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Thème

Taxonomy and pathogenicity of fungal strains isolated from date palm in the region of Ghardaia

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Dedication

I dedicate this work to those who mean the most to me in my life,

those who surround me with love and tenderness,

those who light my way with their kindness and joy in my life,

my **mother** *and* **father**

my brothers **Amine** *and* **Omar Abd Elkafi**

and sisters **Nassiba, Khadidja, Mokhtaria, Nacira**

for their constant encouragement, attention and words of encouragement.

Imane

إهداء

الحمدلله حباً وشكرًا وامتنانا على البدء و الختام

(و آخرُ دَعْواهُمْ أن الحَمْدُللهِ رَبِّ الْعَالَمِينَ)

وبِكل حُب أهدي ثَمرَة نَجاحي و تَخُرجي

إلى من جَرع الكأس فَارغًا ليسقياني قَطرة حُب إلى من حَصدوا الأَشواكَ عن دَربي ليمهدا لي طريق العلم

إلى رُوح أبي و أمي

مهما قلت و مهما فعلت لا أستطيع أن أُعبر عن إمتناني اللامتناهي لما فَعلوه من أجَل نَجاحي عسى أن يكون هذا العمل المتواضع تَحقيقا لأَمانيهم و ثَمرةً لتضحياتهم تَغمدَ الله روحكمَا الطاهِرة بالرحمة و المغفرة

إلى من ساندتني بكل حب عند ضعفي و أزاحت عن طريقي المتاعب ممهدةً لي الطريق زارعًا الثقة و الإصرارَ بِداخلي

إلى زوجةُ أبي الغالية

إلى ملائِكة رزقَني الله بِهن لأَعرف من خِلالهن طُعم الحياة الجميلة ومَفاهيم الحُب والصَداقة

إلى أخواتي

وأخيرًا من قال أنا لها "نالها " وأنا لها إن أبت رغماً عنها أُتيت بها، ماكنت لأفعل لولا تَوفيق من الله. هاهو اليوم العظيم هنا اليوم الذي أُجريت سَنوات دِراسَتي الشَاقة حالمةً بها حتى تَوالت بِمنه و كَرمِه لفرحَة التمام , فالحمدلله الذي ماتيقنتُ به خيرًا و أملاً إلا و أغرقني سُرورًا و فَرحًا يُنسيني مَشقتي

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LIST OF ABBREVIATIONS

ANOVA: analysis of variance .

BC: Before Christ.

BLAST: Basic local alignment search tool.

C°: degree Celsius.

DNA: Deoxyribonucleic acid.

DPM: Date palm mite.

FAO: Food and Agriculture Organization of the United Nations.

FAOSTAT: Food and Agriculture Organization Statistics.

FOA: *Fusarium oxysporum albedinis.*

g: grams.

GAPDH: glyceraldehyde 3-phosphate dehydrogenase .

gDNA: Genomic deoxyribonucleic acid

Ha: Hectare.

ITS: Internal Transcribed Spacer.

LSD: Least Significant Difference .

LSU: the Large Subunit.

m^2: square meter.

MAFFT: Multiple Alignment using Fast Fourier Transform.

ML: Maximum Likelihood.

mm: millimeter.

MP: Maximum parsimony.

mRNA: messenger ribonucleic acid.

NNI: Nearest-Neighbour-Interchange.

PDA: Potato Dextrose Agar**.**

PCR: Polymerase Chain Reaction.

Qx: quintal.

rDNA: ribosomal DNA.

RNA: ribonucleic acid.

SSU: small subunit.

TBR: Tree-Bisection-Regrafting.

TEF: Elongation factor gene.

UAE: United Arab Emirates.

USA: United States of America.

RESUME

Le présent travail a deux objectifs majeurs, d'abord l'identification et la taxonomie de plusieurs souches fongiques isolées à partir de palmiers dattiers, présentant divers symptômes de pathologie dans la région de Ghardaïa ; ensuite l'évaluation de leur pouvoir pathogène sur les plantules de palmiers dattiers. Pour cela, seize (16) isolats fongiques ont été soumis d'abord à une identification morphologique (macroscopique et microscopique), puis à une analyse moléculaire via le séquençage de l'ADN de la région ITS. Une évaluation de la pathogénicité a été réalisée sur des plantules de palmier dattier âgées de deux à quatre mois.

Les résultats révèlent que l'immense majorité des isolats appartiennent au genre *Alternaria*, présenté par 16 isolats groupés en 04 espèces à savoir *A. alternata, A. cumini, A. infectoria* et *A. malorum*.

Des taux variables de pathogénicité ont été exposés par nos isolats sur les jeunes plantules de palmier dattier, dont *A. alternata* étant le plus pathogène et *A. malorum* est le moins pathogène.

Mots-clés : palmiers dattiers, Ghardaïa, souches fongiques, identification, *Alternaria*

ABSTRACT

The current research has two main objectives: firstly, the identification and taxonomy of several fungal strains isolated from date palms causing different pathological symptoms in the Ghardaia region; secondly, the evaluation of their pathogenicity on date palm seedlings. To this end, sixteen (16) fungal isolates were subjected first to morphological identification (macroscopic and microscopic) and then to molecular analysis by DNA sequencing of the ITS region. Pathogenicity was assessed on date palm seedlings two to four months old.

The results showed that most of the isolates belonged to the genus *Alternaria*, represented by sixteen isolates grouped in four species, namely *A. alternata, A. cumini, A. infectoria* and *A. malorum*.

Different ranges of pathogenic ability were shown by our isolates on date palm seedlings, with *A. alternata* being the most pathogenic and *A. malorum* the least pathogenic.

Keywords: date palm, Ghardaia, fungal strains, identification, *Alternaria*

ملخص

يهدف البحث الحالي إلى تحقيق هدفين رئيسيين؛ أولهما تحديد وتصنيف بعض السلالات الفطرية المعزولة من أشجار النخيل في منطقة غرداية، والمرتبطة بأعراض مرضية متنوعة؛ ثم تقييم قدرتها الإمراضية على شتلات النخيل. من أجل ذلك، تم إخضاع ستة عشر (16) عزلة فطرية أولاً للتحديد المورفولوجي (المجهري واللا مجهري)، ثم للدراسة الجزيئية عن طريق تسلسل الحمض النووي لمنطقة ITS. تم إجراء تقييم للقدرة الإمراضية على شتلات النخيل التي يتراوح عمرها من شهرين إلى أربعة أشهر .

كشفت النتائج أن معظم العزلات تنتمي إلى نوع *Alternaria* ممثلة ب 16 عزلة تنتمي إلى أربعة أجناس وهي *A. malorum* , *A. alternata, A. cumini, A. infectoria* *معدلات متفاوتة من القدرة الإمراضية تم إظهارها من طرف العزلات على شتلات النخيل حيث وجد أن الجنس A. alternata هو الأكثر إمراضا في حين أن الجنس A. malorum هو الأقل إمراضا.*

الكلمات المفتاحية : أشجار النخيل، غرداية، السلالات الفطرية، تحديد وتصنيف، *Alternaria*

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Part Ⅱ:Experimental section

Introduction

Date palm (*Phoenix dactylifera* L.), is the symbol of Saharian agriculture, cultivated for millennia in the Middle East and Africa (Zohary *et al.*, 2012). This fruit tree has a vital influence on the history of the Middle East. Without dates, no large human population would have been able to survive the desert conditions of this region. (Gros-Balthazard, 2012). *Phoenix dactylifera*, (2n = 36) is dioecious, from the family *Arecaceae* (Palmae) monocotyledonous, perennial fruit tree (Aly *et al.*, 2022) adapted to arid environments. It has unique biological and developmental characteristics. (Chao and Krueger, 2007).

In the Algerian Sahara, the date palm is the mainstay of oasis ecosystems, where it helps to limit damages caused by silting and protects underlying crops (fruit trees, vegetables and cereals) from intense sunlight. It plays a significant socio-economic role for the populations of these regions, providing products (dates) and sub-products of high nutritional and economic value. In 2022, 9.91 million tonnes of dates were produced worldwide. Algeria is the 3rd date's producer country with 1,25 million tonnes. The region of Ghardaia produces nearly 495 00 tonnes per year of dates and supports nearly 1012 290 productive palm trees (Bouguedoura *et al.*, 2010). However, date palm is subjected to various pathogens, such as insects, bacteria and fungi threatening its productivity and longevity. In fact, fungi are the most dreadful threat of date palm, where some of them were clearly studied and confirmed as pathogens (*Fusarium oxysporum* f. sp. *albidenis*, *Diplodia* sp., *Phytophthora* sp., etc.); while other fungal groups need more detailed studies for their identification and their pathogenic ability on date palm; as well as to establish the link between each group and the symptoms observed on the tree (yellowing, browning and drop of leaves). In this concept, our study comes to clarify the situation regarding the identification and the pathogenicity of fungal groups on date palm.

Our work is conducted based on three main steps:

- Macroscopic and microscopic identification of fungal isolates.
- Molecular analysis by DNA sequencing (ITS region).
- Evaluation of the pathogenicity of isolates on palm seedlings.

Part 1: Bibliographic review

Chapter 1: General information on date palm

1. Presentation

One of the earliest fruit crops to be produced in the dry regions of the Arabian Peninsula, North Africa, and the Middle East is the date palm (*Phoenix dactylifera* L.). The date palm most likely originated in or close to what is now Iraq, although dating back to ancient times, date cultivation was practiced in numerous countries (Chao and Krueger, 2007).

The date palm is a thermophilic and heliophilic plant that prefers a warm, bright environment. It needs high temperatures for growth and production, but it can tolerate cold temperatures as well. Its overall water requirements are estimated to be 183.95 m^3/palm tree/year; it prefers sandy soils with minimal clay content and requires humidity for fruiting (Munier, 1973).

The date palm is a dioecious plant that may grow up to 20 meters tall. If it survives in ideal conditions and is not parasitized by diseases, it can produce dates for as long as 200 years (Toutain, 1996).

2. Historical background

The Miocene is when the first palm trees originated. About 4500 years BC, the date palm was cultivated in the hot regions between the Euphrates and the Nile. Around 2500 BC, its cultivation was brought to Lower Mesopotamia from there. From there, it moved northward, reaching the coastal region of the Iranian plateau and eventually the Indian valley (Munier, 1973). Following Egypt, date palm farming methods made their way to Libya, from which they initially made their way to other Maghreb nations including Tunisia, Algeria, and southern Morocco before arriving to Adrar and Mauritania. In the Northern Hemisphere, date palm agriculture is currently growing, especially in hot, dry, and semi-arid areas (Fernandez *et al.*, 1995).

In 1734, Linné gave the date palm the name *Phoenix dactylifera*. The name of date palm, which the ancient Greeks referred to as the Phoenician tree, is *phoenix*, from whence the term "phoenix" originated (Linné, 1734; Munier, 1973). Zaid & Arias-Jiménez (1999) as saying that date palm is said to have coined the word "*phoenix*" due to its capacity to reappear after being burned, similar to the mythical bird rising from its ashes. Dactylifera is a word that refers to the finger (*dactylus* in Latin, from the Greek *daktulos*) because of the fruit's form.

According to Zaid & Arias-Jiménez (1999), citing Popenoe (1938), the word "*dactylifera*" may have originated from the Hebrew word "*dachel*," which describes the fruit's form.

As shown by the numerous depictions of date palm in iconography, as well as by the creation of man-made palm-shaped islands in Dubai, date palm has been highly considered in North Africa and the Middle East since antiquity (Gros-Balthazard, 2012). It is a sign of fertility and is hence qualified as a tree of life. It is mentioned in several aphorisms and stories, as well as on banknotes and stamps. The Koran, the Bible, and the Torah all make reference to it several times (Musselman, 2003). Eating dates breaks the fast traditionally observed during Ramadan. The faithful carry shortened and blessed palms in processions on the Palm Sunday (Castellana,1998).

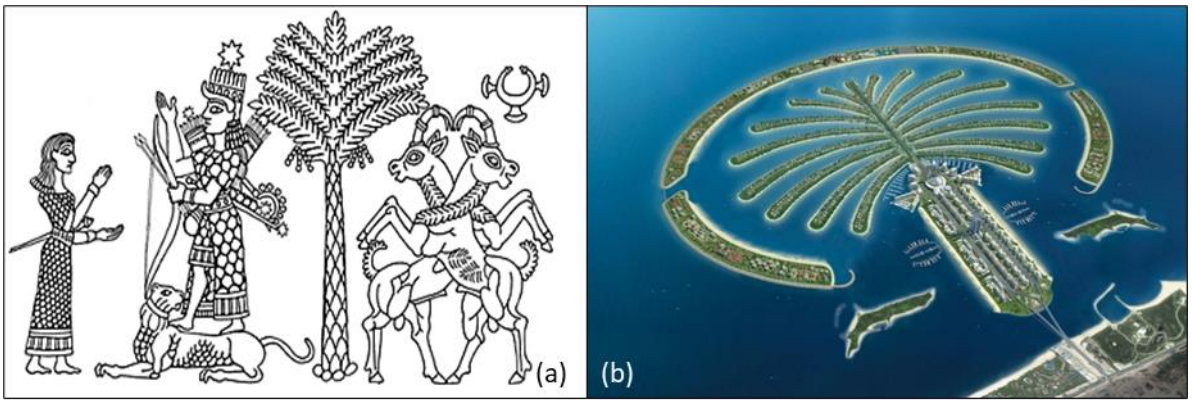


Figure 1 : (a) Neo-Assyrian bas-relief (750-650 BC) showing the goddess Ishtar and a sacred date tree (Image: The British Museum), (b) Artificial island in the shape of a palm tree in Dubai, United Arab Emirates (Image : Google Earth).

3. Taxonomy

According to Dransfield and Uhl, (1986) date palm is classified as follows:

- **Group**: Spadiciflora
- **Order**: Palmea
- **Family**: Palmaceae
- **Sub-family**: Coryphyoideae
- **Tribe**: Phoeniceae
- **Genus**: *Phoenix*
- **Species**: *Dactylifera*L.

Within the *Phoeniceae* tribe, there is just one genus: *Phoenix*. There are 14 species in it (Govaerts& Dransfield, 2005). Some species in the *Phoenix* genus are physically similar to one another and can be challenging to identify (Pintaud *et al.*, 2010). Experts have recognized a varying number of species, (Munier, 1973) took into account 12. This rose to "roughly 17" (Uhl & Dransfield, 1987), and in the most recent monograph on the genus (Barrow, 1998), it was reported to be 13.

4. Botanical description

A stipe covered in a leaf crown, on which branched inflorescences are arranged, makes up the monocular species known as the date palm. Its height can be as much as or more than 20meters (Munier, 1973). It is cespitose, meaning that it generates basal offshoot in response to the entire repetition process.

4.1.Vegetative organs

4.1.1 Root system

The date palm lacks a tap root because it is a monocotyledon. Its fibrous roots and fasciculated root structure resemble those of a maize plant. On the main root that grows straight from the seed, secondary roots emerge. These secondary roots give rise to similar-type lateral roots (tertiary roots, and so on) that have roughly the same diameter over their whole length (FAO, 2024).

Table 1 : Date palm root morphology and distribution (FAO, 2024)

Roots order	Origin	Form	Average length (m)	Average diameter (mm)	Characteristics
Primary	Trunk base	Cylinder	4 (up to 10)	9.5 (7-12.5)	Vertical; adventitious; no root hair; conic tip; called auxirhyzes and also main roots
Secondary	Primary roots	Similar to primary roots	0.20 - 0.25	3.5	Called mesorhyzes
Tertiary	Secondary roots	Similar to secondary roots but thin	0.02-0.1	0.3 - 1.5	Low growth; short; abundant

The breathing organs known as pneumatics are found in all date palm roots. In deep, loamy soil, roots can be detected up to 25 meters from the palm and deeper than 6 meters nevertheless, 85% of the roots are located in a zone between 2 meters and 2 meters on both lateral sides (Munier, 1973). It is important to note that while date roots can tolerate moist soil for several months, prolonged exposure is detrimental to fruit output and root health (FAO, 2024).

4.1.1.1 Zone I : Respiratory roots: Subterranean roots are located in the surface layer of the soil and do not extend 0.20 to 0.25 m deep(Munier, 1973).

4.1.1.2 Zone II: Nutritional roots: Very extensive zone with the highest proportion of roots with numerous rootlets that can develop well beyond the zone where the foliage projects (Munier, 1973).

4.1.1.3 Zone III: absorption roots»: For the function of seeking water, depend on the depth of the water table(Peyron, 200)

4.1.1.4 Zone IV: The largest portion of this zone is dependent on underground water. Zone III and Zone IV might be difficult to differentiate at shorter depths since both types of roots can be found there (FAO, 2024). Very deep roots that can reach great lengths (Munier, 1973). The roots of this zone may extend deeper when the subsurface water is deep. Typically, they are shown as positive geotropism vessels (FAO, 2024).

4.1.2 Trunk

The trunk, more correctly called the "stipe", is cylindrical, i.e. the same diameter from bottom to top, except at the base where the respiratory roots are found (Peyron, 2000).

According to (Djerbi, 1994), the trunk of a young date palm is covered by the Fibrilluim (lif), which persists in the adult state only in the coronal part. At the base of the trunk, we find the respiratory roots that grow by bursting the corona, as well as the shoots (Babahani, 1998).

