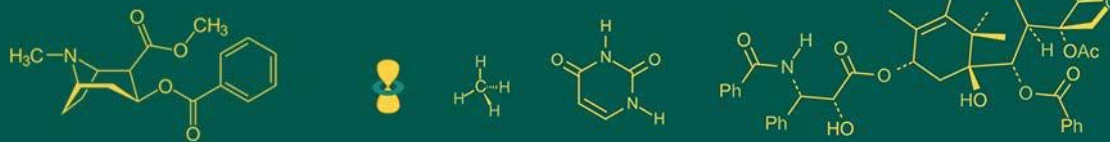


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 IJABR 2025; SP-9(1): 100-106
www.biochemjournal.com
 Received: 14-10-2024
 Accepted: 17-11-2024

Digna A Patel
 Department of Plant
 Pathology, C.P. College of
 Agriculture, Sardarkrushinagar
 Dantiwada Agricultural
 University, Dantiwada,
 Gujarat, India

Tanvi Darji
 Assistant Professor, Swarnim
 College of Agriculture, SSIU,
 Bhoyan Rathod, Gandhinagar,
 Gujarat, India

Krupa Panchal
 Assistant Professor, Swarnim
 College of Agriculture, SSIU,
 Bhoyan Rathod, Gandhinagar,
 Gujarat, India

Tetarwal ML
 Associate Professor,
 Department of Plant
 Pathology, Jodhpur
 Agricultural University,
 Jodhpur, Rajasthan, India

Corresponding Author:
Digna A Patel
 Department of Plant
 Pathology, C.P. College of
 Agriculture, Sardarkrushinagar
 Dantiwada Agricultural
 University, Dantiwada,
 Gujarat, India

Isolation and identification of the different pathogens of date palm diseases

Digna A Patel, Tanvi Darji, Krupa Panchal and Tetarwal ML

DOI: <https://doi.org/10.33545/26174693.2025.v9.i1Sb.3427>

Abstract

So far, it is reported from various countries that several fungi are associated with the decline of the growth and production of date palm. Of them, several pathogenic soil-borne fungi, viz., *Fusarium oxysporum*, *F. solani*, *F. moniliforme*, *Macrophomina phaseolina*; whereas, some fungi like *Thielaviopsis paradoxa* (Black scorch), *Helminthosporium* sp., and *Alternaria* sp. (leaf spots) and *Botrydiplodia theobromae* (Basal rot) are the predominant pathogen associated with date palm foliar diseases. But, unfortunately the aetiology of date palm decline in India is still unknown. To decipher this complex, date palm infected samples were collected from the field of Date palm Research Station, Mundra, Horticulture instructional Farm, and Agroforestry Research Station of Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar; then subjected to the isolation that yielded colonies of *Fusarium* sp., *Macrophomina phaseolina* from the infected root region and *Alternaria* sp. from infected leaf tissues. Purification and identification of the fungal isolates was done based on *in vitro* culture characteristics on Potato Dextrose Agar (PDA) and morphology (micro, macro conidia, and chlamydospores, spore shape and size) under the microscope. Based on cultural characteristics pathogenicity and biological performance of the isolated fungus, the causal agent was identified as *Fusarium* sp., *Alternaria* sp., and *Macrophomina phaseolina* were considered to be cause infection on the leaves and roots of date palm seedlings.

Keywords: Isolation, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Alternaria* sp, date palm

Introduction

The history of date palm cultivation throughout the world dates back approximately to 4000 B.C. or earlier. Date palm (*Phoenix dactylifera* L.) is a monocotyledon, dioecious plant and one of the most cultivated palms around the world (Abass, 2013) [1]. Date palm trees are cultivated in different regions worldwide, especially in Middle East, North Africa, Central and North America, Southern Europe, Pakistan, and India (Zaid, 2002) [23].

