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Rhizosphere and Rhizoplane Mycoflora of Date Palm (*Phoenix dactylifera*) from Saudi Arabia

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

This is the first comparative study of the rhizosphere and rhizoplane mycoflora of *Phoenix dactylifera* (date palm) from three different regions of Saudi Arabia. Samples of roots and rhizosphere soil from the date palm were collected from Jeddah, Jizan and Riyadh. A total number of eighteen samples were collected. Three samples from each region were collected and screened for mycoflora during the month of May 2017. The mycoflora was aseptically cultured on Potato Dextrose Agar plates and Czapek Dox agar plates. A qualitative and quantitative assessment of mycoflora was carried out from the fungal cultures on the petri plates. Slides were identified by microscopic and macroscopic morphological characteristics. A total of 48 fungal isolates represented by 11 fungal genera were isolated from the samples belonging to the three classes of fungi i.e. *Oomycotina, Zygomycotina* and *Ascomycotina.* The rhizoplane and rhizosphere mycoflora was represented by *Aspergillus Chaetomium, Cunnighamella, Fusarium, Mucor, Nigrospora, Oidiodendron, Penicillium, Phytopthora, Rhizopus and Syncephalastrum.* The most predominant genera were *Aspergillus* followed by *Rhizopus.* The rhizoplane mycoflora was lesser than the rhizosphere mycoflora in Jeddah and Jizan. Riyadh recorded equal number of both rhizoplane and rhizosphere mycoflora. The fungal genera isolated are soil borne saprophytic, antagonistic or phytopathogenic fungi of the date palm. The information on the phytopathogenic mycoflora is

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important to plant pathologists and date growers in these regions so that they can take up necessary phytosanitary measures to prevent damage to the date crop. This study can help in controlling the diseases, damage and economic losses to the date crop.

Keywords: Date palm; mycoflora; rhizosphere; rhizoplane; Saudi Arabia.

1. INTRODUCTION

Date palm (*Phoenix dactylifera*) is the commonly grown tree found in all the parts of the country. It deserves to be officially designated as the pride fruit and the national fruit of the country. In Saudi Arabia, date palm is the most important cash and fruit crop grown in different regions. In many ways, dates may be considered as an almost ideal food, providing a wide range of essential nutrients and potential health benefits. Date fruits are cheap to produce preserve and are also very rich in nutrition [1]. Date palm (*Phoenix dactylifera*) is considered the main crop in deserts and arid areas such as Saudi Arabia [2]. The date palm (*Phoenix dactylifera*) is a multi-purpose plant species typically grown in the arid and semi-arid regions of the world and is globally valued for its nutritional and health-promoting fruit [3]. 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 [4].

Rhizosphere: It is the zone/region of soil immediately surrounding the plant roots together with root surfaces, or it is the region where soil and plant roots make contact, or it is the soil region subjected to influence of plant roots and characterized by increased microbial activity.

Rhizoplane: Root surface along with the closely adhering soil particles is termed as rhizoplane. The term "Rhizosphere" was introduced for the first time by the German scientist Hiltner(1904) to denote that region of soil which is subjected to the influence of plant roots (AgriInfo.in, 2015).

This study attempts to find the rhizosphere and rhizoplane mycoflora associated with this very important tree in the country. The wide range of rhizosphere and rhizoplane mycoflora may be pathogenic and damaging to the date crop [4]. Understanding the biology of different fungi in diverse ecosystems, as well as their biotropic interactions with other microorganisms, animals and plants, is essential to underpin effective and innovative technological developments [5].

Further knowledge about the fungal flora that may naturally be associated with healthy palm trees is, however, necessary to (1) improve yield, (2) facilitate the occupation of the ecological "niche" and avoid the random colonization of young plantlets by potential fungal pathogens, and (3) help further plantlet acclimation to arid soil conditions [3].

This is the first comparative study of rhizoplane and rhizosphere mycoflora from the three cities of Saudi Arabia. This study was conducted in order to survey different date palm growing areas of the three cities Jeddah, Jizan and Riyadh and collect soil and root samples.