The stiff, fibrous vascular bundles that make up the trunk are bonded together by a matrix of cellular tissue that is heavily lignified at the area closest to the outside of the trunk. Date palms are monocotyledons, meaning they lack a cambium layer (FAO, 2024).

In young trees the trunk is covered by the bases of the petioles of the old palms and the fibrous padding associated with them. However, these markers disappear with age. The trunk

is bare in older trees, and the fibrous layer is only visible in the terminal part. A single terminal meristem with permanent vegetative activity ensures the growth of the date palm trunk. It produces shoots at the base that help to multiply it. Some date palms can develop axillary buds along their trunks to produce gourmands or aerial branches (Cirad et Gret, 2002).

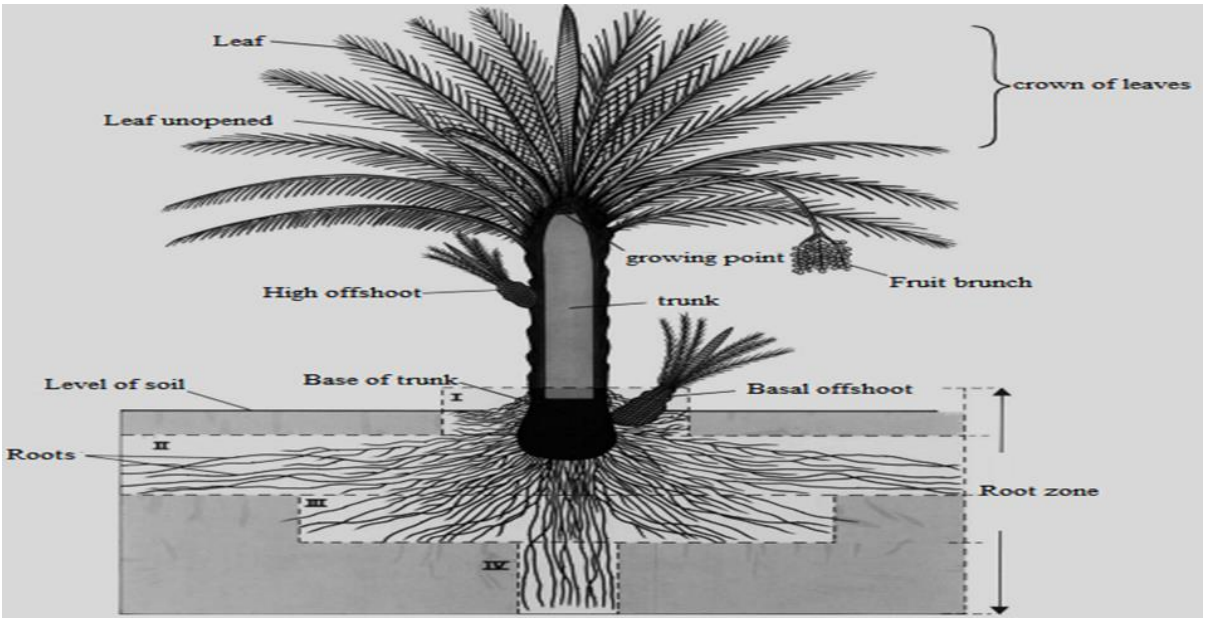


Figure 2: Diagrammatic construction root system (Munier 1973).

4.1.3 Leaves

Leaves consisting of rachis along which leaflets are laid. They are 4 to 7 metres long. Older leaves may remain on the stipe for several months or even years before falling off. All the palms make up the crown, which has a span of 6 to 10 metres (Sbiai, 2011).

As the leaf ages, its functional usefulness to the palm decreases. Moreover, compared to one-year-old leaves, four-year-old leaves are only roughly 65% more efficient in photosynthesis per unit area (Nixon and Wedding, 1956). Older leaves may remain on the stipe for several months or even years before falling off (Sbiai, 2011). Under favorable cultural circumstances, a leaf can support producing one to 1.5 kg of dates. Leaves can be categorized into three groups based on where they are located within the palm canopy:

- Leaves are green and actively photosynthesize on the outside.
- Quickly expanding green leaves in the center.
- Inside, at the palm's.

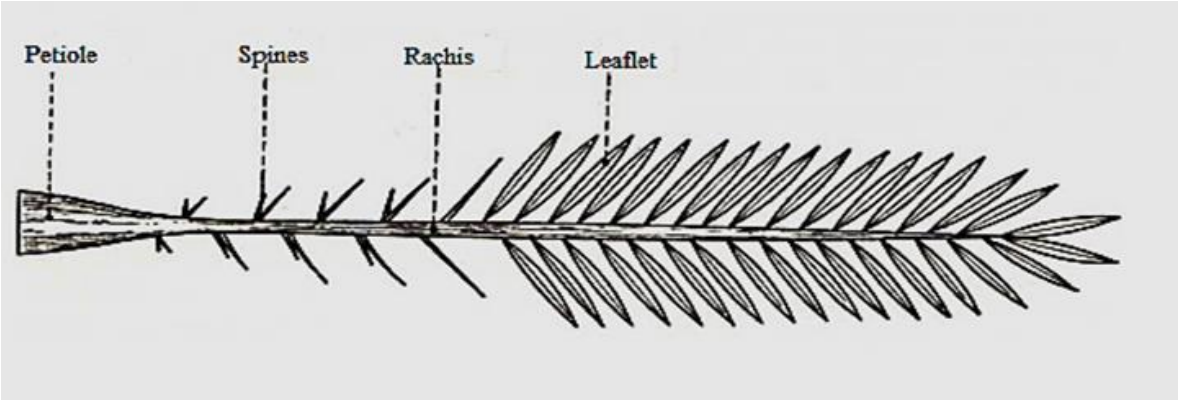


Figure 3: Date palm leaf characteristics (Munier, 1973)

4.2.Reproductive organs

As the date palm is dioecious, distinct people bear the male and female flowers. The sex of the plants may only be ascertained six to eight years after the first flowering (Aberlenc-Bertossi, 2012).

4.2.1 Floral organs

4.2.1.1 Female flower: With three fused sepals, it is globose and has a 3 to 4-mm diameter. Three rounded oval petals and six abortive stamens make up a corolla (FAO, 2024). The gynoecium comprises three separate carpels and one ovule (Munier, 1973) According to Amorsi (1975), depending on the variety and the year, the "*Talâa*" flowering season runs from the end of January to the beginning of May.

4.2.1.2 Male flower*:* Elongated, with three slightly extended petals comprising the corolla and a calyx of three spathes united at the base. The flower comprises three pseudocarpellae and six internally dehiscing stamens (Belhabib, 1995). Each spathe bears 160 sprigs and yields 40 to 45 g of pollen (Belhabib, 1995).

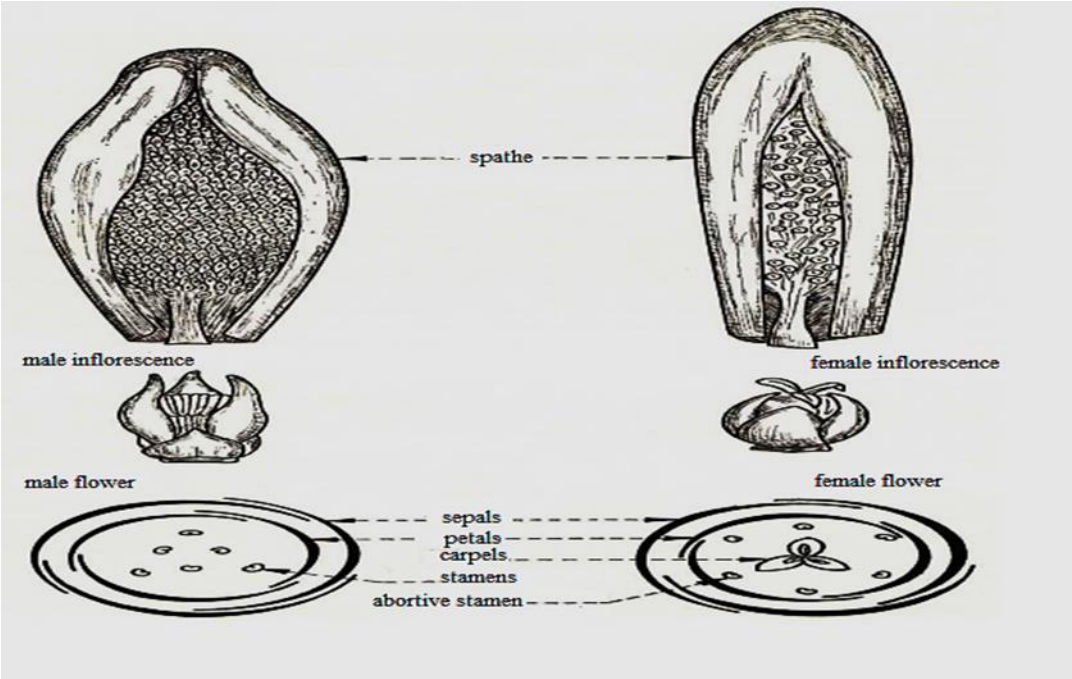


Figure 4: inflorescences and flowers of the date palm (Munier, 1973).

4.2.2 Seeds

The seeds are smooth, oval, and roughly big. They may also have ventral grooves and lateral ridges on the edges of their wings.

Ridges on the ventral furrows and wing edges. They are horny, weigh between 0.5 and 4 grams, and have lengths and widths of 12 to 36 mm and 6 to 13 mm, respectively (Munier, 1973).

5. Date palm ecological requirements

The date palm is grown as a fruit tree in arid and semi-arid regions of the world. Due to its considerable genetic variety, this species is remarkably adaptive despite being ubiquitous in hot, humid regions (Munier, 1973). The date palm is incredibly resilient, yet even with its tolerance, it needs certain, well-defined circumstances (Anonymous ,1989).

5.1.Temperature

According to Munier (1977), the date palm is a thermophilic species that needs a warm, dry, sunny climate. The date palm in Algeria only flowers when the average temperature is between 20 and 25°C; it cannot bear fruit below 18°C (Anonymous, 1993). Toutain (1977), states that different varieties require different temperatures for fruiting, ranging from 37 to

50°C. According to Ben Khalifa (1991), the ideal temperatures for fruit ripening are 26.6°C for soft varieties, 32.2°C for dry varieties, and half-soft varieties in between.

5.2. Light

Date palms are heliophilous plants that are grown in areas with abundant light. While photosynthesis and date ripening are influenced by light, vegetative organ growth, which typically proceeds more slowly during the day is also slowed down or occasionally stopped by light (Babahani, 1998).

5.3.Humidity

During flowering and fruiting, the date palm is susceptible to changes in air humidity. Elevated relative humidity inhibits date transpiration, preventing the dates from ripening (Bouguedoura,1991). When air humidity is 40% or below, the best dates are collected (Bouguedoura, 1991; Bessas *et al.*, 2008).

5.4. Winds

Winds possess both mechanical and desiccating properties. The palm tree enhances transpiration, resulting in the combustion of new branches and desiccation of dates. The distribution of certain date palm predators, such as Ectomyeloisceratoniae, is similarly influenced by winds (Bessas *et al.*, 2008).

5.5.Soil

The cultivation of the date palm encompasses a diverse range of soil types, demonstrating a remarkable ability to thrive in desert and sub-arid environments. According to Munier (1973), palm groves are found in many locations, including fluvial alluvium in the Biskra region, lacustrine alluvium partially covered with aeolian sand in Oued Righ, and aeolian sand in the low-lying areas of the dunes known as Oued Souf. The behavior of date palms varies based on the soil type. The plant exhibits a preference for light soils characterized by a salt level below 10%. In such conditions, the plant demonstrates accelerated growth, earlier onset of production, and superior quality, uniformity, and abundance compared to heavy soils. The plant exhibits tolerance to saline soils under conditions of intensive irrigation and efficient drainage. Date palms thrive and yield optimal results when the soil permits water infiltration to a depth of 2 to 2.5 meters (Toutain, 1996).

5.6. Water requirements

Date palms are often linked to deserts, although despite their integration in hot arid and semi-arid climates, they are consistently found in areas with limited resources.

In arid and semi-arid climates, this phenomenon is consistently observed in areas where the soil possesses ample water supplies to offset inadequate or absent precipitation. Due to its remarkable adaptability, the date palm is capable of flourishing in arid environments, with water serving as its foundation. As the adage suggests, "The date palm thrives by immersing its feet in water and its head in the bright sky" (Munier 1973).

6. Geographical distribution of date palm

6.1.In the world

There are an estimated 122 million palm trees in the world (Ataf and Nadif, 1998; Chaouch Khouane, 2012). The Middle East and Mediterranean regions of Africa are seeing a significant increase in date palm planting. The only nation in Europe that produces dates is Spain, which is home to the well-known Elche palm trees (Toutain, 1996). Asia, with 60 million date palms (Saudi Arabia, Bahrain, United Arab Emirates, Iran, Iraq, Kuwait, Oman, Pakistan, Turkmenistan, Yemen), holds the top spot in terms of spatial distribution, with 32.5 million date palms in Africa (Algeria, Egypt, Libya, Mali, Mauritania, Morocco, Niger, Somalia, Sudan; FAO, 2013).

The date palm was brought to the United States in the 18th century. Around 1900, Iraqi cultivars introduced it to the country, and it became seriously cultivated (Matallah, 2004; Bouguedoura, 1991). In addition, Mexico, Argentina, and Australia grow it on a limited scale (Matallah, 2004).

Figure 5: Geographical distribution of date palms in the world (france-palmier2024).

Egypt is the world's largest producer of dates, with an output of 1.5 million tonnes in 2022, followed by Saudi Arabia, which has produced 1.3 million tonnes. Algeria comes third, with a market share of 13%. Iran, Iraq, Tunisia, Pakistan, Oman, the United Arab Emirates, and Morocco are the other countries in the top 10 largest date producers. According to the (FAOSTAT, 2024), the quantities of date production for the year 2022 for each country are shown on figure 6.

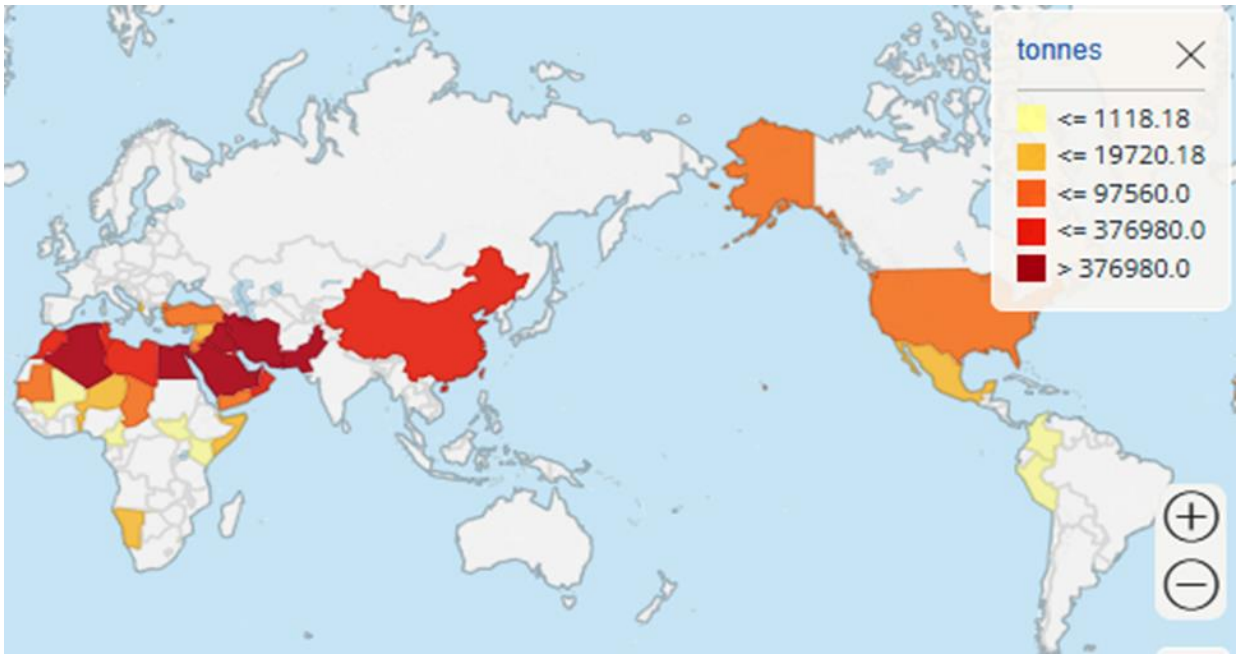


Figure 6: Production quantities of Dates by country 2022 (FAOSTAT, 2024)

6.2. In Algeria

The date palm (*Phoenix dactylifera* L.), a xerophilous species, forms the backbone of oasis agriculture in the Sahara. It can only flower and bear fruit, usually in hot deserts (Amorsi, 1975). Most remote oases in the Algerian desert and a sizable portion of the Sahara's Mediterranean coast are home to its cultivation.