Mainly the cultivation of date palm is aimed for its fruits which are highly nutritious and also have many medicinal uses. Date palm fruits are eaten as raw dates (fresh fruits), dry dates (chhuhara) and soft dates (pink khajoor). Apart from the fruits, other parts of the date palm also have traditional as well as economic usage. The flesh of dates contains a high percentage of carbohydrate (44-88%), protein (2.3-5.6%), dietary fibre (6.4-11.5%) and fat percentage reach to (0.2-0.5%), including 14 types of fatty acids (Alkahtani *et al.*, 2011) [4]. In India, climate of Gujarat, Rajasthan and some areas of Punjab is suitable for the production of date palm. The commercial cultivation of date palm occurs mainly in the western border of India and the coastal belt of Kutch area of Gujarat which contributes about ninety per cent of the total date production in India

Several fungi attach date palm causing root rot, wilt and decline diseases (Abdalla *et al.*, 2000) [2]. Several soil borne fungi were recorded as causal pathogens of date palm, viz., *Fusarium oxysporum*; *F. solani*; *F. moniliforme*; *F. semitectum*; *F. equiseti*; *Phoma* sp.; *Chaetomium* sp.; *Alternaria* sp.; *Cladosporium* sp.; *Macrophomina phaseolina*; *Thielaviopsis paradoxa*; *Diplodia phoenicum*; *Phomopsis phoenicola*, and *Rhizoctonia solani* (Abdalla *et al.*, 2000; El- Zawahry *et al.*, 2000; Sarhan, 2001; Suleman, *et al.*, 2001; El-Morsi, 2004) [2, 13, 20, 22, 12].

The most common disease of date palm was Bayoud disease caused by *Fusarium oxysporum* f.sp. *albedinis*. This disease is the most serious especially in Morocco and Algeria (Elarosi, 1989; El-Hassni *et al.*, 2005) [8, 10].

Fusarium moniliforme and *F. solani* were found associated with declined date palm trees in Egypt. The dominant fungi associated with date palm foliar diseases are: *Helminthosporium* sp. and *Alternaria* sp. (leaf spots) and *Botrydiploia theobromae* (Basal rot), (Al-Sharidy and Molan, 2008) [5].

Materials and methods

The investigation entitled "Identification of diseases in date palm (*Phoenix dactylifera* L.)." was carried out in the Department of Plant Pathology, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar during 2020-21. The materials used and methodology followed for the isolation of pathogen, their pathogenicity, morphological and cultural characteristics, under *in vitro* condition are described as under.

Isolation and identification of the different pathogens of date palm diseases

Collection of diseased samples, isolation and purification of pathogen.

The diseased samples of date palm showing typical symptoms were collected from Date palm Research Station, Mundra, Agroforestry Research Station and Horticulture Instructional Farm in S. D. Agricultural University, Sardarkrushinagar. The infected samples were brought to the laboratory for further studies.

The pathogens were isolated from diseased specimens using the "tissue isolation technique". The typically infected leaves and root portion were washed in running tap water and then air dried. The diseased part along with some healthy portion were cut into small pieces with the help of sterilized razor and again washed with sterilized water. These pieces were kept for one minute in one per cent sodium hypochloride solution for disinfection. These pieces were washed thrice in sterilized distilled water to remove residues of sodium hypochlorite. The pieces were transferred aseptically onto sterilized Potato Dextrose Agar (PDA) medium in Petri-plates. The Petri-plates were incubated at $27 \pm 20^\circ\text{C}$ temperature for seven days in BOD incubator.

Purification of spore forming fungal culture was done by using single spore suspension method. In this method, water agar (2%) was poured into Petri-plates of 90 mm diameter and allowed to solidify. The spore suspension was prepared by diluting 5 mm sized mycelial disc into 5 ml sterilised distilled water. 5 μl of spore suspension was transferred onto the water agar using a micro pipette. Spore suspension was spread uniformly over the water agar by streaking with L-shaped inoculating needle/spreader. The plate was incubated at $27 \pm 1^\circ\text{C}$ for 2 to 3 days. Germinating conidia on the culture plate were located using low power microscope. A portion of the media with an isolated germinating conidium was excised from the water agar medium using a sterilized needle and was transferred to PDA medium for incubation at $27 \pm 1^\circ\text{C}$ temperatures (Niemeyer and Andrade, 2015) [17]. Purification of non-spore forming fungal culture was done by using hyphal tip method. Culture of the test pathogen was grown on water agar (2%). Medium was poured in less quantity to the 90 mm diameter Petri-plate so as to limit the growth of the test pathogen. The individual hyphal tip from the margin of sparsely grown culture was excised using a sterilised needle and transferred to Petri-plates containing

PDA medium (Leslie and Summerell, 2006) [15]. The plates were incubated at $27 \pm 1^\circ\text{C}$ temperatures for further growth.