The species isolated during the study are not only phytopathogenic but are also saprophytic and are responsible for decomposition of the soil organic matter. Species of *Oidiodendron* are known as saprobes and come from a variety of living and decomposing plant, animal, and fungal substrates, including peat, soil, humus, wood, lichens, marine sediments and holothurians, human skin (incidental contamination only) and decomposing human hair [6].

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). F. solani and F. oxysporum were predominant in the rhizosphere of date palm (Baraka et. al. 2011). One of the most serious fungal diseases in North Africa is the Bayoud disease incited by *Fusarium oxysporum* f. sp. *albedinis*. This disease has caused large losses of date palms, and research concerning Bayoud is one of the most important areas of date palm research [7]. *Phytophthora* sp. was reported by several workers which caused Balaat disease in Date palm [4].

The causal organism responsible for bayoud (Common local name of the disease caused by Fusarium- white fungal disease) is a microscopic fungus which belongs to the mycoflora of the soil

and is named *Fusarium oxysporum forma specialis albedinis* (Killian and Maire, 1930; Malencon, 1934 and 1936). *Fusarium oxysporum f. sp. albedinis* is preserved in the form of chlamydospores in the dead tissues of infected palm, especially in the roots which have been killed by the disease and in the soil [8]. Fungi associated with plants are known to exert their beneficial effects by helping them in absorption of water and nutrients and protecting them against harmful microorganisms. Protective effect is generally mediated by performing antagonistic action on pathogens and pests [9]. Diverse mechanisms are involved in the suppression of plant pathogens, which is often indirectly connected with plant growth. When the plant pathogens are suppressed it indirectly leads to healthy growth of plants.

The present study aims to find the diverse range of mycoflora associated with the rhizophere and rhizoplane of date palm in the three cities. The mycoflora isolated may be saprophytic and phytopathogenic. This study is a source of information to plant pathologists and date growers in these areas so that they can take up necessary phytosanitary measures to prevent damage to the date crop. The isolated fungi release the mycotoxins which help in controlling the growth and proliferation of other pathogens by being antagonistic .Hence this study can add up further information to researchers to carry out further studies on antagonism and other types of interactions between the mycoflora and root exudates in relation to the soil physico - chemical properties.

2. MATERIALS AND METHODS

2.1 Isolation and Identification

2.1.1 Isolation of rhizoplane mycoflora

Fresh roots for rhizoplane mycoflora from below the soil from Date palms from Jeddah, Jizan and Riyadh were collected in sterile polythene bags and stored in the laboratory refrigerator. Altogether nine rhizoplane root samples, three samples from each of the locations were collected during the month of May 2017. 1 cm pieces of roots from the date palm were cut using sterile blades and washed with sterile distilled water. Then the pieces were surface sterilized with 1% $Ca(OCl)_2$ and plated on PDA and Czapek Dox Agar plates with 10 ml of the media under aseptic conditions. The plates were incubated in triplicate for five days.

2.1.2 Isolation of rhizosphere mycoflora

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 (PDA) and Czapek Doz Agar media supplemented with streptomycin (0.2 g) to inhibit bacterial growth. The plates were incubated in triplicate at 28 ± 2°C for five days. Fungal colonies growing on plates were counted and identified [4].

Lactophenol and cotton blue in lactophenol were used as mounting and staining media for preparing semi permanent slides which were sealed with DPX mountant. The fungal isolates were counted and identified by macroscopic and microscopic characters using standard procedures. Research microscope with adequate high power has been used throughout the study. Identification was carried out by using standard manuals and keys to the identification. Colony characters on media like the colour, size , texture etc along with morphological characters like nature of mycelium, its colour,sexual and asexual structures and their characters , conidiophore nature and spore nature has been used as criteria for identification .Spore types and sporangial structures were also considered as criteria for identification of myco taxa. Photographs were taken for the samples and the fungal cultures on the petriplates. Photomicrographs of the slides were also taken under the microscope with a camera for the rhizoplane and rhizosphere samples for each of the three locations.