Due to its vast 160,000 hectares and more than 2 million gardens, Algeria leads the Maghreb in date farming and ranks sixth in the world (Aberlaance-Bertossi, 2010). In Algeria, date palm is found in many oases in the south of the country, characterized by hot, dry environment known as the Sahara. Algeria's topography allows the description of multiple date palm-growing zones . To the east are Ouargla, Oued Righ, Zibans (Biskra), and Oued Souf (El Oued), especially with the highly valuable Deglet Nour cultivar. In the foothills of the Atlas Mountains (Ksour Ouled Naïl, Zibans, and Aures), a chain of oases marks the entry to the Sahara. In the West, the palm groves of Saoura (Béni Abbés), Touat (Adrar), Gourara (Timimoun), and Tidikelt (Reggane) have cultivars that are comparatively low in quality for commercial use El Golea, M'Zab (Ghardaia), and Laghouat are in the middle (Bougoudoura *et al*., 2015).

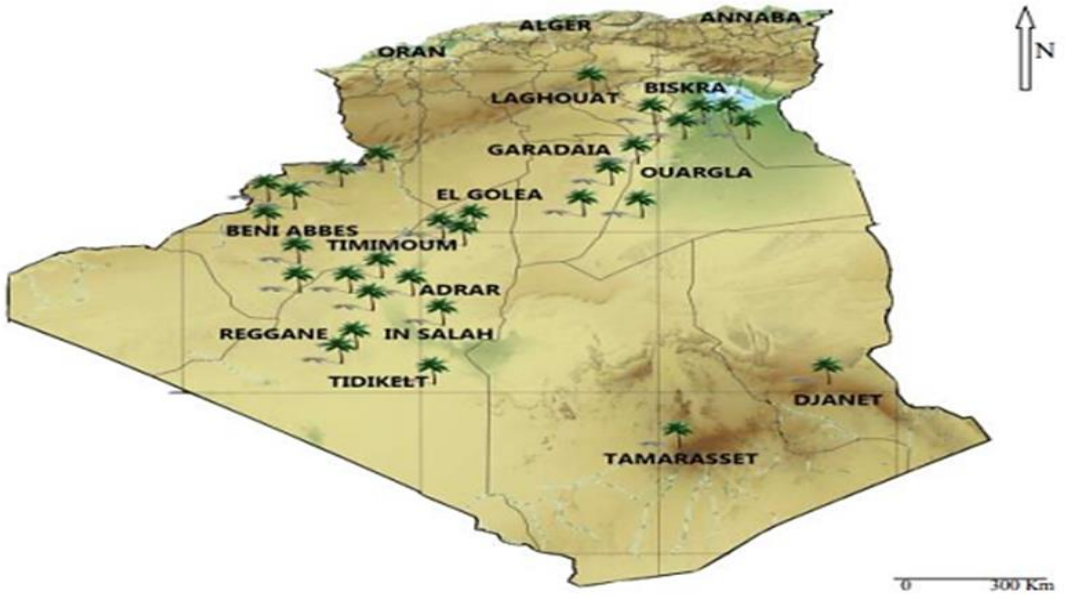


Figure 7: Palm trees in Algeria (Boulanouar, 2015).

Algeria is one of the world's leading date producers, and it is among the top ten countries in terms of global date production (Statista, 2024).

According to a report published by the Food and Agriculture Organization of the United Nations (FAO), Algeria produced more than 1.4 million tonnes of dates in 2022, an increase of 7% from the previous year.

6.3. In Ghardaia

As reported by the agriculture services of Ghardaia province in 2022/2023 (Table 2).

Table 2 : Date palm trees (Area occupied, number of existing palms, number in production) (DSA, 2024)

	"datters in mass" area occupied (ha)	**Number of existing palm trees**				**Date production (Qx)**			
		deglet nour (No)	ghers and analogues (No)	deglet beida and analogues (No)	TOTAL (No)	deglet nour (No)	ghers and analogues (No)	deglet beida and analogues (No)	total (No)
TOTAL Exploitation	8547,00	40906	203796	399434	1012290	185 000	86 800	223 200	495 000

No: number ; Qx: quintal

7. Date Palm cultivars in Algeria

The recognition of cultivars is made difficult by the fact that for the same cultivar, it is possible to note morphological differences from one palm grove to another. Only date palm growers are still able to distinguish between cultivars in their own gardens (Bouguedoura, 1991). Date palms show great morphological diversity. In general, morphological characteristics vary from one cultivar to another. Rhouma (1994) believes that these characteristics may vary for the same cultivar depending on growing conditions, maintenance and the age of the cultivar; the general appearance of the plant and especially the fruit are the only valid criteria for recognizing and distinguishing between cultivars. Just by looking at the morphological characteristics of the fruit (shape, colour and size), we can see that there is considerable diversity, reflecting the richness of the plant-growing heritage, which varies from one plant-growing basin to another and from one terroir to another, and even from one farm to another when the maintenance factor is predominant.

Table 3. Cultivar of the three Algerian date palm areas (Benkhalifa, 1998).

Area	Region	Location	Names of cultivars identified
East	Zibans	Biskra Tolga	Arechti, Degla Beida, Deglet Noor, Ghars, Ghazi, Mech Degla, Tantboucht, Tinicine, Zoggar Moggar
	Oued-Souf	El Oued El-Meghaier Djamaa	Arechti, Degla Beida, Deglet Noor, Ghars, Ghazi, Mech Degla, Tantboucht, Tinicine, Zoggar Moggar, Halimi-Halwa (Halwaya), Kesba, Khodri, Loulou, Masri-okrya, Tachelilt, Tacherwint, Tachlikt, Takermust, Takhedrayt, Tantbucht, Taoudent, Tarmount, Zaghraya, Zehdi, Deglet Noor, Ghars, Takermoust, Tanslit
	Oued-Righ	El Arfiane, Ouargla Touggourt	Aliyane, Beidh H'mam, Bentqbala, Bouldjib, Degla Beida, Deglet G'rara, Deglet Mechta, Dguel El Hadj, El Caber, El Kid, Ghars-Halwa, Hamraya, Tafezwin, Akermoust, Tanetboucht,, Tanslit, Taoudanet, Tawragha, Tazegakht, Tinicine
	Aures	Khenchela	Buzrur, 'Alig, Buhles, Mech Degla, Tanghimen, Tabanist, Khadaji
	Tassili	Batna	
Center	M'zab	Ghardaïa, Berriane, Guerrara, Zelfana	Tamezouaret, Tanaguarout, Tanetboucht, Tawragha, Tazerzayt, Tazizawt, Timdjouhart, Timedwel, Tinnaser, Tissibi, Adham Bent Q'bala, Ajujil, Baydir, Bent Q'bala, Bouarous, Chikh, Degla Beida, Deglet Noor, Gachouch, Ghars, Naser Ou Salah, Oucht, Sab'a Bedraa, Taddela (El Dala), Tademamt, Tafezwin, Taqerbucht (Akerbouch)
West	Touat	Adrar, Timimoun	Bamekhlouf, Feggus, Hmira, Ouarglia, Taqerbucht, Takerbucht Beida, Takerbucht Hamra, Taqerbucht Safra
	Saoura	Bechar Béni-Abbes	Adham Boula, Adham Tirnou, Adhamet El Rob, Cherka, Deglet Talmine, Feggus, Hmira, Hartan, Kenta, Khomira, M'charet, Taqerbucht, Timliha, Tinnaser
	Tidikelt		Tgazza, Taqerbucht, Cheddakh, Agaz

Chapter 2: Date palm diseases

1. Physiological disorders

1.1. Blacknose

Fruit damages due to water, mostly caused by overhydration of the skin or flesh. Such overhydration may harm the fruit immediately by breaking the peel or interfering with the natural dehydration process that leads to ripening (Chao and Krueger, 2007). According to Fawcett and Klotz (1932), Deglet Nour and Hayani appear to be the types most vulnerable to this physiological condition.

Excessive checking of the epidermis causes blacknose, particularly when it manifests as several tiny transverse checks or cracks near the fruit's stylar end. The degree of shrivelling and discoloration is directly correlated with the checks' abundance (Chao and Krueger, 2007).

Since rains and excessive humidity are the main causes of checking, precautions against conditions that tend to raise humidity should be taken. Avoidable situations include too much moisture in the soil, weeds, and intercrops, especially when the fruit is still in the vulnerable stage of development. blacknose checking was discovered to be inhibited by bagging the fruits in brown wrapping paper (Nixon, 1932). Over thinning can also increase the incidence of checking and subsequent development of blacknose.

1.2. Black scald

Unlike blacknose, black scald is a mild condition that occurs in the United States and has an unknown cause (Djerbi, 1983). It comprises a sunken and blackened area with a well-defined boundary line. Affected tissues have a bitter favour; the disease typically manifests on the fruit's sides or tip. Although the disorder's appearance suggests exposure to high temperatures, the precise origin is still unknown (Nixon, 1951).

1.3. Bastard offshoot

Date palm vegetative buds, particularly those of offshoot fronds, are growing distorted, as seen in Figures 8. According to (Mohamed and Al-Haidari 1965), the date palm bud mite *Makiella phoenicis* K is the cause of the bastard condition. It might also result from growth regulators being out of balance, reducing growth.

Figure 8: Bastard offshoots on a tissue culture-derived Barhee palm (FAO, 2024)

2. Disease caused by *Phytoplasma*

2.1. Al Wijam

Al Wijam means "poor fruitful" in Arabic. Nixon (1954) first observed the disease in the Al Hassa oasis in eastern Saudi Arabia. The disease's primary signs and symptoms are yellow streaks and leaf stunting. There was also a noticeable decrease in fruit and stalk size. Leaves lose some of their vitality and get choritic. As the leaves age, they get more stunted and yellowed, ultimately resulting in their demise. Compared to healthy spathes, diseased spathes are shorter and split open before fully emerging. Fruits and fruit stalks displayed a 36-40% drop in size. The identification and molecular characterization of phytoplasma linked to Al Wijam in Al Hassa, Saudi Arabia, was documented by (Alhudaib *et al.*, 2007) Placing the phytoplasma discovered from 28/40 date palms exhibiting characteristic Al Wijam symptoms distinctly in the 16SrI group "Ca.P.asteris" is corroborated by the Phylogenetic and sequencing data.

Furthermore, phylogenetic analysis revealed that the phytoplasma found in the date palm exhibiting Al Wijam symptoms and the leafhopper *Cicadulina bipunctata* Melichar were 100% identical. As a result, it has been identified as a potential disease vector (Alhudaib *et al.*,2007).

Figure 9: Symptoms of stunted dates and leaf rachis yellow streaks from an "Al-Wijam" affected palm (Alhudaib *et al.*, 2007).

2.2. Leathal yellowing

The disease's significance was initially recognized in the United States (Florida), where it killed almost half a million coconut palms (McCoy, 1976).

Phoenix dactylifera L, *P. canariensis* Hort, and *P. reclinata* Jacq, and other hosts are among the many species spread by the disease (Thomas, 1974). The first signs are inflorescence necrosis and fruit loss; the crown will die entirely in three to six months (McColl, 1992). Al Awadhi et *al*. (2002) published their findings on a phytoplasma linked to date palm yellowing disease in Kuwait. The disease exhibited symptoms resembling those of Al Wijam on the leaves, spathes, and bunches of date palms. Ammar et *al*. (2005) found phytoplasma linked to the streaking and yellowing disease in date palms prevalent in Egypt.

According to Harrison et *al*. (2002), phytoplasma from the 16SrIV group, subgroup D, is responsible for the deadly yellowing drop in Texas Canary Island date palms.

Figure 10: a) Leaf yellowing symptoms on *Cocos nucifera*. Photo by N. A. Harrison, University of Florida. b) Spear leaf of this *Phoenix sylvestris* has collapsed and is hanging down out of the canopy on the right side of the trunk. Very few of the oldest leaves have discolored. Photo by M. L. Elliott.

2.3.White tip die-back

This disease, which affects immature date palms (*Phoenix dactylifera* L.). The disease is present in isolated foci in northern Sudan . 5–8-year-old palm trees started to show symptoms, which pass away 6–12 months after the onset of symptoms. There is severe chlorosis of the juvenile leaf and at the tips of the older fronds' pinnae, which rapidly turn from green to dry white without exhibiting crown fading. The causative pathogen has been identified as Plantoplasma by molecular methods (Cronje *et al*., 2000a).

2.4.Slow decline

The disease attacks mature date palms in the North Sudanese region between Dongola and Mero-Karem. Palm death happens one to two years after symptoms first manifest and results in losses that are estimated to reach 6%. The symptoms started with yellowing of the outermost fronds and then moved into the young leaves and center fronds that were just starting to emerge. The fronds eventually dry up to a light brown color and shed, leaving a few immature leaves at the top of the trunk that may eventually break off and leave the tree alone. The slow-declining Phytoplasma 16S/235rDNA intergenic spacer sequence exhibited 99% similarity with similar Phytoplasma sequences linked to White tip-dieback disease in immature date palms (Cronje *et al.*, 2000b).

3. Insect and Arcarian pathology

3.1. Boufaroua (*Oligonychus afrasiasticus*)

The date palm mite (DPM) is a type of spider mite found in the Acari: Tetranychidae family. It is a major cause of disease for date palms throughout the Middle East and North Africa. It spreads quickly and is challenging to identify in its early stages. It destroys the crop and results in severe financial losses (El-Shafie and Hamadttu, 2022).

The date mite has different names in different countries, as follows:

- Boufaroua in Algeria.
- Boufazroua in Tunisia.
- Goubar in Iraq.
- Goubash in Libya.

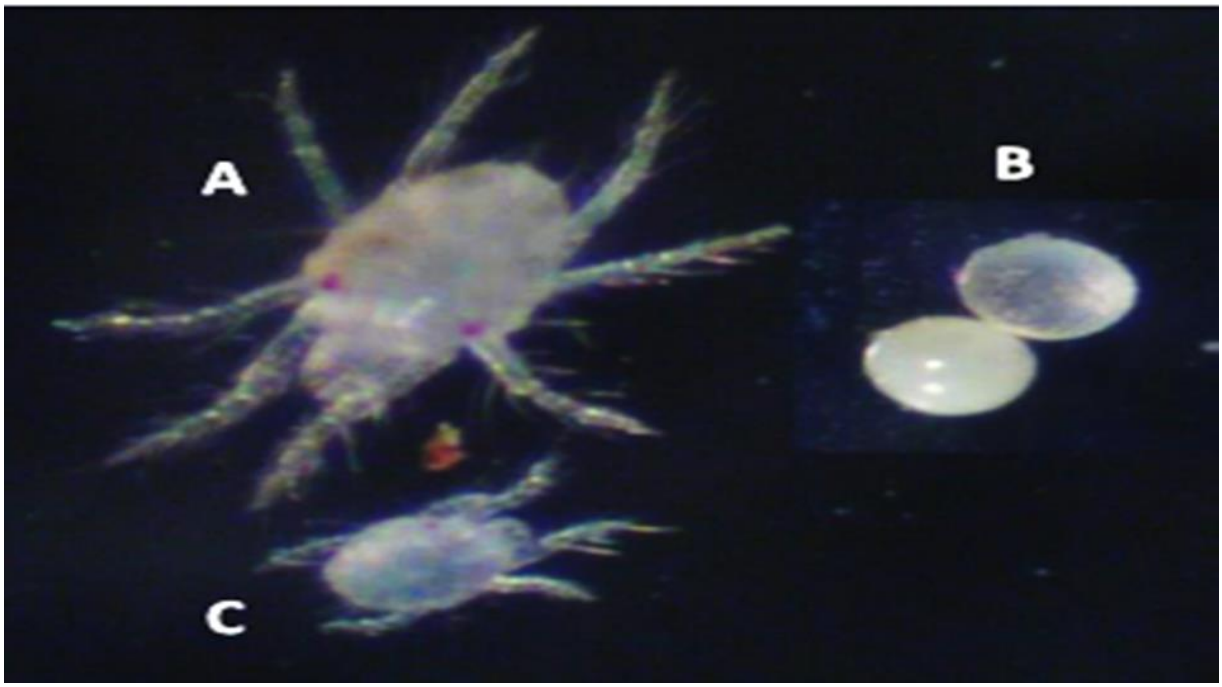


Figure 11: Old world date palm dust mite, *Oligonychus afrasiaticus*, different life stages: (A) adult, (B) egg and (C) larva (El-Shafie & Hamadttu, 2022).

The four developmental phases the *Oligonychus afrasiaticus* undergo are egg, larva, nymph, and adult. When measuring an adult, the width is between 0.17 and 0.2 mm, while the length is between 0.2 and 0.5 mm. The round egg has a diameter of approximately 0.1 millimeters and is colored either red, pink, or yellow (El-Shafie & Hamadttu, 2022).

Growing or unripe fruits (kimri stage), which contain low sugar, high acidity, and high moisture content, are the main source of DPM reproduction. In Arabic, date fruits are divided into five phases: hababook, kimri, khalal, rutab, and tamr. The kimri period is when web spinning and egg depositing are busiest. In the kimri and pre-khalal stages, the date fruit is the main food source for *O. afrasiaticus* larvae, nymphs, and adults. Date fruits grow silvery-gray and eventually become reddish-brown due to mite consumption. Infected fruits are damaged and covered in the thick, silken webs of the mites. The fruits seem dusty due to these webbings' collection of dust particles (El-Shafie & Hamadttu, 2022).