Identification

Identification of pathogens grown on PDA medium were examined visually as well as microscopically for cultural and morphology characters. The cultural characters were recorded right from initiation of growth up to 15 days. The morphological characters viz., length and width of spores, mycelium etc, were measured under high power magnification from 10 days old culture of pathogens and were compared with those given in the literature or manual. The photomicrographs of the same were also be taken. The identification was based on taxonomic identification keys.

To prove the pathogenicity

Root dip inoculation method of root infecting pathogen

Date palm seedlings grown in autoclaved sand filled in pots were uprooted, and washed in running tap water and clipped from the distal one-third root system. These seedlings were dipped for 10 minutes in the spore suspension (1×10^6 spores/ml). Inoculated seedlings were then transplanted into earthen pots filled with autoclaved soil. The seedlings treated without inoculation were kept as control. The test was conducted in three replicates.

Foliar spraying of spore suspension of leaf infecting pathogen

The pathogenicity of the foliar microbes was tested on the leaves of date palm seedlings grown in the earthen pots (30 cm diameter) filled with sterilized soil and FYM in the ratio of 3:1. Date palm seedling were grown in earthen pots, covered with plastic to avoid any air-borne infection. The pots were labelled, watered gently and arranged in line. The pots were watered up to saturation in the morning. The leaves were surface sterilized with 1 per cent sodium hypochlorite (NaOCl) solution and washed thoroughly with sterilized distilled water to remove the traces. Three months old date palm seedlings were used for inoculation. The spore suspension of the microbes were sprayed on the leaves in the evening with newly fresh inoculum. The control pots without inoculation were sprayed with sterilized distilled water. The inoculated and uninoculated pots were covered with polyethylene bags for 48 hour to provide high humidity. The observations on the disease development on leaves were recorded periodically from the initiation of the disease. Re-isolation were made from the artificially inoculated plants showing typical leaf spot symptoms by tissue isolation method and the identity of the fungus were confirmed as per the original description. The culture obtained by re-isolation was transferred on PDA slants for comparison with the original culture and further investigations.

Morphological and cultural studies of isolated pathogen from date palm

All the isolates established and maintained on PDA were studied for their cultural and morphological characters. In the study of solid medium, the discs were inoculated in the centre of 90 mm Petri-plate containing 20 ml of PDA medium. Observations on fungal radial growth, colony character, sporulation and pigmentation were recorded after 7 days of incubation. The radial growth was determined by measuring the colony diameter in mm along the two

diagonals passing through the centre of colony and the average were worked out. For recording sporulation in each isolate, the spore suspension was prepared by randomly cutting five uniform discs (4 mm) of fungal colony, raised on solid media, putting it and shaking it well in 5 ml of water in 50 ml flasks. The droplet of the spore suspension was placed on a clean glass slide and spores were checked at low power (10x) field of microscope. The sporulation were counted with the help of haemocytometer, whereas length and breadth of conidia were measured by ocular micrometre.

Results and Discussion

Isolation and identification of the different pathogens of date palm diseases

The diseased samples of date palm showing typical symptoms were collected from Date palm Research Station, Mundra, Agroforestry Research Station, and Horticulture Instruction farm in Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The infected samples were brought to the laboratory for further studies. In order to analyse, the occurrence of pathogen in the host, tissues from each part (leaves and roots) of the diseased samples were subjected to isolation on PDA. The results revealed that samples yielded the isolate of *Fusarium* sp. and *Macrophomina phaseolina* from root portion and *Alternaria* sp. from leaves.