The soil moisture content for each of the replicate samples was calculated by using the following equation:

% moisture content (MC) =

$$\frac{\text{weight of moist soil (M)} - \text{weight of dry soil (D)}}{\text{weight of dry soil (D)}}$$

(dry wt. basis) [10].

pH values of the soils tested were all in the alkaline side (7.05 -7.6). The soil dilutions used for the estimation of cellulose (Abdel Hafez, 1982). All the soil samples showed pH values above 7 indicating that the soil samples were alkaline. The soil sample from Jeddah showed highest alkalinity of 7.93. The samples collected

from Riyadh showed highest moisture content due to precipitation at the time of sampling (Table 2). 40–80% soil water content was the best level for fungal multiplication [11].

Soil samples were collected from five localities in Jizan city, Saudi Arabia, and analysed for metals and fungal flora. Soil type is sandy and alkaline; [12].

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 [4].

3. RESULTS AND DISCUSSION

This is the first study of rhizoplane and rhizosphere mycoflora of date palm from the three cities of Saudi Arabia. Total of 48 fungal isolates were obtained belonging to 11 genera. *Aspergillus* was the predominant genera with 11 isolates followed by *Rhizopus* with 8 isolates (Fig. 8). The rhizoplane and rhizosphere of Jeddah had 4 and 5 isolates respectively whereas the rhizoplane and rhizosphere of

Fig. 1. Map of Saudi Arabia showing the sampling sites- Jizan, Jeddah and Riyadh

Fig. 2. Date Palm from the Sampling Site

Fig. 3. Roots of date palm

Table 1. Temperature and rainfall of Jeddah, Jizan and Riyadh on different sampling dates

Name of the sampling site	Date of Sampling	Temperature of the place in C		Precipitation%
		Maximum	Minimum	
Jeddah	7 May 2017	35	26	Nil
Jizan	1 May 2017	37	29	Nil
Riyadh	6 May 2017	29	21	42

Table 2. pH, moisture content and wind speed of Jeddah, Jizan and Riyadh at the time of sampling

Name of the sampling site	pH of the Soil	Moisture content of the soil		Moisture %	Wind speed in km/hr
		Initial weight in gms	Final weight in gms		
Jeddah	7.93	50	46.08	7.84	27
Jizan	7.52	50	42.50	15.00	18
Riyadh	7.72	50	21.03	57.94	10

Jizan showed 8 and 9 isolates each. Riyadh recorded the maximum isolates from rhizoplane and rhizosphere with 11 isolates each (Fig. 7). This may be attributed to the high moisture content of the soil at the time of sampling due to precipitation.

3.1 List of Rhizoplane and Rhizosphere Mycoflora of Date Palm

Rhizoplane Mycoflora of Jeddah: *Rhizopus* and *Aspergillus* were isolated (Fig. 4a, 4b).

Rhizosphere Mycoflora of Jeddah: *Rhizopus, Aspergillus* and *Chaetomium* were isolated (Fig. 4c, 4d).

Rhizoplane Mycoflora of Jizan: *Rhizopus*, *Aspergillus, Penicillium* and *Syncephalastrum* were isolated (Fig. 5a, 5b, 5c, 5d, 5e).

Rhizosphere Mycoflora of Jizan: *Phytopthora, Aspergillus, Oidiodendron* and *Nigrospora* were isolated (Fig. 5f, 5g, 5h, 5i).

Rhizoplane Mycoflora of Riyadh: *Phytopthora*, *Cunnighamella, Rhizopus, Fusarium*, *Aspergillus* and *Penicillium* were isolated (Fig. 6a, 6b, 6c, 6d, 6e).

Rhizosphere Mycoflora of Riyadh: *Phytopthora, Mucor, Aspergillus*, *Fusarium* and *Penicillium* were isolated (Fig. 6f, 6g, 6h, 6i).

The above listed mycoflora are common soil saprophytes, parasites and antagonist. *Aspergillus* (12 species + 4 varieties), *Alternaria* (4 species), *Stachybotrys* (3 species + 1 variety), and *Penicillium* (12 species) were of high frequency of occurrence, [13]. Oididendron is an uncommon species isolated during this study. Species of *Oidiodendron* have been isolated worldwide, mostly from soil and decaying plant materials [6]. *Aspergillus* were predominant and represented by four species. *Fusarium* and *Penicillium* were represented by 3 species. While *Auerobasidium*, *Mucor* and *Monascus* were represented by one species each [14].