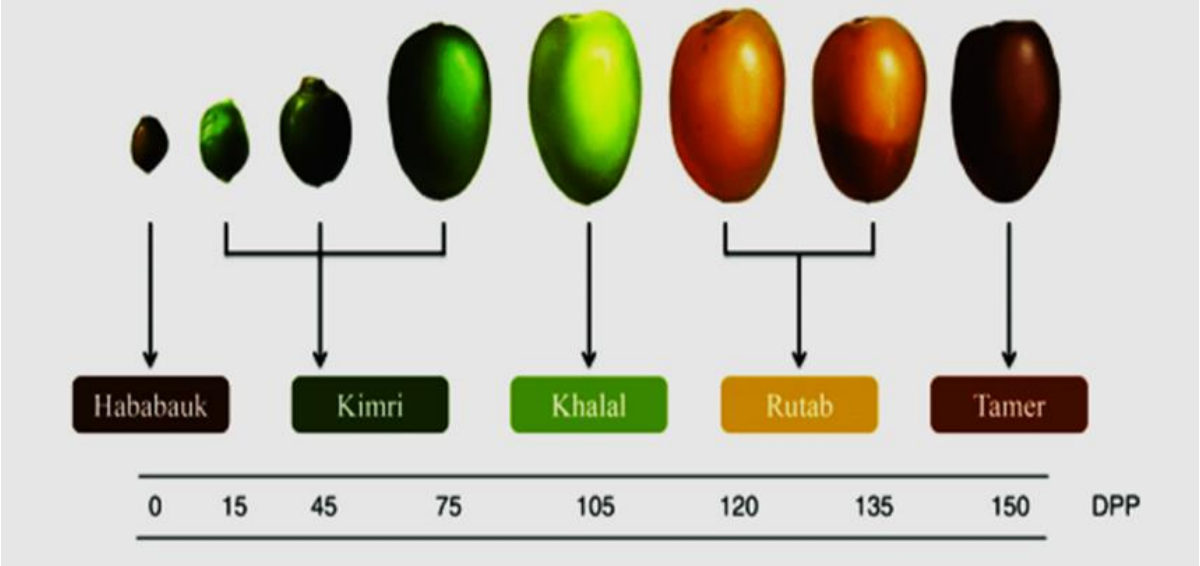


Figure 12: The five growth stages of a date fruit by days post pollination (DPP) (Al-Mssallem *et al.*, 2013).

Figure 13: Clumped dust mite infestation with highly infested bunch next to uninfested one (El-Shafie, Hamadttu 2022).

3.2.White scale (*Parlatoria blanchardi*)

It can be found everywhere dates are grown. It is locally known as Djereb or Sem in Algeria, Rheifiss in Mauritania, and Nakoub and Guelma... in Morocco (Vilardebo, 1973).

The insect injects a poison that changes the plant's metabolism while feeding on the plant's sap.moreover, respiration and photosynthesis are decreased by the leaves' crusting. It is also present in fruit, where it stops developing. Scale insects may result in A fruit yield decrease of more than half, making the fruit unfit for human eating (Bounaga and Djerbi1990). Mealybugs are insects that only consume sap, among other things. Their only food source is incredibly developed sap (Balachowsky, 1932).

It mostly affects the palms of the Ghars cultivar, though other cultivars will also be affected if that doesn't work. In the case of a severe infestation, it can establish itself on the dates of all cultivars (Dakhia *et al.*, 2013).

Figure 14: White scale infestation of date palm leaflets (Ben Aissa & Ben Sahla 2018).

3.3. Carob moth (*Ectomyelois ceratoniae*)

This is the date worm's name; they belong to the Pyralidae family of lepidopterans. The caterpillar, which lives between the pulp and the stone and feeds on it, is the one who ruins dates (Vilardebo, 1975). Le Berre (1978) says that dates in Algeria have been dated since 1904 due to the moth's presence. He also claims that soft dates like ghars are more infected than semi-soft dates rather soft dates.

The species' polyphagy is shown as extensive dispersal throughout space, and ability to eat a wide range of hosts make it challenging to create efficient management strategies. Its extensive dissemination over a range of hosts and in space (Zouioueche, 2012).

Figure 15 : *Ectomyelois ceratoniae* damage on dates (Zouioueche, 2012).

3.4. Bougassass (*Apate monachus*)

A beetle called *Apate monachus* is indigenous to tropical Africa, feeding on coffee trees. There have been reports of the palm borer in Ouargla and the Ziban (Bordj Ben Azzouz/Tolga). Owing to their prevalence, Deglet Nour dates are damaged. This pest chops off the date palm's leaves when it attacks them (Bensalah *et al.*, 2000).

Figure 16 : Pest Palm borer (Dakhia *et al.*, 2013)

4. Fungal diseases of date palms

4.1.Inflorescence rot of date palm

4.1.1 Origin and importance

For the first time in Libya, Cavara (1925) documented the presence of inflorescence rot disease, commonly known as Khamedj in North Africa, caused by *Mauginiella scaettae* Cav. The disease was then documented in It has also been reported from the Arabian Peninsula (Abu Yaman and Abu Blam, 1971; Djerbi,1982;Al Shridia & Al Shahwan, 2003) and Iraq (Allison,1952; Abdullah *et al.*,2006) in addition to other North African countries (Taxana & Larous, 2003). In southeast Spain, it has recently reported cases of the disease (Abdullah *et al.*, 2005).

Inflorescence rot is sometimes called Khamedj disease in North Africa. One of the main factors limiting productivity is inflorescence rot, which, in extreme circumstances, can kill between 50 and 80% of date palm inflorescences. The disease is most destructive when there have been prolonged, intense rainstorms for up to two months before the spathes or bracts cover the emerging budding flowers. Early in the young inflorescence, when the spathe is still

concealed in the leaf bases, infections arise, and the symptoms are more noticeable on the internal face of the spathes. Male inflorescences are contaminated during the pollination phase, which is how the disease is transmitted. For several years, disease can linger on the trees, resulting in rotten inflorescences (Bensaci *et al.*, 2023).

4.1.2 Disease symptoms

Young spathes exhibit the disease's initial outward signs on their tissues as they emerge. as elongated or elliptical russet dots, followed by brownish ones. In a light attack, only a portion of the Blossom buds deteriorate and drop off. The other buds grow in a typical manner. When there is a severe attack, the inflorescences dry out and get coated in a mycelial felting, preventing the spathe from opening due to the complete destruction of the flowers and pedicels (Bellkacem, 2005).

4.1.3 Détection of the pathogen

The fungus *Mauginiella scaettae* Cav. is thought to be the primary cause of inflorescence rot (Cavara, 1925; Djerbi, 1983;Abdullah *et al.*, 2005). But other fungus, like Additionally, *F.oxysporum, F.moniliforme, F.solani, Trichothecium roseum, Botrytis aclada, Thielaviopsis paradoxa, Acremonium strictum*, and *Memmoniella sp*. were discovered to be connected to date palm rotten inflorescences. These are thought to be of modest significance.

It is simple to isolate *Mauginiella scaettae* from decaying inflorescence by surface disinfecting tiny sections with a 5% sodium hypochlorite solution and plating them on appropriate culture media, such as potato agar or malt extract agar. Potato-carrot agar, also known as dextrose agar. It is also possible to achieve isolation by incubating disinfected pieces in moist chambers, picking up conidia that have proliferated profusely, and streaking them on an appropriate medium. It is recommended to incubate inoculated plates at 25 °C. White colonies with submerged and exposed mycelia are the fungus's growth form. A detached inflorescence free of disease can be subjected to a pathogenicity test. Symptoms common to the infection were observed four days after the inoculation with spore suspension (Bensaci *et al.*, 2023).

Sequencing of this fungus's internal transcribed spacer (ITS) region revealed as reported by Abdullah *et al.* (2005), that it is closely related to *Phaeosphaeria* I and confirmed last year by Bensassi *et al.* by using the a combined ITS-LSU alignment performed a more thorough phylogenetic reconstruction, which provided compelling evidence for placing *Mauginiella*

within the *Phaeosphaeriaceae*. The placement of *Mauginiella* was near the genera *Phaeosphaeriopsis*, *Leptospora*, and *Populocrescentia*.

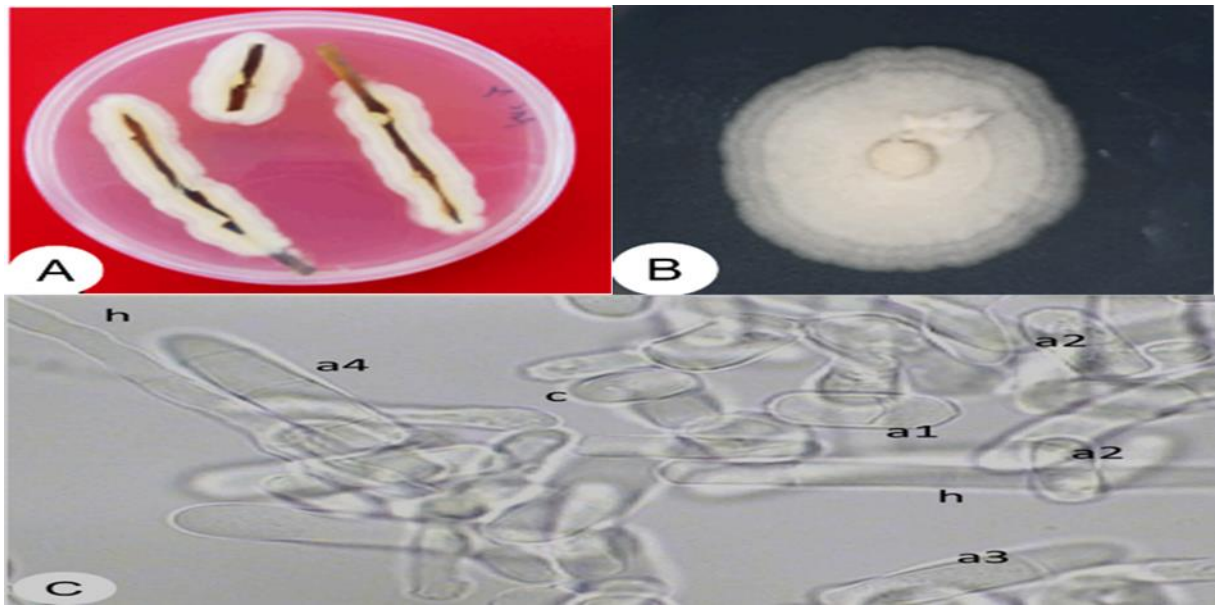


Figure 17: Culture and light microscopic analysis of fungal strains isolated from diseases male and female date palm spathe. (A) The emergence of white fungal mycelia from explants of inflorescence rot exhibiting spathe. (B) After many rounds of sub-culture, single strains of white fungal colonies were isolated. (C) light microscopic analysis of fungal strain morphologies showing hyphae (h), and one (a1), two (a2), three (a3) and four-celled (a4) arthroconidia. Magnification 300x (Bensaci *et al.*, 2023).

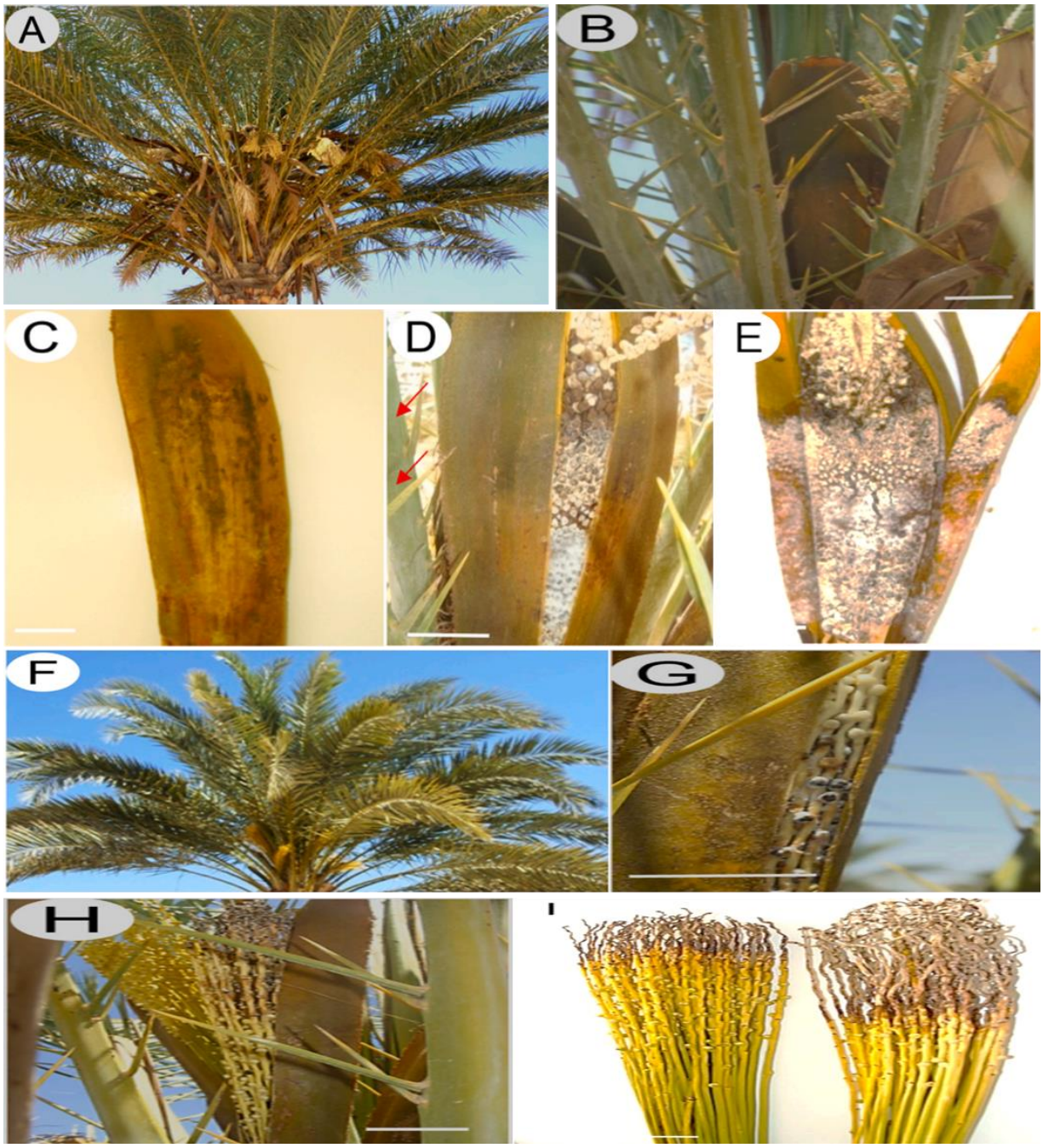


Figure 18: Symptoms of date palm inflorescence rot caused by *Mauginiella scaettae*. (A) Male date palm with (B) male inflorescences with the spathe cover (bar = 10 cm). (C) Necrotic symptoms symptomatic of inflorescence rot (arrowed), as seen on the outside of the male spathe (bar = 5 cm) with (D) white mycelial development within the male spathe (bar = 2 cm). (E) white mycelial development and necrosis is eventually observed both within and outside the infected male spathe (bar = 5 cm). (F) healthy female date palm exhibiting the emergence of female flowers. (G) white mycelial development within inflorescence rot exhibiting female spathe (bar = 2 cm) which (H) is associated with necrotic symptoms as the flowers emerge (bar = 10 cm) and (I) kills the flowers at the tips (bar = 5 cm).

4.1.4 Control

The first stage in stopping the inflorescence rot disease is accomplished by practicing appropriate management, which includes gathering and burning all diseased inflorescences and cutting leaves, as well as applying various fungicides, such as 4% thirame spray or 3% dichlone spray, at a rate of 8 liters per each palm (Al Hassan *et al.*, 1977).

4.2.Belaat disease

The disease, known as "belaat," or bud rot, was initially identified in Algeria by Maire and Malençon (Bellkacem, 2006). The disease is sporadic and of minimal consequence. According to Calcat (1959) and Toutain (1996), it originated in North African nations. *Phytophtora* sp. is the disease's causative agent. It is caused by a phycomycete, a fungus with a siphoned thallus of the order Péronosporales (Bellkacem, 2006).

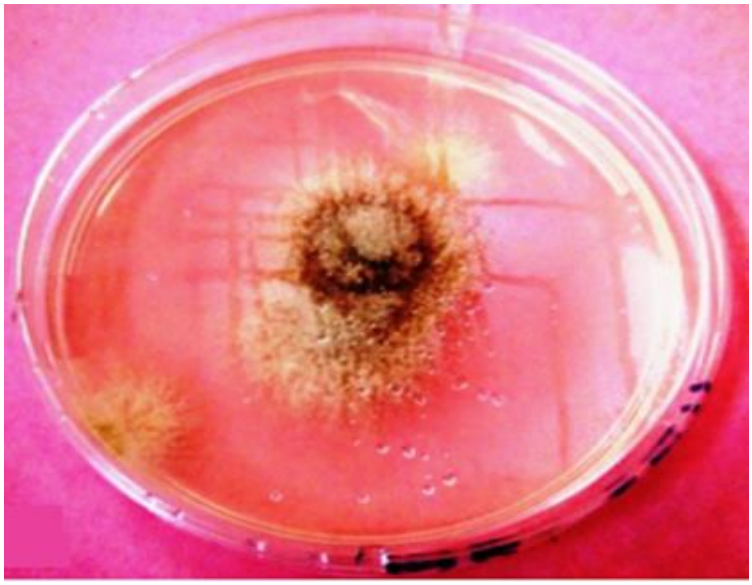


Figure 19: Macroscopic observation of *Phytophtora infestans* (Chacha 2017).