Infected roots of date palm



Infected leaf

Colonies of fungus emanated from the tissues were subjected for isolation on PDA medium. Growing hyphal tips from the margin of these white colonies were transferred to fresh PDA medium and incubated at $27 \pm 1^\circ\text{C}$. The culture so obtained was purified using different techniques and used for further investigation.

Results obtained further suggested that *Fusarium* sp. and *Macrophomina phaseolina* caused root rots diseases and *Alternaria* sp. caused leaf spot diseases on date palm. Ahmed (2018) [3] mentioned that some soilborne fungal diseases viz., *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina* affect the roots of the

date palm. Elhassan (2016) [9] isolated *Fusarium solani* causing the wilt disease in date palm trees. However, Baraka *et al.* (2011) [7] isolated *Fusarium* sp. most frequently from the root rot on date palm. Leaf spot diseases are the most widely spread foliar disease in date palm. Similarly, Ammar and El-Naggar *et al.* (2011) + isolated leaf spot causing *Alternaria alternata* from the leaves of date palm.

Identification of pathogen

The purified culture of the fungal isolates responsible for the wilt, root rot and leaf spot pathogen on date palm seedlings were examined for initial identification of the fungus. Based on the growth pattern in Petri-plates as well as mycelial and conidial structures under a microscope, the fungal culture was identified. The identification of the fungus isolate was made based on *in vitro* culture characteristics on Potato Dextrose Agar (PDA) and morphological features (micro, macro conidia and chlamydiospores shape and size) under the microscope (Ellis, 1971) [11]. Based on cultural characteristics, pathogenicity and biological performance of the isolated fungus, the causal agent was identified as *Fusarium* sp. (Lesile and Summerell, 2006) [15] constituting by that the first report of *Fusarium* wilt disease on date palm in the Sudan (Elhassan *et al.*, 2016) [9].

The isolates were classified based on their morphological characters on PDA plates. *Alternaria* isolates grew rapidly and produced colonies with different shapes. The colony was flat and was covered with grayish, short, aerial hypha. Its surface was grayish-white at the beginning which later darkens and becomes greenish-black or olive-brown with a light border. The reverse side was typically brown to black due to pigment production. Hyphae were septate and brown. Conidia and proconidia were appeared brown with an elongated beak-like apical cell, often in chains and sometimes solitary (Badawy *et al.*, 2019) [24].

Based on this information, the fungal cultures were identified *Fusarium* sp., *Macrophomina phaseolina* and *Alternaria* sp. and was considered to be cause infection on the root and leaves of date palm seedlings.

To prove the pathogenicity

Pathogenicity test was attempted by different inoculation method (root dip inoculation method and spore suspension method) for different pathogens on healthy date palm seedlings grown in pots. Plants were incubated in the mist house, where relative humidity (80%) and optimum temperature ($27 \pm 2^\circ\text{C}$) were maintained for the further development of symptoms.

Root dip inoculation method of root infecting pathogen

Root dip inoculation method of root infecting pathogens were performed to confirm the pathogenicity of the test pathogens. The seedlings grown in autoclaved sand filled in pots were uprooted, and washed in running tap water, and clipped from the distal one-third root system. These seedlings were dipped for 1 hr in the spore suspension (1×10^6 spores/ml). Inoculated seedlings were then transplanted into earthen pots filled with autoclaved soil. The seedlings without inoculation were kept as control. In *Fusarium* sp. and *Macrophomina phaseolina* proved pathogenicity by this method. Symptoms on seedlings infected with *Fusarium* sp. was observed on the leaves of the inoculated seedlings of the date palm resulted in drying and general death of the seedling. In *Macrophomina phaseolina* mortality of plant was observed.



Inoculated Control

Pathogenicity of *Fusarium* sp.

Inoculated

Control

Pathogenicity of *Alternaria* sp.