3.2 Symptoms of the Phytopathognic Genera Isolated during This Study

1. *Phytopthora*: It is responsible for the damage of the roots of the date palm by rotting them which indirecty leads to crown decline.
2. *Cunninghamella*: It causes the black root rot diseases which leads to decay of the roots.
3. *Rhizopus*: The roots show soft rot due to the presence of the fungus on ther surface.
4. *Mucor*: It causes black root rot diseases which causes slow plant growth and effects the quality of the fruit.
5. *Syncephalsatrum*: The surface of the root shows black moldy growth and it damages the roots by causing root rot.
6. *Aspergillus*: It causes brown, black and powdery rots of infected roots. The root surface surface is paler, discoloured and turns brown, black or powdery.
7. *Chaetomium*: It is found in the rhizosphere soil in the present study.
8. *Fusarium*: White fungal mycelia growth on the surface of the infected roots with multitude of symptoms which leads to decline of the tree. It causes rot rot.
9. *Nigrospora*: It is found in the rhizosphere soil in the present study.
10. *Oidiodendron*: It is found in the rhizosphere soil in the present study.
11. *Penicillium*: The infected roots show blue or green moldy growth. The root tissue rots and softens and emits mouldy smell.

Different magnifications were used for different pictures, picture clarity was the main criteria of photomicrographs.

3.3 Statistical Analysis

The rhizoplane and rhizosphere of Jeddah had 4 and 5 isolates respectively whereas the rhizoplane and rhizosphere of Jizan showed 8 and 9 isolates each. Riyadh recorded the maximum isolates from rhizoplane and rhizosphere with 11 isolates each (Fig. 7).

Total of 48 fungal isolates were obtained belonging to 11 genera. Aspergillus was the predominant genera with 11 isolates followed by Rhizopus with 8 isolates (Fig. 8).

The rhizoplane mycoflora was lesser than the rhizosphere mycoflora in Jeddah and Jizan whereas Riyadh recorded equal number of both rhizoplane and rhizosphere mycoflora. Also, fungi were more abundant in the rhizosphere than in the rhizoplane [15].

4a. Photoplate - Rhizoplane of Jeddah | **4b. *Rhizopus*** | **4c. Photoplate – Rhizosphere of Jeddah** | **4d. *Chaetomium* from Rhizosphere of Jeddah**

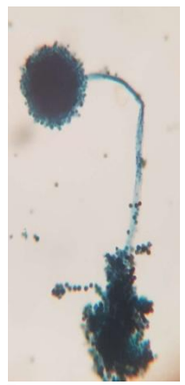
Fig. 4. Photomicrographs of rhizoplane and rhozosphere mycoflora from Jeddah

Table 3. Rhizoplane and rhizosphere mycoflora of Jeddah, Jizan and Riyadh

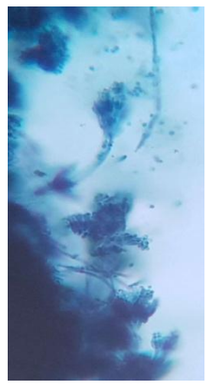
Sl. no.		*Oomycotina*	*Zygomycotina*				*Ascomycotina*					
		Phytopthora	***Cunninghamella***	***Rhizopus***	***Mucor***	***Syncephalastrum***	***Aspergillus***	***Chaetomium***	***Fusarium***	***Nigrospora***	***Oidiodendron***	***Penicillium***
Jeddah	Rhizoplane 1	-	-	+	-	-	-	-	-	-	-	-
	Rhizosphere 1	-	-	+	-	-	-	+	-	-	-	-
Jizan	Rhizoplane 1	-	-	-	-	-	+	-	-	-	-	+
	Rhizosphere 1	+	-	-	-	-	+	-	-	+	+	-
Riyadh	Rhizoplane 1	-	+	+	-	-	+	-	-	-	-	-
	Rhizosphere 1	-	-	-	+	-	+	-	+	-	-	+
Jeddah	Rhizoplane 2	-	-	+	-	-	-	-	-	-	-	-
	Rhizosphere 2	-	-	-	-	-	+	+	-	-	-	-
Jizan	Rhizoplane 2	-	-	-	-	+	+	-	-	-	-	+
	Rhizosphere 2	+	-	-	-	-	-	+	-	+	-	-
Riyadh	Rhizoplane 2	-	-	+	-	-	+	-	+	-	-	-
	Rhizosphere 2	-	-	-	+	-	+	-	+	-	-	-
Jeddah	Rhizoplane 3	-	-	+	-	-	+	-	-	-	-	-
	Rhizosphere 3	-	-	-	-	-	-	+	-	-	-	-
Jizan	Rhizoplane 3	-	-	+	-	+	-	-	-	-	-	+
	Rhizosphere 3	-	-	-	-	-	-	-	-	+	+	-
Riyadh	Rhizoplane 3	+	+	+	-	-	+	-	-	-	-	+
	Rhizosphere 3	+	-	-	+	-	+	-	+	-	-	-
Total Fungal Isolates -48		4	2	8	3	2	11	4	4	3	2	5