4.2.1. Symptoms

The youngest leaf near the heart becomes discolored and eventually turns white as a result of this disease, which damages the terminal bud, Start with the youngest leaf becoming discolored and turning white in the center, followed by a wet rot that spreads quickly. The tissues beneath the terminal bud become reddish and fully delignify, transforming. Change into a mushy, wet, greenish-yellow meat that smelled strongly of acetic acid. Several mucorales and yeasts speed up this breakdown. The palm tree cannot be salvaged after the bud is damaged, yet occasionally, it can recover by growing a lateral sub-terminal bud to replace the lost terminal bud (Zaid *et al.*, 2002).

4.2.2. Control

Drain the soil and disinfect the affected area with copper salts, good growing conditions and elimination of intercropping. They are destroying diseased plants by burning them (Bellkacem, 2006).

4.3.Black scorch disease

The fungus *Thielaviopsis paradoxa* (De Seyeres) Hohn or *Thielaviopsis punctulata* (Hennebert) Paulin, Harrington, and McNew is the cause of black scorch disease, which is also referred to locally as Medjnoon or Fool (saeed *et al.*,2016). Date palm are impacted by these soil-borne wound infections palm tissues throughout every developmental stage. According to some reports, *T. paradoxa* has been linked to black scorch disease on date palm in Kuwait (Mubarak *et al.*, 1994), Iraq (Abbas *et al.*, 1997). *Thielaviopsis punctulata* is the primary causative agent of date palm black scorch disease in Oman, and qatar (Al-Naemi *et al.*, 2014) , as well as in the UAE (*Saeed et al.*, 2016). A disease spread by the air is black scorch. In addition, it can spread through contaminated offshoots.

4.3.1. Symptoms

Hard black lesions on leaves, inflorescence blight, and trunk and bud rot are common signs of black scorch, depending on the time of infection and the stage of the disease's development (Al-Raisi *et al.*, 2011). A common sign of severe symptoms is "neck bending" of the fungal invasion-affected terminal bud and heart areas, which ultimately results in the death of the tree (Saeed *et al.*, 2016).

Stressful environments and poor horticultural can potentially accelerate the onset of disease. Research on palm tissues colonized by *T. paradoxa* and *T. punctulata* under saline and drought stressors revealed heightened black scorch intensity, which ultimately led to plant mortality (Suleman *et al.*, 2001).

It is frequently discovered that both fungi, either by themselves, in combination, or in connection with other fungal infections like *Phoma* and *Alternaria* spp., are responsible for the disease symptoms on palm trees (Al-Raisi *et al.*, 2011).

Figure 20: Black scorch disease symptoms and microscopic confirmation of the causal agent *Thielaviopsis punctulata.* Date palm plants showing neck bending (A,B) leaf drying under natural field conditions. (Alhudaib *et al* 2022).

4.3.2. Control

The primary line of defense against black scorch is good sanitation. It is necessary to trim, gather, and burn the impacted fronds, leaf bases, and inflorescences right away. Bordeaux should be sprayed on the pruning cuts to protect the surrounding tissues, combination, lime-sulfur solution, copper sulfate lime mixture, dichlone, thiram, or any other recently developed fungicides based on copper (Suleman *et al.*, 2001).

4.4.Leaf spot diseases

Leaf spot diseases are often not very significant economically. Various fungal species showing leaf spot symptoms have been identified from palm leaves. All countries that grow date palms have a high prevalence of leaf spot infections on their trees (Fayad & Mania, 2006; El Deeb *et al.*, 2007). In general, infection affects lower whorls and old leaves more severely than it does upper young leaves, and both the rate and severity of infection rise with increasing palm age (Fayad and Mania 2006).

Other fungi caused leaf spot symptoms on palm trees include *Alternaria alternata*, *Bipolaris australiensis*, *Drechslera* sp., *Helmnthosporium* sp., *Colletotrichum* sp., *Stemphylium* sp., *Pestalotiopsis palmarum* , *Chaetosphaeria* sp., *Phomopsis* sp., *Phoma* spp., (Fayad and Mania,2006; El Deeb *et al.*,2007).

4.4.1 Symptoms

Symptoms of the disease occur on the rachis, pinnae and spines as dark lesions with well-defined margin on green leaves and on drying leaves, the margin of the lesion remains reddish brown as the center becomes pale (Abdullah, 2010).

4.4.1. Control

The following combined management package is advised in order to control the disease:

a) **Cultural control:** It is recommended to prune old diseased leaves annually and to burn them right away. By doing this, the tree's affected areas become smaller and the spores' spread to immature leaves is prevented (El Bouhssini, 2018).
b) **Chemical control:** If it is necessary and in order to avoid the disease dissemination at an early stage of the disease, it is advised to spray with mancozeb, mancozeb added to copper or other fungicides used for the control of black scorch disease, like Methylthiophanate added to Maneb. In the case of low levels of infection, chemical treatments are not recommended and only good annual pruning is sufficient (El Bouhssini, 2018).

4.5.Omphalia root rot

Two species of *Omphalia* (*O. tralucida* Bliss and *O. pigmentata* Bliss) are the cause of the disease. The disease, which is known to exist in the USA (California) and in Muritania, has little economic impact for date palm. (Fawcett & Klotz, 1932; Bliss, 1944).

4.5.1. Symptoms

The disease is characterized by the early mortality of the fronds, which is followed by the plant's growth being retarded or stopped, necrosis, and root damage.

4.5.2. Control

As a chemical control, Sachs (1967) advised using one spray of dexon or brestan fungicide every two weeks for a period of eight weeks.

4.6.Vascular fusariosis of the date palm or Bayoud

It is the most dreaded disease affecting the palm groves of North Africa. It appeared around 1870 in the Draa valley north of Zagora (Morocco), spread to Algeria and then to Mauritania. Fusarium wilt of the date palm was first reported in Algeria at Béni Ounif in

1898. It subsequently spread to most palm groves in western Algeria, reaching the M'Zab region in 1949 (Mezaache, 2012).

Fusarium tolerates aerated, moist soil and temperatures that can exceed 35°C. However, in unsuitable conditions, the spores of the fungus transform into chlamydospores to enable it to preserve itself and better resist environmental conditions. Chlamydospores can be preserved in plant debris and soil and can withstand high temperatures of 60°C (Sedra, 2006).

The first phase is contact between the date palm and the pathogen, involving adhesion and surface recognition, followed by a second phase involving penetration through the roots via the raw sap, with the appearance of disease symptoms. Finally, when the mycelia invade the palm's terminal bud, they cause dieback and death (El Modafar, 2010). After the tree dies, FOA persists in the form of chlamydospores in the tissues of certain organs, such as roots and leaf rachis (Sidaoui *et al.*, 2018).

4.6.1. Symptoms

4.6.1.1.External symptoms

Bayoud attacks both young and mature palms, as well as their basal shoots. The first visible external symptoms of the disease appear on one or more leaves in the middle crown. Affected leaves take on a leaden tinge (ash-grey) and then fade in a particular way: the pinnae on one side of the leaf begin to turn white, hence the Arabic name "*Bayoud*", derived from Abiad = White, and the disease progresses from the base towards the apex. When the whole of this side has been affected, wilting begins on the other side, this time in the opposite direction, from the tip of the leaf towards its base, until the leaf dies. This vascular disease causes wilting by blocking the circulation of water in the conducting vessels, resulting in browning and the formation of tyloses. In all cases, the disease always advances towards the heart of the tree and the tree dies when the mycelium reaches the terminal bud. The palm may die 6 months to 2 years after the first symptoms appear, depending on the cultivar and planting conditions (Boulenouar *et al.*, 2009).

4.6.1.2.Internal symptoms

A diseased palm that has been uprooted will reveal a small number of diseased, reddish roots, out of all proportion to the damage observed on the tree. These diseased roots correspond to several groups of vascular bundles in the stipe, which have taken on a reddish-

brown color, as have the surrounding parenchyma and sclerenchyma. Towards the base of the stipe, the spots are large and numerous. As they ascend the tree, the colored vascular bundles separate, and the fronds showing external symptoms have a reddish-brown color and highly colored vascular bundles when cut. There is therefore a continuity of vascular symptoms from the roots to the apical leaves of the palm. Symptoms on peduncles, flowers or fruit have never been reported (Boulenouar *et al.*, 2009).

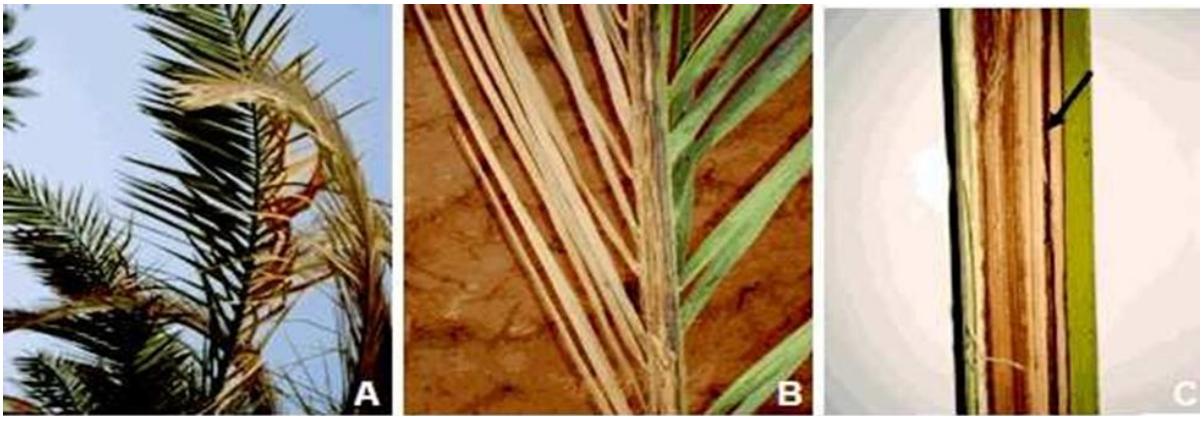


Figure 21: Symptpmatology of fusariosis of the date palm (Chihat 2011).

4.6.2. Control

4.6.2.1.Chemical control

Chemical control may, however, be feasible in the event of the discovery of primary sources of infection in a healthy area. Sources of infection in a healthy area. Antifungal trials undertaken with two powerful pesticides, Chloropicrin and Methyl Breomide, have given good results, a mixture of Benomyl and Methyliophanat can inhibit the growth of the fungus (Zaid *et al.*, 2002).

4.6.2.2.Biological control using micro-organisms

The use of microorganisms is one of the promising alternatives to fungicides, given the ubiquity of these microorganisms, their great diversity and their dissemination in rhizospheric soils (Berg *et al.*, 2005).

The existence of suppressive soils preventing the development of Bayoud disease has been attributed to microorganisms antagonistic to F.o.a., in particular the genera *Pseudomonas*, *Bacillus* and fungi of the genera *Aspergillus* and *Penicillium* (Chakroune *et al.*, 2008). It has been shown that inoculating date palm roots with hypoaggressive strains of F.O.A. (El

Hassani *et al.*, 2004) or mycorrhising them improves the palm's resistance to the pathogen (Jaiti *et al.*, 2008).

The search for micro-organisms living in the same environment, the same ecological niche and the same plant, as in the case of microbial endophytes, represents an alternative to pesticides and other micro-organisms used in biological control to date. Natural colonization by endophytic organisms could be at the origin of the date palm's adaptation to different types of biotic and abiotic stress (Mahmoud, 2017).

Chapter 3: Molecular methods of fungi identification

Fungi are a kingdom unto themselves, with a high degree of biodiversity. Nevertheless, there are fewer species of fungi known to exist than there should be, mainly because fungal species are still identified using antiquated techniques based on morphological traits.

The development of molecular techniques has made significant progress in the study of these organisms' phylogeny possible. (Messaoud and Dhib, 2021). Nucleic acid extraction is the first step in the genetic identification process of the many types. Next, specific regions of the DNA or RNA are amplified by PCR, and finally, fingerprinting techniques are used to analyze the diversity. Molecular techniques are universally applicable and enable the analysis of polymorphism at different levels (differences between strains, species, taxa, etc.) (Ben Slimane, 2016).

1. Genes analyzed to characterize fungal species

1.1 ITS genes

The most often used region in fungus molecular characterization and identification research is ribosomal DNA (rDNA). All organisms include this gene since it is a part of the processes that convert mRNA into proteins (Beaulieu, 2007).

The main advantages of using this region are, firstly, that it is well characterized and documented. Secondly, in eukaryotes, rDNA genes are present in multiple copies present in several copies repeated in tandem in one or more chromosomes, which facilitates amplification. Thirdly, the rDNA gene has loci that evolve at different rates. Each copy is simply made up of three units transcribed into ribosomal RNA a small 18S subunit, a large 28S subunit and a very small 5.8S subunit, and two untranscribed and more variable regions ITS1 and ITS2 that separate the three genes. Since the ribosomal subunit genes are more conserved, they are targeted for the manufacture of primers specific to this region. In addition, the primers targeting them are universal, meaning that they can be used for more than one species (Beaulieu, 2007).

The ITS (Internal Transcribed Spacer) regions are usually quite variable, it is in these regions that markers can be found to distinguish species. However, in some cases, the ITS regions are not variable enough to distinguish species (Beaulieu, 2007).

1.2 The actin gene

Codes for a protein involved in the structure of the cytoskeleton of fungi cells. This gene is a single copy in the genome of most fungi (Beaulieu, 2007)

1.3 The ß-tubulin gene

Part of the tubulin gene family. It codes for the manufacture of a protein essential for the proper functioning of the eukaryotic cell machinery during mitosis. This gene may be present in several copies in the genome (Beaulieu, 2007).

1.4 Elongation factor gene (TEF)

Code for a protein that controls the rate and fidelity of protein synthesis. It is generally present in one copy in the fungal genome (Beaulieu, 2007).

1.5 Histone H3 gene

Codes for one of the four core histone proteins. These play an important role in the organization of eukaryotic DNA and also in gene regulation via post-translational modifications of histone tails. Among these core histones, the gene coding for H3 is used to construct a phylogenetic tree of species that are closely related species. In protists and fungi, the peptide sequence of H3 shows a high degree of interspecific divergence compared with higher organisms. From nucleotide variation in this gene, which is sufficient to differentiate several fungal to differentiate several fungal species (Ozaki, 2017).

1.6 LSU (the Large Subunit)

The LSU "the Large Subunit" region in particular has begun to gain ground. This is because this tool speeds up analyses and gives access to a tool for annotating sequence data (Brown *et al.*, 2014). It contains two hypervariable regions, designated D1 and D2, which are flanked by relatively conserved sequence regions in most fungi. This arrangement allows LSU gene sequences to be aligned for phylogenetic analysis (Liu *et al.*, 2012).

Historically, the D1/D2 region has been used, with or without the corresponding ITS sequence, to identify yeast species (Liu *et al.*, 2012). These divergent domains, or expansion regions, can present a large variation in sequence and length between species (Porter and Golding, 2012)

1.7 SSU (smal subunit)

SSU sequence variation has been used to classify a fungal crop above the genus level and it has done well taxonomically at high levels (Porter and Golding, 2012), SSU rRNA genes were amplified using primers that overlap with the universal primers NS1 and NS8 (Schwarzott and Schüßler, 2001).

Taxonomic identification based on these sequences is more problematic, because it is being generally limited to the genus or family level. This is mainly due to the relative lack of variation within the 18S rRNA genes between closely related fungal closely related fungal species due to the relatively short evolutionary period of kingdom fungi kingdom compared with bacteria (Anderson and Cairney, 2004).

1.8 Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH or gpd) is a gene encoding an enzyme involved in glycolysis and gluconeogenesis (Alhawatema and AlTawaha, 2019). There are several reasons why this gene was selected; Firstly, GAPDH is a crucial enzyme in glycolysis, The gene is known as a housekeeping gene - a gene that is constitutively expressed and is required for cell survival (Explorer, 2009).

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH or gpd), which has been a target for amplification using primers gpd1 and gpd2 (Alhawatema and Al Tawaha, 2019).

Part 2: Experimental section

Chapter 1: Materials and methods

1. Materials

1.1 Plant materials

The fungal strains were previously isolated from date palms exposing divers symptoms.

For the pathogenicity test, we worked on healthy date palm seedlings, aged from two to four months.

1.2 Fungal material

Sixteen (16) fungal strains isolated by M^{r} Y. Djellid from date palms show symptoms of leaves and fruit pathology (necrosis of various colors and shapes).

2. Samples

The samples were taken during the month of November 2018, from date palms showing symptoms of pathology on the leaves (Jride) (necrosis of different colors and shapes). Sampling concerned 06 palm cultivars in the Wilaya of Ghardaïa.

Figure 22 : Examples of symptoms observed at the sampling site.

3. Isolation and identification of fungi

3.1 Preparation of samples

The palm samples are prepared according to the following main steps:

- Preparation of small sticks (3 to 4 mm) under aseptic conditions.
- Disinfection of the sticks in 5% bleach for 3 minutes.
- Rinse with sterile water (twice for 2 minutes) then dry.
- Refresh the sticks to allow contact between the fungus in the necrosis and the culture medium.

3.2 Isolation and purification of fungi

Isolation of the fungal flora from the palm sticks was performed out on PDA agar.The dishes were then incubated at 25°C in an incubator (Memmert),for 5 to 8 days.The isolates were then aseptically transferred to a new PDA medium to maintain them in pure culture.