A

B

C

Noculated

Control

Pathogenicity of *Macrophomona phaseolina*

in the early study, Elhassan (2016) [9] tested pathogenicity against *Fusarium oxysporium* caused wilt disease on date palm. Two months old plants were inoculated with 2 ml of inoculums suspension, the early symptoms observed on the leaves of the inoculated seedlings of the date palm appeared as brown spots, which later resulted in drying and general death of the seedling. Similarly, Purwati *et al.* (2008) [19] compared different inoculation methods of *Fusarium oxysporium* f. sp. cubense (Foc) in Abaca. In the previous literature Sukanya *et al.* (2016) [21] tested seedling roots were dipped for 2 min in *M. phaseolina* spore suspension of 10^5 spores per ml and these seedlings were kept for germination. The early mortality of the plant was observed

Foliar spraying of spore suspension of leaf infecting pathogen

Pathogenicity test was attempted by spraying the spores on healthy three month-old date palm seedlings grown in pots. Plants were incubated in the mist house, where relative humidity (80%) and optimum temperature ($27 \pm 20^\circ\text{C}$) were maintained for the further development of symptoms. Results revealed that *Alternaria* sp. was pathogenic to date palm seedling causing Alternaria leaf spot. After 30 days of incubation, typical symptoms of Alternaria leaf spot on the foliage of artificially inoculated date palm seedling were observed. However, the control plant sprayed with only sterilized distilled water remained healthy and did not produce any kind of symptoms throughout the observation. The fungus was re-isolated from the inoculated leaves and was compared with the original culture of the test pathogen. The same was found identical to that of the original culture, thereby confirming the pathogenicity.

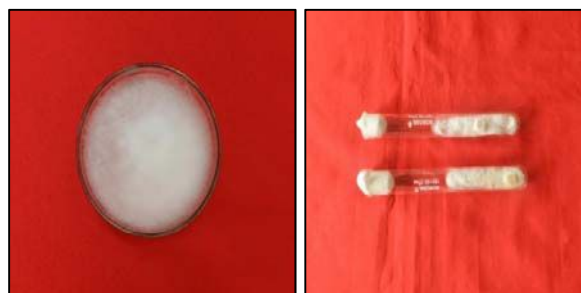
In early study, Badawy *et al.* (2019) [24] tested the pathogenicity of foliar pathogens on date palm seedling. The isolated fungus was inoculated via spraying spore suspension results showed that the pathogen is capable of making infection. This can be seen via the increasing length of lesions. Black coloured lesions were noticed in all inoculated plants. Moreover, black spots were noticed on leaves long distance from the inoculated area on leaves' both sides.

Cultural and morphological study of the isolated pathogen from date palm

The cultural and morphological characters of the isolated pathogens from date palms were studied by growing them on potato dextrose agar (PDA) medium at $27 \pm 2^\circ\text{C}$ after 7 days incubation. Macroscopic growth features viz., colony diameter (mm), cultural characteristics, and microscopic growth features, viz., size of spores and septation in spore, micro and macroconidia and chlamydospores on potato dextrose agar medium in Petri-plates were recorded after 7 days of incubation.

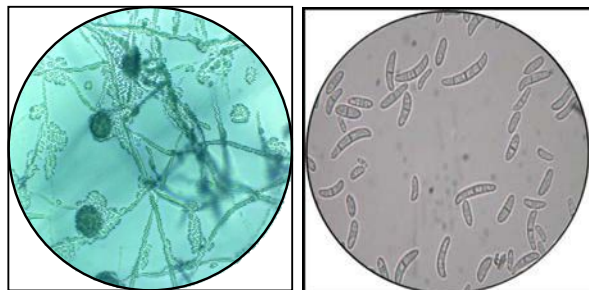
Cultural and morphological study of *Fusarium* sp.

Cultural characters of the isolates were identified as white fluffy colonies which gradually turned orange after ten days of incubation. Orange pigmentation was visible at the bottom side of the Petri-plate within seven days of incubation as well as in the culture incubated at 4°C . The radial growth of mycelium attained an average size of 90 mm with a circular margin in seven days of incubation. The culture produced aerial mycelium without any undulations.