5a. Photoplate - Rhizoplane of Jizan

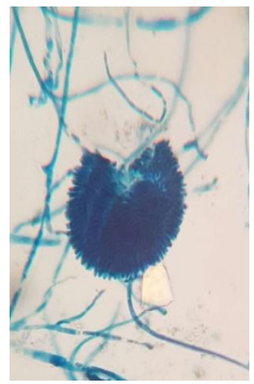
5b. *Aspergillus*



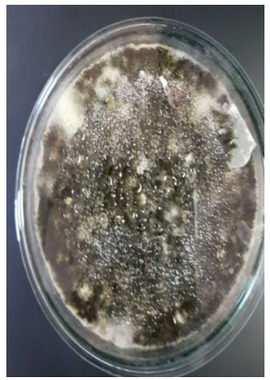
5c. *Aspergillus*



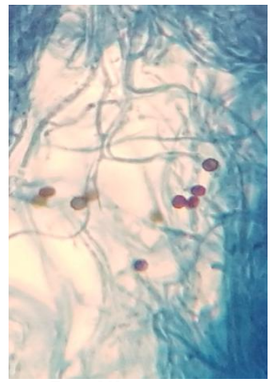
5.d. *Penicillium*



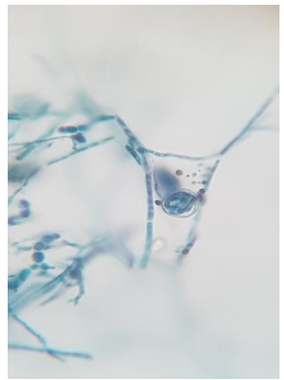
5.e. *Syncephalastrum*



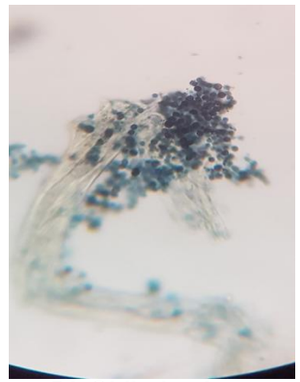
5.f. Photoplate – Rhizosphere of Jizan



5.g. Nigrospora



5.h. Phytopthora



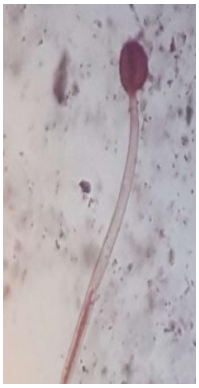
5.i. Oidiodendron

Fig. 5. Photomicrographs of rhizoplane and rhozosphere mycoflora from Jizan

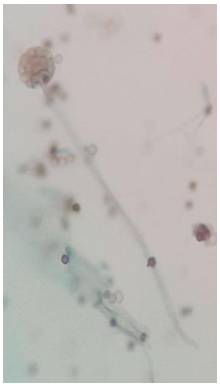
6a. Photoplate - rhizoplane of riyadh

6b. *Aspergillus*

6c. *Aspergillus*

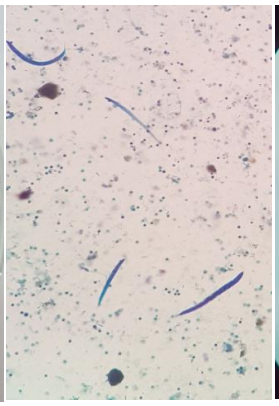


6d. *Rhizopus*



6e. *Cunninghamella*

6.f. Photoplate – Rhizosphere of Riyadh



6.g. *Fusarium*

6.h. *Mucor*

6.i. *Phytopthora*

Fig. 6. Photomicrographs of rhizoplane and rhozosphere mycoflora from Riyadh

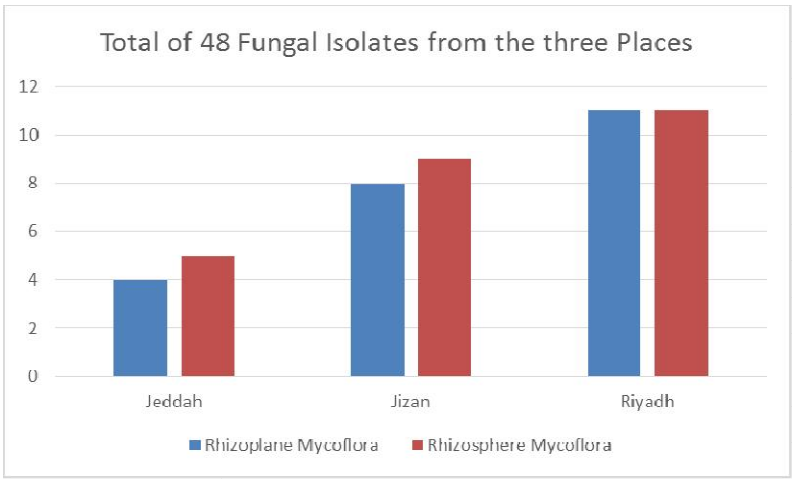


Fig. 7. Total of 48 fungal isolates from the three places

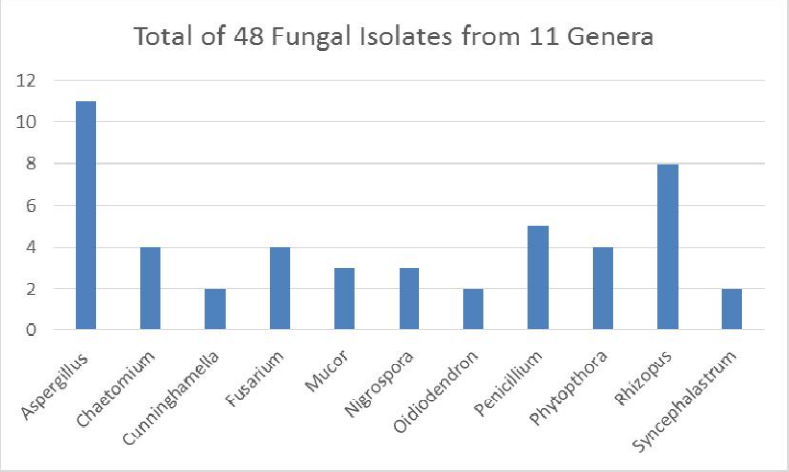


Fig. 8. Total of 48 fungal isolates from 11 Genera

4. CONCLUSION

The present study has isolated rhizosphere and rhizoplane mycoflora in date palm *(Phoenix dactylifera)* which is the most important and commonly grown fruit crop in Saudi Arabia. A total of 48 fungal isolates represented by 11 fungal genera were isolated from eighteen samples under study. The fungal genera isolated are soil borne saprophytic, antagonistic or phytopathogenic to the date palm. This study adds valuable information to the plant pathologists and date growers of the sampling sites so that they can utilize the beneficial effects of the mycoflora and also take necessary phytosanitary measures to control the phytopathgenic genera. Hence this study can add up further information to researchers to carry out further studies on antagonism and other types of interactions between the mycoflora and root exudates in relation to the soil physico - chemical properties. Hence this study will be useful in controlling the phytopathogens of date palm thereby helping in protection and prevention of damage and economic losses .This leads to better yield, good health and resistance in the date crop.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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