4. Methods

Our adopted protocol has three main steps, namely isolates identification, pathogenicity tests and phylogenetic analyses (Figure 23).

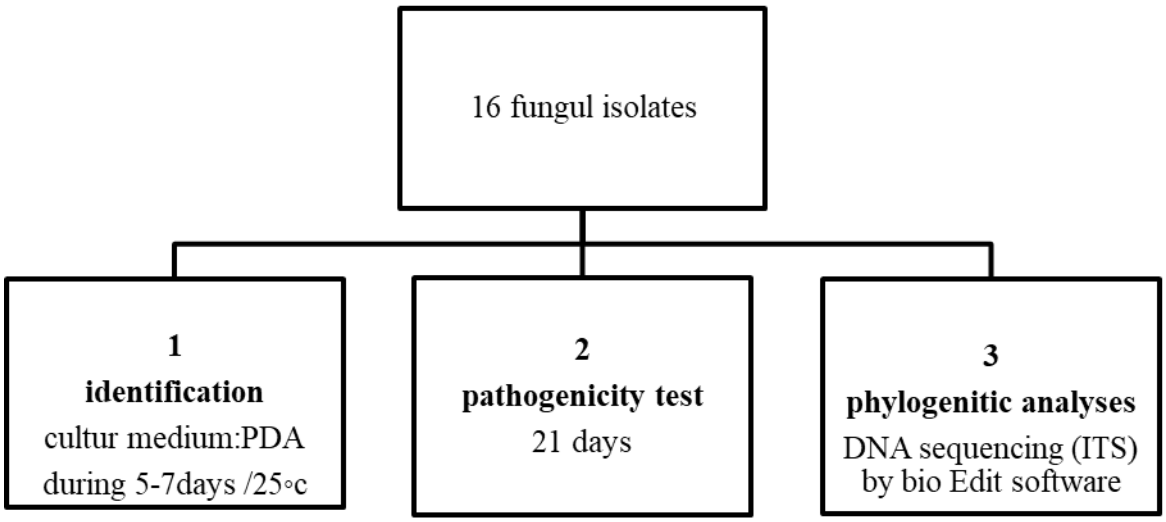


Figure 23: Protocol workflow.

4.1 Culture medium preparation

The culture medium used is PDA (Potato Dextrose Agar). Common microbiological culture, a product based on potato infusion and dextrose. It is used to cultivate and stimulate the growth of various isolates of fungi. 39g of culture medium powder per liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until

complete dissolution, and then sterilize it in autoclave at 118-121°C for 15min. After cooling to 45_50°C; the medium is well mixed and dispensed into Petri plates.

Figure 24: Main steps of culture medium preparation.

4.2 Transplanting

Using a sterile Pasteur pipette, the isolates were selected and transferred into Potato Dextrose Agar (PDA). After five (5) to seven (7) days at 25 °C in the darkness.

Figure 25: Transplanting processes.

2.1 Macroscopic and microscopic identification

a- Macroscopic identification: The PDA fungal cultures of 7 days have been exanimated for the consistency (fluffy, woolly, cottony, flaky, powdery, etc.), the color, the size, the pigmentation and the shape of contour of their colonies.

b- Microscopic identification: The microscopic examination of the fungal isolates has been performed by spreading a small fragment of the mycelium between slide and coverslip, using lactic acid for a clear observation; using (NOVEX) optic microscope.
The shape, the size and the organization of the conidia and the spores have been noted

5 Molecular methods

5.1 DNA extraction method

According Möller et *al.*, 1992 the method consists of collecting the mycelium and placing it in a 2.2 ml eppendorf tube containing a 500 µl volume of sterile TES buffer (100 mM Tris, pH 8.0, 10mM EDTA,2% SDS).This buffer facilitates solubilisation and protection of the DNA.The eppendorfs are heated to 100°C for 3 min and then transferred directly to ice to cause a thermal shock that releases the DNA from the cell.The next step is to add 10 µg of proteinase K at a concentration of 10 mg/ml to destroy the contaminating proteins and those bound to the DNA.The mixture is heated to 65°C for 30 minutes with moderate agitation during heating.Next, the salt concentration was increased by adding 140 µl of 5M NaCl solution and 65 µl of 10 CTAB.The tube should be incubated at 65°C for 30 min with occasional moderate agitation.Next, 1 ml of a chloroform-alcohol-isoamyl solution (24:1) was added to the mixture, followed by homogenisation by inversion for 1 min and incubation for 30 min in ice.Le mélange est centrifugé à une vitesse de rotation de 12 000 rpm à 4 °C durant 10 min. At this point, the supernatant with a volume of approximately 800 µm is transferred to a new 1.5 ml eppendorf tube.To this is added 225 µl of 5 M NH4OAc, mixed thoroughly and incubated on ice for 30 min.After a second 10 min centrifugation at 12,000 rpm at 4°C, the supernatant (1000 µl) was transferred to a new 1.5 ml tube.To precipitate the DNA, 800 µl of cold isopropanol was added and homogenized before being incubated for 30 minutes on ice.This step is followed by centrifugation (12,000 rpm at 4°C for 10 min) to precipitate the DNA and discard the supernatant.The precipitated DNA is rinsed twice with cold 76% ethanol and centrifuged for 10 min.Finally, the DNA is dried and dissolved in 50 µl of TE.The concentrated DNA solution is kept at -20°C, while its dilutions are kept at 4 or even -20°C.

5.2 Amplification of the ITS region and sequencing

To amplify the ITS region, the primer pair ITS1 and ITS4 is used (White *et al.*, 1990). Amplification is performed in a final volume of 21 µl which consists of a mixture of 50 to 100

ng of gDNA, 1 PCR buffer, 50 pmol of each primer, 200 µM of each dNTP, 2 mM MgCl2, 1 U Taq DNA polymerase and ultrapure water.The mixture is well homogenised and a witness containing all the components of the mixture, except the gDNA, is also prepared.All amplification reactions were performed in a BIO-RAD MJ Mini Thermal CyclerTM (Bio-Rad, Hercules, USA).The DNA amplification conditions for 35 PCR cycles are presented in the table below.

After amplification, the PCR products were analyzed by electrophoresis on a 1.8% agarose gel containing a solution of GelRed (2 µl / 80 ml of gel).The purpose of this step is to separate the DNA fragments and estimate their size.For each amplificate, a volume of 4 µl is deposited in gel wells already immersed in TAE buffer (1×) (40 mM Tris, 40 mM acetate, 1 mM EDTA, pH 8).Migration is ensured by an electric current of 100V for 1 h.The size of the DNA fragments separated is determined by comparison with the standard concentration range of "1 Kb DNA Ladder" size marker DNA deposited at a rate of 10 µl in each gel.

The amplification bands produced were visualised under UV illumination using the“Molecular Imager Gel Doc XR System”(Bio-Rad, Hercules, California, USA).

The amplifiates obtained are submitted to Eurofins (Germany) for purification and sequencing.

5.3 Sequence editing and searching using BLASTN

After receipt of the raw sequences, they were visualised and corrected using the BioEdit v 7.0.4.1 sequence alignment editor (Hall, 1999).all sequences are manually checked and arrangements of nucleotides at ambiguous positions are clarified.

Preliminary taxonomic positioning of isolates is ensured by a sequence search using BLASTn (Basic local alignment search tool) (Altschul *et al.,* 1990).In addition, other reference sequences closely related to the sequences of our isolates were removed from the GenBank platform. These sequences are included in the alignment matrix.

Phylogenetic analysis

DNA Sequences of ITS were checked and manually adjusted were necessary using BioEdit v.7.0.4.1 (Hall, 1999). Sequences alignment were conducted through the online version of the

multiple sequence alignment program (MAFFT) v.7 (Katoh *et al.*, 2019) using the default settings. The phylogenetic analysis was conducted through Maximum Likelihood (ML) method using MEGAX (Kumar *et al.*, 2018). The best-fit evolutionary model was determined by the software. ML analysis was conducted using heuristic searches consisted of 1000 step utilizing the Nearest-Neighbour-Interchange (NNI) algorithm with a Neighbour-Joining starting tree automatically generated. MP analysis, the Tree-Bisection-Regrafting (TBR) algorithm was applied. One thousand (1000) bootstrap replications were conducted to evaluate the generated MP trees robustness. *Stemphylium botryosum* ATCC 42170 and *S. vesicarium* ATCC 18521 were used as outgroup taxa.

6. Pathogenicity test

The pathogenicity test was performed according to the protocol cited by Sezer and Dolar, (2015) and Al-Sadi (2016) on healthy date palm seedlings. The plant tissue was inoculated by placing a mycelial disc from an actively growing edge of the 5–7-day old fungal culture. Leaves were kept at room temperature 25°C for 21 days, with a 12h photoperiod and regular watering. Trials were carried out in triplicate. The development of necroses was monitored by measuring every 7, 14 and 21 days. The Koch's postulate was confirmed by the re-isolation of the pathogenic strains.

Lesion length data were subjected to statistical analysis to determine the homogeneity of the variance of the dataset by performing analysis of variance (ANOVA) and means comparison by Fisher's Least Significant Difference (LSD) test at $P \leq 0.05$.

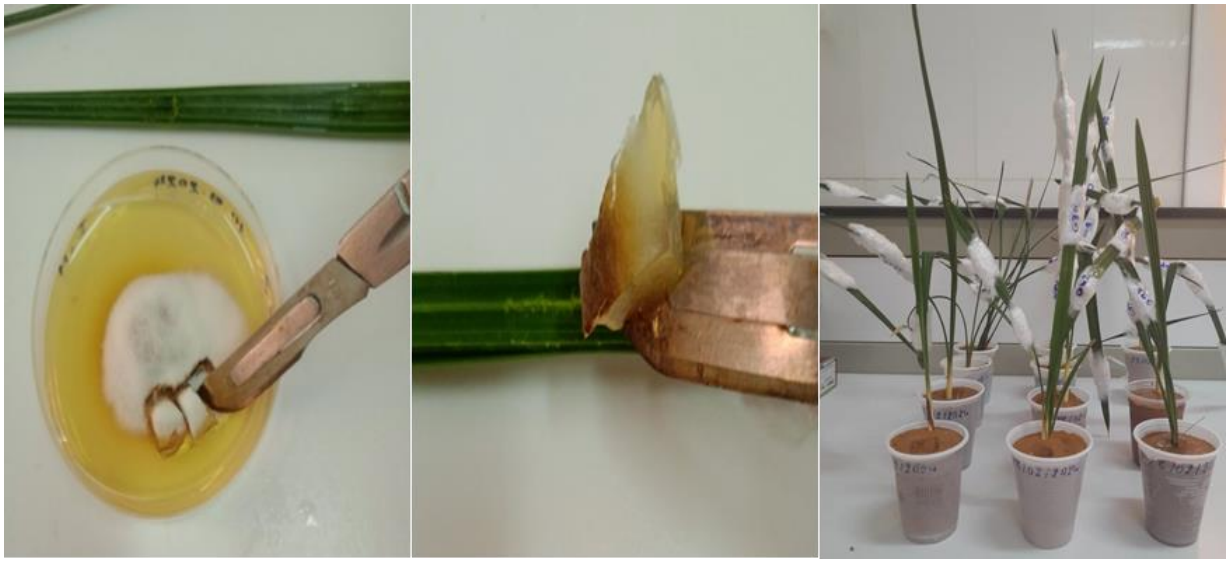


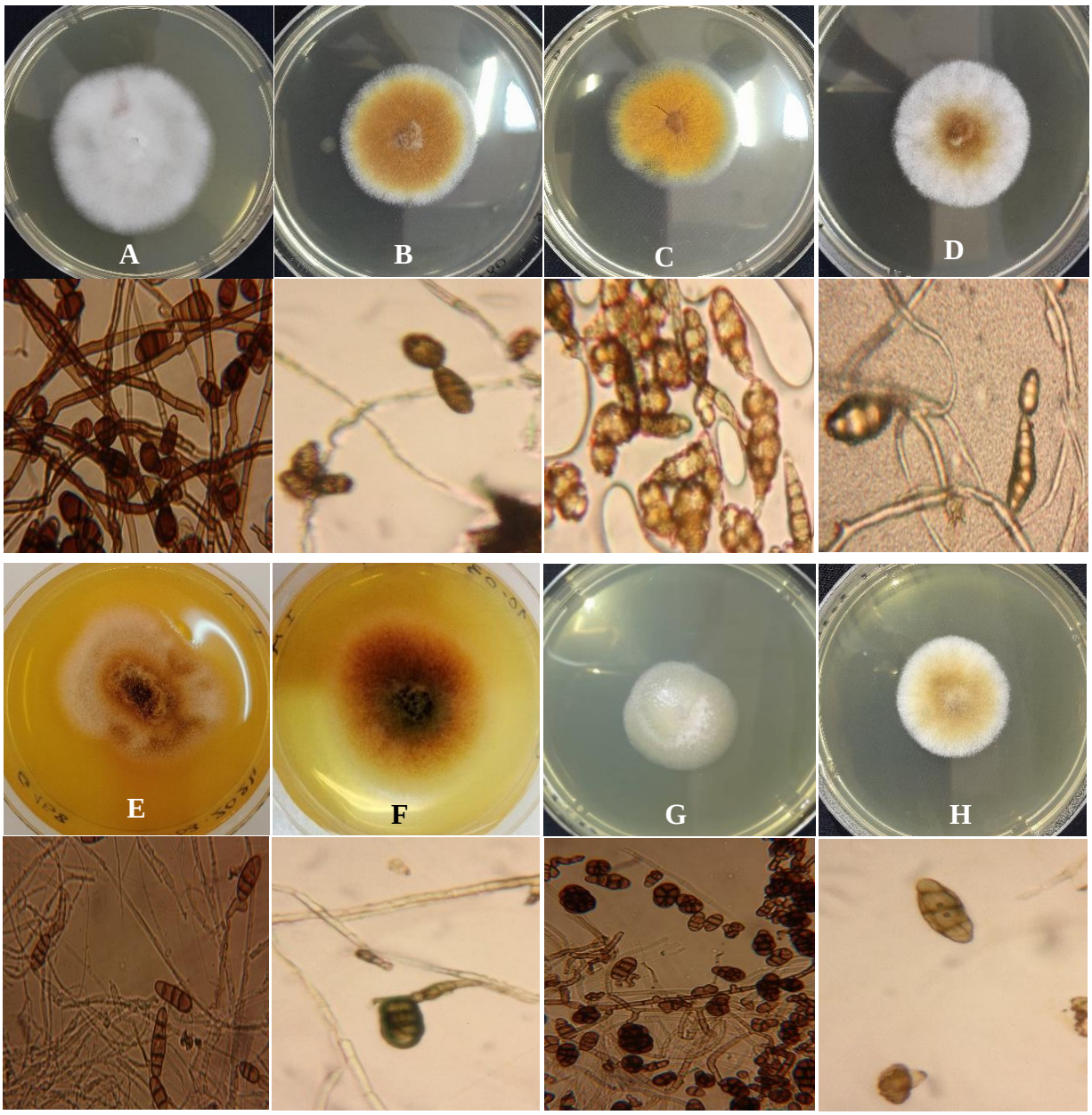
Figure 26 : Pathogenicity test processes.

Chapter 2: Results

1. Isolates identification

1.1 Morphological identification

The fungal isolates were identified based on macroscopic characteristics, appearance of the colonies (color, outline, texture, etc.) and the microscopic characteristics of the mycelia and conidia. Allowing us to identify the fungal groups associated with the different palm tree decline.