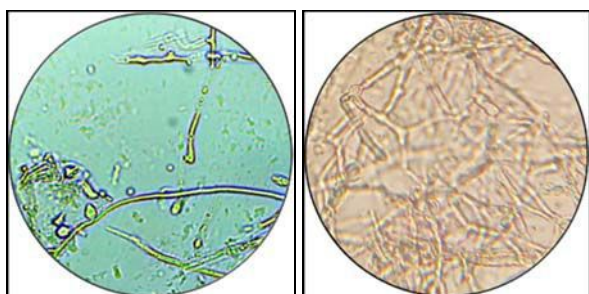


Microscopic characteristics showed the formation of a fusiform, slightly curved macroconidia, with 3-4 septate. The microconidia were ellipsoidal to cylindrical, abundant, without septate and formed in chain. Microconidia were the most abundant structure in the culture media. All isolates

also formed intercalary or terminal chlamydospores on hyphae, which had a thickened cell wall. Microconidia ranged in size from 9.50 to 11.50 μm , macroconidia varied from 18.7 to 48.9 μm and chlamydospores had a diameter from 7.5 to 8.4 μm . The fungal macroscopic characteristics and variations observed in the size and shape of the microconidia, macroconidia and chlamydospores suggested that the isolates collected may have corresponded to *Fusarium* sp.



Micro-Macro conidia



Chlamydospore

Hypha

Cultural and morphological characters of *Macrophomina phaseolina*

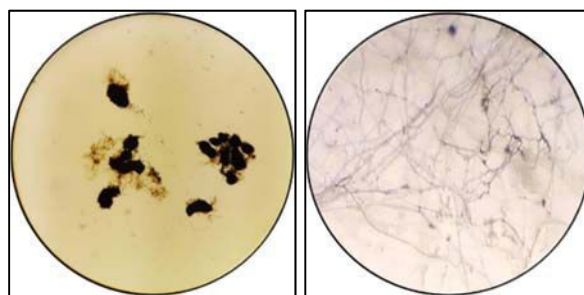
Initially, the mycelium of the fungus was white in colour and gradually turned greyish black to black in colour in the fungal culture.

The isolated fungus grew fast on PDA and attained a diameter of 90 mm within 10 days at 30 °C temperature with profuse mycelial growth. Initially fluffy white mycelia were produced by the fungus on PDA. The mycelium was grey, hyaline, branched, septate and width ranging from 1.22 to 6.60 μm . The average width of mycelium recorded in the present study was 3.80 μm . Previously, identification of *Macrophomina phaseolina* was done on the basis of cultural characteristics and morphological characters (Lakhran *et al.*, 2018) [14].

The fungus produced profuse white aerial mycelium later turns into brown to black on PDA. Black hard sclerotia were formed after 10-15 days at the periphery of the colony. The young hyphae of the fungus were observed to be hyaline, thin-walled light brown to dark brown in colour, and branches arised from the main hyphae at a right angle to parent hyphae with constriction of the point of origin. The colour of the sclerotia was light brown at the beginning which became darker with age and finally brown to black, sclerotia varied in shape irregular or spherical or oval or oblong, measuring from 90-120 $\times$ 60-150 μm with an average of 82.5 to 105 μm in size.

But in the present study sclerotia on the fungal culture was not observed. The young hyphae of the fungus were

observed to be hyaline, thin-walled light brown to dark brown, and branches arose from the main hyphae at a right angle to parent hyphae with constriction of the point of origin. Based on the cultural and morphological character, this fungus had been identified as *Macrophomina phaseolina*.

Cultural characters of *M. phaseolina*Morphological characters of *M. phaseolina*

Cultural and morphological study of *Alternaria* sp.

After seven days of incubation, the colony diameter of *Alternaria* sp. was recorded as 85.00 mm. The growth of mycelia was slightly fluffy, olive greenish in colour with raised margins in a uniform concentric fashion.

