1.3% in Nazini and *D. hawaiiensis* with the mean value of 1% (Table 4).

DISCUSSION

Fungi cause different diseases in various parts of date palms- fruit, root spathe and shoot. Many factors involved to spoilage the dates as rain and humidity but the fungi involved to cause calyx end rot by *A. niger* (Zaid *et al*., 2002). The date palm orchards of district Khairpur are suffering from a sudden decline disease of date palm since last couple of years that is threatening to the date palm industry of the region (Maitlo *et al*., 2013). Matilo *et al.* (2014) investigated the different date palm varieties from Khairpur and isolated *Fusarium solani, Phoma ucladium, Helminthosporium sativum, Alternaria alternata, Aspergilus nige*r and *Penicillium chrysogenum* from different date palm varieties. Abul-Soad (2011) reported the soil-borne fungus *Fusarium solani, P. ucladium* and *H. sativum* from different date palm varieties in Pakistan.

In the present study, five fungi genera were isolated from affected dates of fifteen varieties among them *A. niger* was predominate as compared to *A. flavus, Penicillium* sp., *Cladosporium* sp. and *Rhizopus* sp. Thus showed that *A. niger* infected dates were affected by fruit rot. *F. solani* and *F. oxysporum* were most abundant fungi in date palm which cause root rot and main reason for degeneration of date palm plantations. El-Arose *et al.* (1982) reported that several soil borne fungi attack on date palm and causing root rot, wilt and decline diseases. According to Sarhan (2001) *F. solani* and *F. oxysporum* are significantly decline date palm production in different parts of Iraq. In addition, several workers from different date palm growing areas of the world reported various fungal pathogens associated with similar kind of date palm diseases such as *F. oxysporum, F. monliforme, F. proliferatum* and *F. solani* (Abdalla *et al*., 2000; Sarhan, 2001; Rashed and El-Hafeez, 2001).

Similar type of fungal pathogens on date palm are also reported losses in Khairpur, Pakistan (Maitlo *et al.*, 2013; 2014), Egypt (Rashed and El-Hafeez, 2001; Rasheed, 1998), Saudi Arabia (El-Arosi *et al*., 1982), Libya (Edongali *et al*., 1985; Khalil *et al*., 1986) and Iraq (Sarhan, 2001).

CONCLUSION

The present study suggested that in district Turbat, the most infected date palm varieties are Gokna and Koroch. Thus farmer of Turbat should avoid cultivation of these varieties and focus on cultivation of Begham Jangi, Shakar and Husaini due to their less susceptibility to fungal diseases.

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