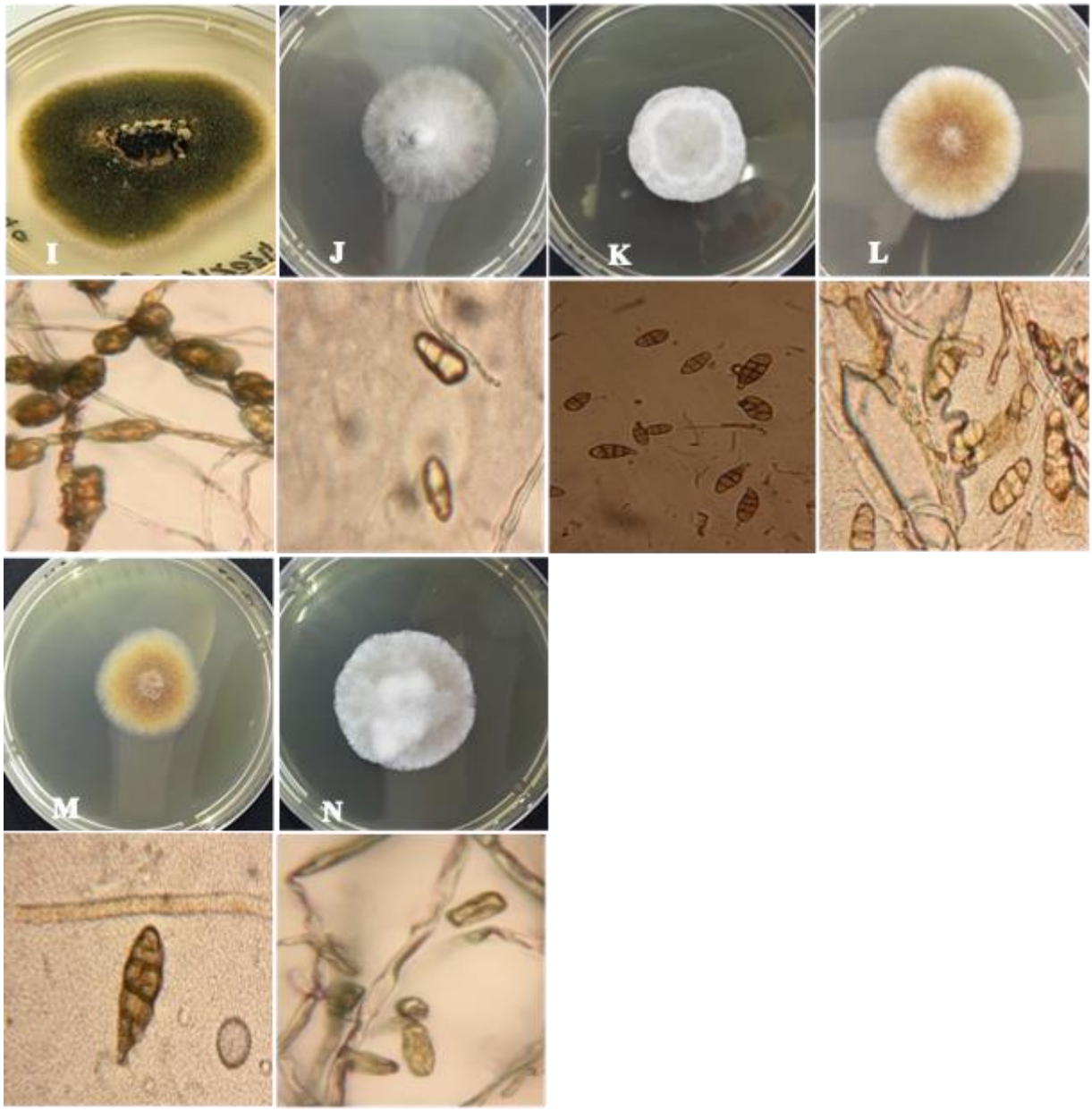


Figure 27: Marco- and micro morphological identification.
A:GH12,**B**:GH5,**C**:GH13,**D**:GH6,**E**:GH16,**F**:GH9,**G**:GH8,
H:GH7,**I**:GH15,**J**:GH10,**K**:GH4,**L**:GH11 **M**:GH14,**N**:GH3

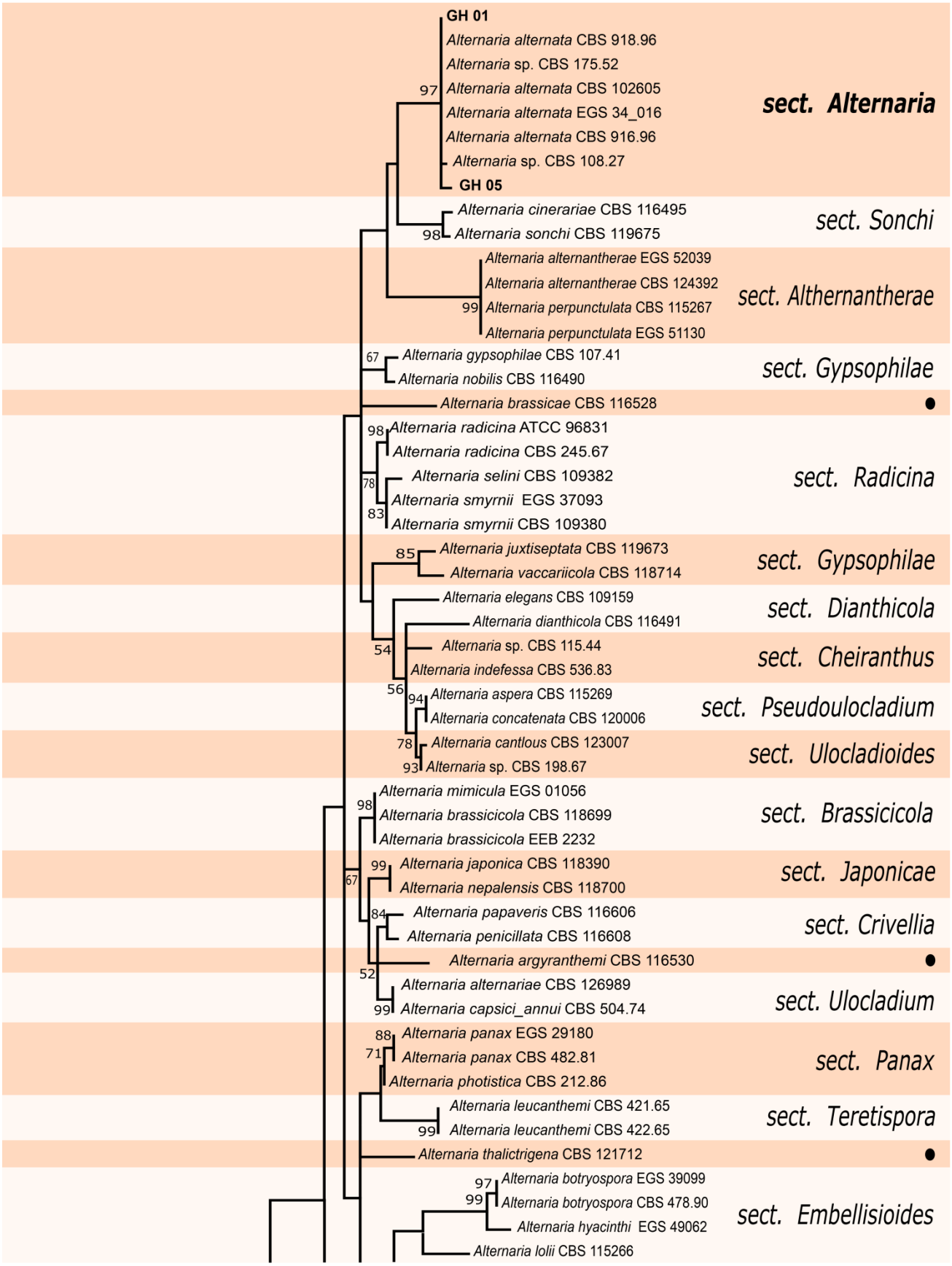
1.2 Phylogenetic analysis

The sequence alignment ITS region contained 590 characters of which 411 were conserved, 8 were excluded, 31 were variable and parsimony-uninformative and 140 were parsimony-informative. Maximum parsimony (MP) analyses of the dataset produced a single most parsimonious tree (CI= 0.373, RI= 0.835 and HI=0.627), which resulted in the identification of the strains. Furthermore, maximum likelihood analyses on concatenated dataset yielded a phylogenetic tree (Figure 26) which was similar with maximum parsimony tree.

Based on the results of phylogenetic analyses, the isolated *Alternaria* strains grouped within the species *A. Alternata* (4 isolates), *A. Malorum* (4isolates), *A.Infectoria* (3isolates) and *A. Cumini* with 5 isolates .

Table 4 : List of *Alternaria* species isolated from date palms

Isolate	Species
GH1 GH5 GH10 GH15	*A. alternata*
GH6 GH9 GH4 GH12	*A. malorum*
GH2 GH14 GH3	*A. infectoria*
GH7 GH8 GH11 GH13 GH16	*A. cumini*


GH 01
Alternaria alternata CBS 918.96
Alternaria sp. CBS 175.52
97
Alternaria alternata CBS 102605
Alternaria alternata EGS 34_016
Alternaria alternata CBS 916.96
Alternaria sp. CBS 108.27
GH 05
sect. Alternaria
Alternaria cinerariae CBS 116495
98
Alternaria sonchi CBS 119675
sect. Sonchi
Alternaria alternantherae EGS 52039
Alternaria alternantherae CBS 124392
99
Alternaria perpunctulata CBS 115267
Alternaria perpunctulata EGS 51130
sect. Althernantherae
67
Alternaria gypsophilae CBS 107.41
Alternaria nobilis CBS 116490
sect. Gypsophilae
Alternaria brassicae CBS 116528
98
Alternaria radicina ATCC 96831
Alternaria radicina CBS 245.67
78
Alternaria selini CBS 109382
Alternaria smyrnii EGS 37093
83
Alternaria smyrnii CBS 109380
sect. Radicina
85
Alternaria juxtiseptata CBS 119673
Alternaria vaccariicola CBS 118714
sect. Gypsophilae
Alternaria elegans CBS 109159
Alternaria dianthicola CBS 116491
sect. Dianthicola
54
Alternaria sp. CBS 115.44
Alternaria indefessa CBS 536.83
sect. Cheiranthus
56
94
Alternaria aspera CBS 115269
Alternaria concatenata CBS 120006
sect. Pseudoulocladium
78
Alternaria cantlous CBS 123007
93
Alternaria sp. CBS 198.67
sect. Ulocladioides
Alternaria mimicula EGS 01056
98
Alternaria brassicicola CBS 118699
Alternaria brassicicola EEB 2232
sect. Brassicicola
99
67
Alternaria japonica CBS 118390
Alternaria nepalensis CBS 118700
sect. Japonicae
84
Alternaria papaveris CBS 116606
Alternaria penicillata CBS 116608
sect. Crivellia
Alternaria argyranthemi CBS 116530
52
Alternaria alternariae CBS 126989
99
Alternaria capsici_annui CBS 504.74
sect. Ulocladium
88
Alternaria panax EGS 29180
71
Alternaria panax CBS 482.81
Alternaria photistica CBS 212.86
sect. Panax
Alternaria leucanthemi CBS 421.65
99
Alternaria leucanthemi CBS 422.65
sect. Teretispora
Alternaria thalictrigena CBS 121712
97
Alternaria botryospora EGS 39099
99
Alternaria botryospora CBS 478.90
Alternaria hyacinthi EGS 49062
Alternaria lolii CBS 115266
sect. Embellisioides

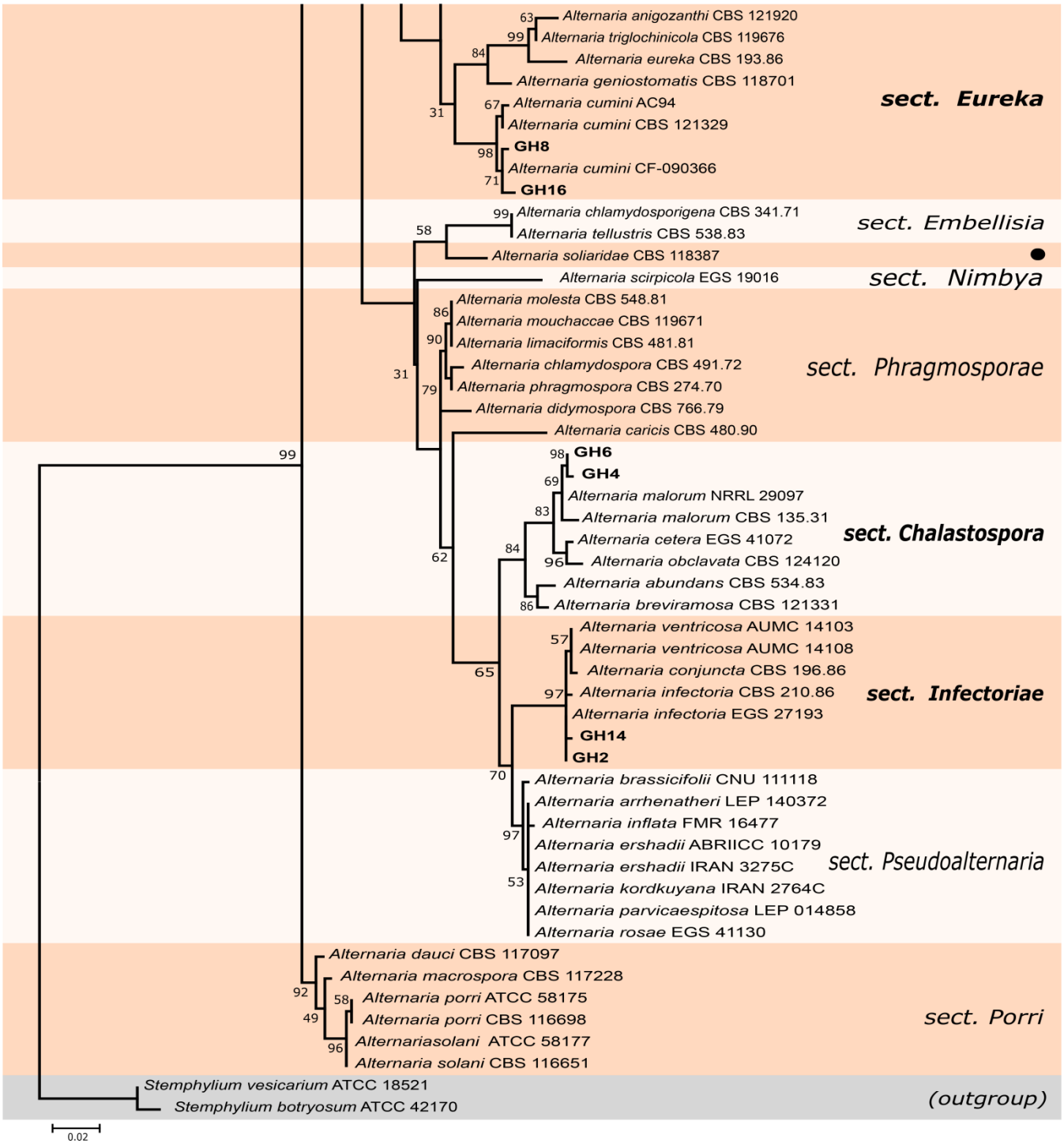


Figure 28: Maximum Likelihood phylogenetic tree inferred from ITS sequence data with 1000 rapid bootstrap inferences. Bootstrap support values are indicated near the corresponding nodes. The scale bar indicates the expected changes per site. Identified species are indicated in bold face. The tree is rooted to *Stemphylium botryosum* ATCC 42170 and *S. vesicarium* ATCC 18521.

2. Pathogenicity test

2.1 A. alternata: Significant differences were detected between *A. alternata* strains and inoculation period (F=5.694; P<0.0001) based on one-factor ANOVA and Fisher's (LSD) *post-hoc* test (LSD-value = 10.584) with α = 0.05.

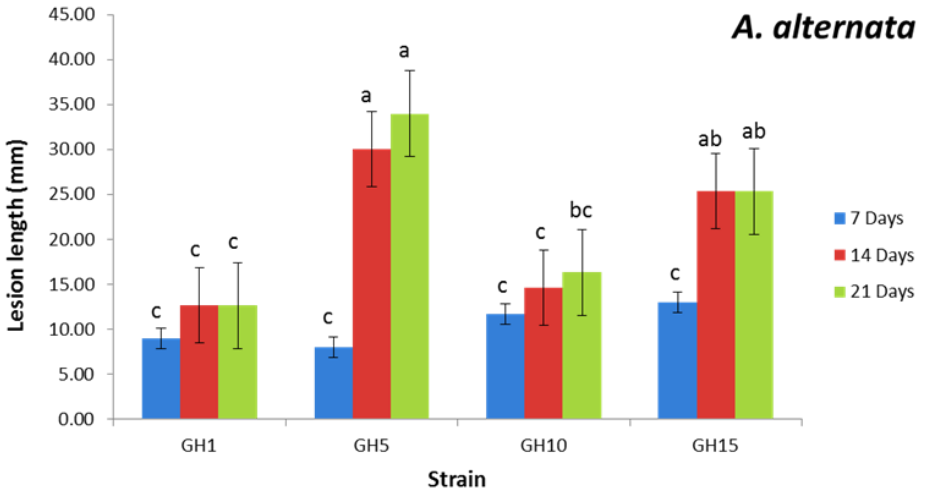


Figure 29 : Means lesion length (mm) caused by *A. alternata* strains (GH1, GH5, GH10 and GH15) associated to date palm disease. Error bars represent the standard error of means. Significant differences are represented with different letters above columns according to Fisher's Least Significant Difference test (α ≤ 0.05).

A. cumini: No significant differences were detected between *A. cumini* strains and inoculation period (F=0.948; P=0.528) based on one-factor ANOVA and Fisher's (LSD) *post-hoc* test (LSD-value = 13.466) with α = 0.05.

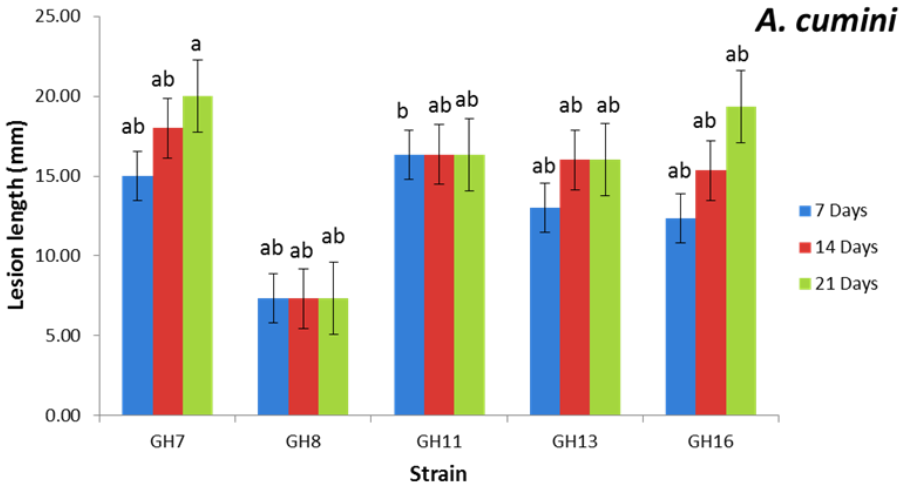


Figure 30 : Means lesion length (mm) caused by *A. cumini* strains (GH7, GH8, GH11, GH13 and GH16) associated to date palm disease. Error bars represent the standard error of means. Significant differences are represented with different letters above columns according to Fisher's Least Significant Difference test (α ≤ 0.05)

2.2 A. infectoria: No significant differences were detected between *A. infectoria* strains and inoculation period (F=1.047; P=0.439) based on one-factor ANOVA and Fisher's (LSD) *post-hoc* test (LSD-value = 16.354) with α = 0.05.