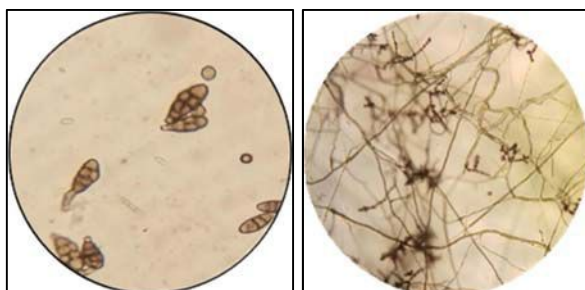
The microscopic study of *Alternaria* sp. revealed that the colony was flat and was covered with grayish, short, aerial hypha. Its surface was grayish-white at the beginning which later darkens and becomes greenish-black or olive-brown with a light border. The hyphae were septate and irregularly branched. The average length and width of the spores were 30.39 and 12.18 μm , respectively. Likewise, the average beak length was 3.97 μm with 4-5 transverse septa and 1-2 longitudinal septa. Thus, it is confirmed that *Alternaria* sp. was responsible for the leaf spot on date palm seedlings.

Descriptive information on *Alternaria* sp. was presented by Badawy *et al.* (2019) [24] who found similar results on cultural characteristics of *A. alternata* as were recorded in the present study. They recorded that the colony was flat, and was covered with grayish, short, aerial hypha. Its surface was grayish-white at the beginning which later darkens and becomes greenish-black or olive-brown with a light border. The reverse side was typically brown to black due to pigment production. Hypha septate were brown. Conidia and proconidia appeared brown with an elongated beak-like apical cell, often in chains and sometimes solitary. A similar study by Mirhosseini *et al.* (2015) [16] cultures gave rise to initially white colonies which turned to grayish-black later due to abundant sporulation. Conidia were produced in long chains, pale to light brown, with a beak one to seven transverse and up to three longitudinal septa, and measured 10 to 45 μm long $\times$ 7 to 22.5 μm wide. Thus, the results obtained in the present investigation corroborate the findings of the earlier research workers. However,

Prathima *et al.* (2018) ^[18] observed brownish mycelia, conidiophores were septate, olive-brown, branched. Conidia measured about 96.22 - 49.59 µm in length and 26.53-15.90 µm in-width, they also produced beak and beakless conidia, produced in chain.



Cultural characters of *Alternaria* sp.



Morphological characters of *Alternaria* sp.

Conclusions

Samples of date palm yielded the colony of different fungi. Of them, *Fusarium* sp. and *Macrophomina phaseolina* was isolated from the infected root, whereas, *Alternaria* sp. was isolated from leaf spot. The identification of these fungi was made based on cultural and morphological characteristics that are compared with standard taxonomic identification keys of *Fusarium* sp., *Alternaria* sp. and *Macrophomina phaseolina*. Based on the characters, the fungal cultures were identified as *Fusarium* sp., *Macrophomina phaseolina* and *Alternaria* sp.

References

1. Abass MH. Microbial contaminants of date palm (*Phoenix dactylifera* L.) in Iraqi tissue culture laboratories. *Emirate Journal of Food and Agriculture Science*. 2013;25(11):875-882.
2. Abdalla MY, Al-Rokibah A, Moretti A, Mulè G. Pathogenicity of toxigenic *Fusarium proliferatum* from date palm in Saudi Arabia. *Plant Diseases*. 2000;84:321-324.
3. Ahmed MFA. Management of date palm root rot diseases by using some biological control agents under organic farming system. *Novel Research in Microbiology Journal*. 2018;2(2):37-47.
4. Alkahtani M, El-Naggar MA, Omer SA, Eman M, Abedl-Kareem, Ammar MF. Effect of toxic *Fusarium moniliforme* on some biochemical components of some date palm cultivars. *American Journal of Food Technology*. 2011;6:730-741.
5. Al-Sharidy A, Molan Y. Survey of fungi associated with black scorch and leaf spots of date palm in Riyadh Area. *Saudi Journal of Biological Science*. 2008;15:113-118.
6. Ammar MI, El-Naggar MA. Date palm (*Phoenix dactylifera* L.) fungal diseases in Najran, Saudi Arabia. *International Journal of Plant Pathology*. 2011;2:126-135.
7. Baraka MA, Radwan FM, Shaban WI, Arafat KH. Efficiency of some plant extracts, natural oils, biofungicides and fungicides against root rot disease of date palm. *Egypt Journal of Biological Chemistry Environmental Science*. 2011;6(2):405-429.
8. Elarosi H. Studies on plant diseases affecting date palm trees at the Eastern Province of Saudi Arabia. *King Abdul Aziz City Science and Technology*. 1989;26:79-109.
9. Elhassan AM. Isolation, identification and characterization of *Fusarium oxysporum*, the causal agent of Fusarium wilt disease of date palm *Phoenix dactylifera* L. in Northern State, Sudan. *International Journal of Current Microbiology and Applied Sciences*. 2016;5(8):381-386.
10. El-Hassni M, Verdeil JL, El-Hadrami I. A hypoaggressive *Fusarium oxysporum* isolate induces polyphenol oxidase activity in the date palm seedlings allowing their protection against the bayoud disease. *Plant Pathology Journal*. 2005;4:96-102.
11. Ellis MB. *Dematiaceous hyphomycetes*. Kew, England: Commonwealth Mycological Institute; 1971.
12. El-Morsi MA. Studies on certain fungal diseases of date palm offshoots in New Valley, Egypt [PhD Thesis]. Assiut University; 2004. p. 110.
13. El-Zawahry A, El-Morsi MA, Abd-Elrazik AA. Occurrence of fungal diseases on date palm trees and off-shoots in New Valley governorate and their biological control. *Indian Journal of Agriculture Science*. 2000;31(3):189-212.
14. Lakhra L, Ahir RR, Choudhary M, Choudhary S. Isolation, purification, identification and pathogenicity of *Macrophomina phaseolina* (Tassi) goid causing dry root rot of chickpea. *Journal of Pharmacognosy and Phytochemistry*. 2018;7(3):3314-3317.
15. Leslie JF, Sumerell BA. *Fusarium Laboratory Manual*. Manhattan: Blackwell Publishing; 2006. p. 388.
16. Mirhosseini HA, Babaeizad V, Basavand E. Identification and detection of agent of loquat leaf spot and fruit rot in north of Iran. *Journal on New Biological Reports*. 2015;4:134-137.
17. Niemeyer JL, Andrade M de. A basic procedure for single spore isolation of *Fusarium verticillioides* and *Fusarium subglutinans*. *International Journal of Environmental Biology Research*. 2015;3(1):145-148.
18. Prathima P, Thiruvudainambi S, Kalpana K. Molecular characterization of *Alternaria alternata* (Fr.) Keissler causing leaf blight disease of marigold. *International Journal of Pure and Applied Bioscience*. 2018;6(6):1286-1291.
19. Purwati RD, Hidayah N, Sudjindro S. Inoculation methods and conidial densities of *Fusarium oxysporum* f.sp. cubense in Abaca. *Hayati Journal of Biosciences*. 2008;15(1):1-7.
20. Sarhan ART. A study on the fungi causing decline of date palm trees in middle of Iraq. *United Arab Emirates*. 2001;424-430.

21. Sukanya R, Jayalakshmi SK. Response of inoculation technique to seed and seedling infection by *Macrophomina phaseolina* in sorghum. International Journal of Environment, Agriculture and Biotechnology. 2016;1(3):2456-1878.
22. Suleman P, Al-Musallam A, Menezes CA. The effect of solute potential and water stress on black scorch caused by *Chalara paradoxa* and *Chalara radiculicola* on date palms. Plant Diseases. 2001;85:80-83.
23. Zaid A, editor. *Date Palm Cultivation*. Rome: FAO; 2002.
24. Badawy ME, Marei GI, Rabea EI, Taktak NE. Antimicrobial and antioxidant activities of hydrocarbon and oxygenated monoterpenes against some foodborne pathogens through *in vitro* and *in silico* studies. Pesticide biochemistry and physiology. 2019 Jul 1;158:185-200.