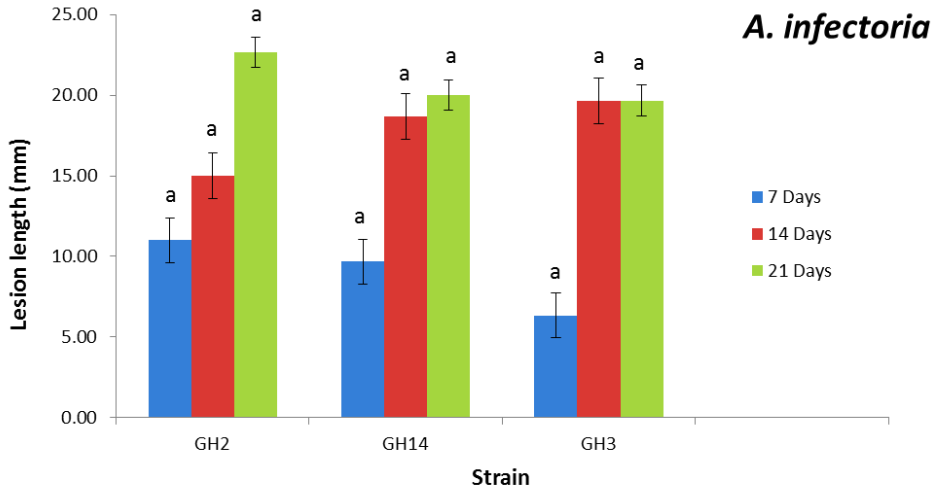


Figure 31. Means lesion length (mm) caused by *A. infectoria* strains (GH2, GH14 and GH3) associated to date palm disease**.** Error bars represent the standard error of means. Significant differences are represented with different letters above columns according to Fisher's Least Significant Difference test (α ≤ 0.05)

2.3 Alternaria malorum: No significant differences were detected between *A. malorum* strains and inoculation period (F=1.275; P=0.296) based on one-factor ANOVA and Fisher's (LSD) *post-hoc* test (LSD-value = 14.548) with α = 0.05.

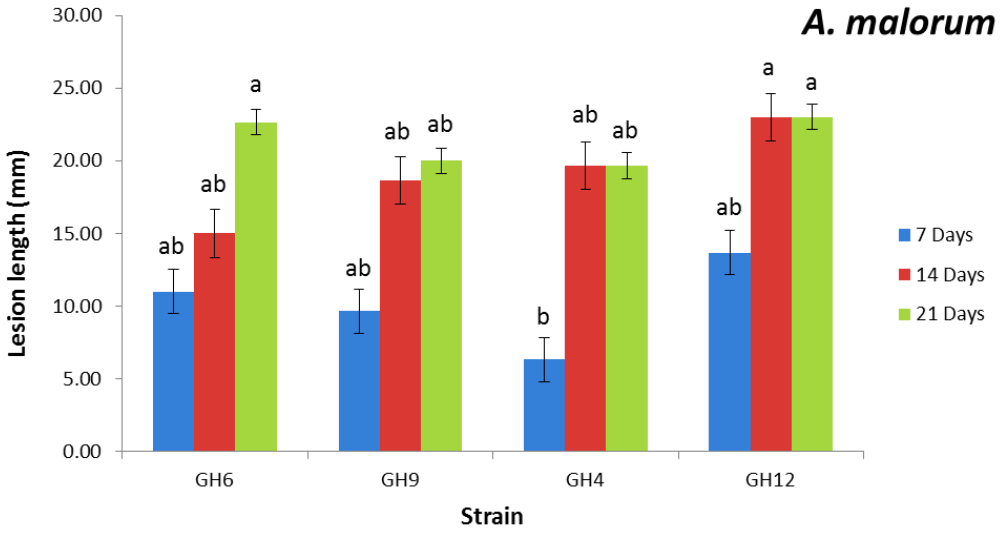


Figure 32. Means lesion length (mm) caused by *A. malorum* strains (GH6, GH9, GH4 and GH12) associated to date palm disease. Error bars represent the standard error of means. Significant differences are represented with different letters above columns according to Fisher's Least Significant Difference test (α ≤ 0.05).

Chapter 3: Discussion

Sixteen (16) fungal isolates have been molecularly studied for their pathogenicity for the objective of knowing their taxonomic position. In the palms examined, we have different pathological symptoms characterized by leaves with brown and white spots, also observed by Al-Nadabi *et al.*, (2020) in Oman and not previously observed in other regions of Algeria. Based on molecular analysis and macroscopic and microscopic identification, we found that all isolates belonged to the genus *Alternaria*, grouped into four genera, *A. cumini*, the genus with the highest number of isolates.

Alternaria was recognized as the most abundant plant pathogen (Delgado-Baquerizo *et al.*, 2020). *Alternaria* species are ubiquitous pathogens, reported as a major causing agent of leaf spot disease for many crops worldwide, such as cowpea leaf spot disease and wheat and barley black head in Iran (Atashi and Fotouhifar, 2022; Poursafar *et al.*,2018), Kangi Palm (*Cycas revoluta*) in Pakistan (Faraz *et al.*, 2020). *Alternaria* species has also been reported as a fungus associated with Leaf spot of Aloe vera in different parts of the world; namely-leaf spot of *A. barbadensis* in India (Chavan and Korekar, 2011) , in Lousinia, USA (Silva and Singh, 2012), in Pakistan (Bajwa *et al.*, 2010). In Algeria, *Alternaria* was recorded by Bessadat *et al.* (2017) as agent of early blight epidemics on tomato and other Solanaceae crops.

Alternaria genius was reported in man researches as a common pathogen, causing several diseases including leaf spot, leaf blight, black point and many other diseases on date palm in Saudi Arabia (Bokhary,2010), Oman (Al-Nadabi *et al.*,2018, 2020), Qatar (El Badawy 2019), Iraq (Khudhair *et al.*, 2015) and Tunisia (Rabaaoui *et al.*, 2022). While, the presence of *Alternaria* species on date palm has been not reported yet in Algeria.

Alternaria alternata was recorded in many researches as pathogen of various crops such as brown spot disease of potato and tomato (Schmey *et al.*,2024). In addition, *A. alternata* may infect other organs of the pomegranate tree: in Turkey, and Spain, by causing lesions on leaves, flowers, and young fruits (Ezra et al. 2010; Pala et al. 2009;Tziros et al. 2008). In Algeria

A. alternata was reported as cause of leaf spot and blight disease of Solanaceae (Bessadat *et al.*,2019). This species was also isolated from Leaf Spot of date palm in Iraq (Manea *et al.*, 2023), Oman (Al-Nadabi *et al.*, 2020) and Saudi Arabia (Alghanem *et al.*, 2023). Particularly, *A. alternata* was proved as the major pathogen species of date palm in Tunisia (Rabaaoui *et al.*, 2022).

Alternaria cumini was reported as major causal agent of leaf spot disease of broccoli in Pakistan and (Javaid, 2018).

Alternaria malorum, was mentioned as a causal agent of cowpea leaf spot disease and declining Persian oak trees in Iran (Atashi and Fotouhifar, 2022; Alidadi et *al*., 2018). It was also isolated from barely straw and stored grain in Pakistan and Turkey (Goetz and Dugan, 2006) and linked with black head disease of wheat in Kazakhstan.

Alternaria infectoria was isolated from many cereals such as barley, maize and wheat grain and also from ornamental plants (Andersen *et al*., 2009; Perelló *et al*.,2008) and pyrethrum (Moslemi *et al*., 2017).

Conclusion

Date palm is the most economically important fruit tree in drylands, where it is cultivated in a large area in many countries including Algeria. This tree is attacked by many ravagers, where fungi represent the major threat for the life and productivity of the whole palm groove. The identification of these fungal pathogens is the first step for their control.

The main objective of this work was to identify the fungal groups responsible for different disease's symptoms visible on date palm in the region of Ghardaia, using molecular analysis by DNA sequencing (ITS region) followed by the confirmation of their pathogenicity on date palm seedlings.

The major fungal group isolated was *Alternaria* presented by sixteen isolates grouped in four species, namely *A. alternata, A. cumini, A. infectoria* and *A. malorum*. All the isolates showed a pathogenic ability on date palm seedlings, with variable ranges, where *A. alternata* revealed the most pathogen and *A.malorum* is less pathogen.

This study deserves to be completed firstly by enlarging the study area to cover other phoenicicole regions in Algeria, then by extending the research to identify the other fungal groups linked to different disease's symptoms of date palm and finely Deepening the taxonomic study by sequencing other key genes (GAPDH, TEF, LSU, SSU…).

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Annexes

Table 5 results of pathogenicity test lesion length (mm)

Code	lesion length (mm)								
	7Days			14 Days			21 Days		
GH1	*9*	*8*	*10*	*15*	*12*	*11*	*15*	*12*	*11*
GH2	*18*	*11*	*17*	*20*	*13*	*26*	*25*	*37*	*15*
GH3	*5*	*9*	*4*	*15*	*10*	*20*	*28*	*18*	*25*
GH4	*7*	*8*	*4*	*30*	*25*	*4*	*30*	*25*	*4*
GH5	*5*	*9*	*10*	*40*	*15*	*35*	*43*	*22*	*37*
GH6	*9*	*13*	*11*	*15*	*20*	*10*	*19*	*12*	*37*
GH7	*14*	*16*	*15*	*16*	*21*	*17*	*16*	*17*	*27*
GH8	*7*	*7*	*8*	*7*	*7*	*8*	*7*	*7*	*8*
GH9	*12*	*10*	*17*	*29*	*10*	*17*	*33*	*10*	*17*
GH10	*12*	*10*	*13*	*15*	*15*	*14*	*17*	*15*	*17*
GH11	*12*	*15*	*22*	*15*	*22*	*12*	*15*	*22*	*12*
GH12	*12*	*15*	*14*	*30*	*20*	*19*	*30*	*20*	*19*
GH13	*12*	*15*	*15*	*12*	*15*	*21*	*12*	*15*	*21*
GH14	*7*	*9*	*10*	*14*	*13*	*13*	*14*	*13*	*13*
GH15	*13*	*13*	*13*	*30*	*15*	*31*	*30*	*15*	*31*
GH16	*10*	*11*	*16*	*13*	*14*	*19*	*17*	*20*	*21*

ITS sequences:

GH1

ATCATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTGCTG
AATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCCACCA
CTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAATA
ATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAAC
GCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTAC
CCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCC
TTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTC
TCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTTCAACTTTTGACCTCGG
ATCAGGTAGGGATA

GH14

TACACAATAACAAGGCGGGCTGGACACCCCCCGCTGGGCACTGCTTCACGGCGTG
CGCGGCGGGGGCCGGCCCTGCTGAATTATTCACCCGTGTCTTTTGCGTACTTCTTG
TTTCCTGGGTGGGCTCGCCCGCCCTCAGGACCAACCACAAACCTTTTGCAATAGC
AATCAGCGTCAGTAACAACGTAATTAATTACAACTTTCAACAACGGATCTCTTGG
TTCTGGCATCGATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGA
ATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAG
GGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTC
TTTTGTCTCCAGTTCGCTGGAGACTCGCCTTAAAGTCATTGGCAGCCGGCCTACTG
GTTTCGGAGCGCAGCACAAGTCGCGCTCTTTGCCAGCCAAGGTCAGCGTCCAGCA
ACCCTTTTTTTTCAACCTTTGACCTCGGATCAGGTAGGGATACC

GH16

ACACAATAAGAAGGCGGGCTGGGCCCCCTCCGGGGGACCAGCCTTGCTGAATTA
TTCACCCGTGTCTTTTGCGTACTTCTTGTTTCCTGGGCGGGCTCGCCCGCCACCAG
GACAAACCATAAACCTTTTGCAATTGCAATCAGCGTCAGTAACAAACATAATAAT
TACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAA
ATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
ACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACCC
TCAAGCTCTGCTTGGTGTTGGGCGTCTTTGTCTTCCCCAGAAGACTCGCCTTAAAG
TCATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCGCTCTCTTCC
AGCCAAGGTCAGCGTCCACAAAGCCTTTTTTCAACTTTTGACCTCGGATCAGGTA
GGGATACC

GH2

AGCTGGACACCCCCCGCTGGGCACTGCTTCACGGCGTGCGCGGCGGGGCCGGCCC
TGCTGAATTATTCACCCGTGTCTTTTGCGTACTTCTTGTTTCCTGGGTGGGCTCGCC
CGCCCTCAGGCCAACCACAAACCTTTTGCAATAGCAATCAGCGTCAGTAACAACG
TAATTAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAAC
GCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCT
TTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCA
TTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTTTGTCTCCAGTTCGCTGGA
GACTCGCCTTAAAGTCATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAG
TCGCGCTCTTTGCCAGCCAAGGTCAGCGTCCAGCAAGCCTTTTTTTTTCAACCTTT
GACCTCGGATCAGGTAGGGATACC

GH4

TTTATTACACAATACGAAGGCGGGCTGGACAAACCCCCCTAGCTGGGCACTGCTT
CACGGCGGTGCGCGGTTTGGGTGGCCGGCCCTGCTGAACTATTCACCCGTGTCTT
TTGCGTACCTCTTGTTTCCTGGGCGGGCTCGCCCGCCACCAGGACCAACCCATAA
ACCTTTTTGTAATAGCAATCCGCGTCAGTAAACAATGTAATCAATTACAACTTTCA
ACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATACGT
AGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
TTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGC
TTGGTGTTGGGCGTCTTTTGTCTCCAGCTTGCTGGAGACTCGCCTTAAAGTCATTG
GCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCGCTCTCTTCCAGCCAA
GGTCAGCGTCCAGCAAGCCTTTTTTTCAACCTTTGACCTCGGATCA

GH5

ATCATTACACAAATATGAAGGCGGGCTGGACCCTCCCGGGGTTACAGCCTTGCTG
AATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCCACCA
CTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAATA
ATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAAC
GCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTAC
CCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCC
TTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTC
TCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTCAACTTTTGACCTCGGA
TCAGGTAGGGATACC

GH6

ATTACACAATACGAAGGCGGGCTGGACAAACCCCCCTAGCTGGGCACTGCTTCAC
GGCGGTGCGCGGTTTGGGTGGCCGGCCCTGCTGAACTATTCACCCGTGTCTTTTGC
GTACCTCTTGTTTCCTGGGCGGGCTCGCCCGCCACCAGGACCAACCCATAAACCT
TTTTGTAATAGCAATCCGCGTCAGTAAACAATGTAATCAATTACAACTTTCAACA
ACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATACGTAGT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTT
GGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTG
GTGTTGGGCGTCTTTTGTCTCCAGCTTGCTGGAGACTCGCCTTAAAGTCATTGGCA
GCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCGCTCTCTTCCAGCCAAGGT
CAGCGTCCAGCAAGCCTTTTTTTCAACCTTTGACCTCGGATCA

GH8

ATCATTACACAATAAGAAGGCGGGCTGGGCCCTCCCCGGAGGACCAGCCTTGCTG
AATTATTCACCCGTGTCTTTTGCGTACTTCTTGTTTCCTGGGCGGGCTCGCCCGCC
ACCAGGACAAACCATAAACCTTTTGCAATTGCAATCAGCGTCAGTAACAAACATA
ATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA
GCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
AACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTT
GTACCCTCAAGCTCTGCTTGGTGTTGGGCGTCTTTGTCTTCCCAGAAGACTCGCCT
TAAAGTCATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCGCTCT
CTTCCAGCCAAGGTCAGCGTCCACAAAGCCTTTTTTTCAACTTTTGACCTCGGATC
AGGTAGGGATACC

Potato Dextrose Agar Composition

Diced potatoes.....300.0 g

Glucose..... 20.0 g

Agar……..... 15.0 g

DI Water……. 1000.0 mL

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قسم البيولوجيا

Université de Ghardaïa

Ghardaïa le : 01/07/2024

Rapport : Correction du mémoire

Enseignant (e) (s) Chargé (e) de la correction :

Nom et prénom l'examinateur 1 et Signature	Nom et prénom de l'examinateur 2 et Signature	Nom et prénom du président de Jury et Signature
Mme Maidi Leila 		

Thème :

Taxonomy and pathogenicity of fungal strains isolated from date palm in the region of Ghardaia

Après les corrections apportées au mémoire, L (es)'étudiant (s) (es) :

LAKEHAL Imane et KHOUDIRI Meriem

Est (sont) autorisé (es) à déposer le manuscrit au niveau du département.

Président du Jury

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		BAKLI Mahfoud

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Président du Jury