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TO OBTAIN THE MASTER'S DIPLOMA IN BIOTECHNOLOGY

Domain: SNV **Sector:** BIOTECHNOLOGY

SPECIALITY: Microbial biotechnology

THEME

Phytochemical and microbiological study of one traditional variety of date palm (*Phoenix dactylifera L.*) from southwest Algeria (Taghit national park)

Presented by:

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Defended on 03/07/2024 before the jury composed of :

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Thanks

We begin by thanking God the most powerful for giving us the will, the courage and the patience necessary to carry out this modest work.

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Finally, we would like to express our sincere thanks to all the people who contributed, whether directly or indirectly, to the completion of this work. May they find here our deep gratitude and our sincere thanks.

Dedication

To my mother,

To my father,

You have spent yourselves for me without counting. I thank you for all the sacrifices that everyone has made to allow me to reach this stage of my life, with all my tenderness.

To my dear brother and my dear sisters,

All my friends,

I would like to express my deep gratitude.

Finally, I dedicate this thesis to all my teachers and my colleagues in my class (Microbial biotechnology).

Ayoub

Abstract

The providence tree of desert regions is the date palm (*Phoenix dactylifera L.*). It offers a variety of products, such as dates, a food with a rich energy value, and its seeds are rich in various important biochemical and mineral substances. The aim of this study was to analyze the phytochemical and microbiological characteristics of three individuals, made up of seeds and fruits of the Khalte variety date palm, as well as the germination of seeds from these three individuals with different pretreatments to assess germination rates and speed.

The results showed that the highest germination rate and germination speed were obtained from seeds that had undergone mechanical pre-treatment (scarification).

The results of the phytochemical study showed that the seed is richer than the fruit in phenolic compounds (total polyphenols, Anthocyanidins) and the opposite for (Aglycones and C-glycosides). According to the DPPH method, both parts of the date (fruit and seed) have an interesting antiradical potential.

The microbiological study carried out to identify and isolate the fungi present on the surface of the germinated seeds yielded the following results: two species belonging to two genera: *Aspergillus fumigatus*, *Aureobasidium pullulans*. These two fungal strains were compared, using the double culture method for the in vitro antagonism test against an antagonistic Trichoderma sp strain, to assess the latter's antifungal activity against the phytopathogenic strains isolated. The results of this study showed that the *Trichoderma sp* strain influenced the radial growth of both *Aspergillus fumigatus* and *Aureobasidium pullulans* strains (inhibitory effect).

Key words: Date palm, seed germination, phenolic compounds, DDPH test, phytopathogenic fungi.

Résumé

L'arbre providence des régions désertiques est le palmier dattier (*Phoenix dactylifera L.*). Il offre une variété de produits, tels que la datte, un aliment d'une riche valeur énergétique, et ses graines sont riches en diverses substances biochimiques et minérales importante. L'objectif de cette étude visait à analyser les caractéristiques phytochimiques et microbiologiques de trois individus, composés de graines et de fruits du palmier dattier de la variété Khalte, ainsi que la

germination des graines de ces 3 individus avec des différents prétraitements pour évaluer les taux et la vitesse de germination.

Les résultats montrent que le taux de germination et la vitesse de germination le plus élevé est des graines qui ont subi un prétraitement mécanique (scarification).

Les résultats de l'étude phytochimique ont mis en évidence que la graine est plus riche que le fruit en composés phénolique (Polyphénols totaux, Anthocyanidines) et le contraire pour les (Aglycones et C-glycosides). Selon l'évaluation par la méthode du DPPH, les deux parties de la datte (fruit et graine) présentent un potentiel antiradicalaire intéressant.

L'étude microbiologique ce fait pour l'identification et l'isolement des champignons présent à la surface des graines germées, on a trouvé les résultats suivent 2 espèces appartiennent à 2 genres sont : *Aspergillus fumigatus, Aureobasidium pullulans*. Ces 2 souches fongiques ont été confrontées, par l'utilisation de la méthode de double culture pour le test d'antagonisme in vitro contre une souche antagoniste *Trichoderma sp*, pour évaluer l'activité antifongique de ce dernier vis-à-vis des souches phytopathogènes isolées. Les résultats de cette étude montre que la souche de *Trichoderma* sp a impacté la croissance radiale des deux souches d'*Aspergillus fumigatus* et d'*Aureobasidium pullulans* (effet inhibiteur).

Mot clés : Palmier dattier, germination des graines, composés phénoliques, teste DDPH, champignons phytopathogène.

ملخص

الشجرة الأساسية في المناطق الصحراوية هي نخيل التمر .(*Phoenix dactylifera L.*) وهي تقدم مجموعة متنوعة من المنتجات، مثل التمر، وهو غذاء ذو قيمة عالية من الطاقة، كما أن بذورها غنية بالعديد من المواد الكيميائية الحيوية والمعدنية الهامة. كان الهدف من هذه الدراسة هو تحليل الخصائص الكيميائية النباتية والميكروبيولوجية لثلاثة أفراد، مكونة من بذور وثمار نخيل التمر من صنف الخلط، وكذلك إنبات بذور هذه الأفراد الثلاثة بمعالجات مسبقة مختلفة لتقييم معدل الإنبات وسرعته.

أظهرت النتائج أنه تم الحصول على أعلى معدل إنبات وسرعة إنبات من البذور التي خضعت للمعالجة الميكانيكية المسبقة (الخدش)

أظهرت نتائج الدراسة الكيميائية النباتية أن البذور أغنى من الفاكهة بالمركبات الفينولية (البوليفينول الكلي، والأنثوسيانيدين) والعكس بالنسبة لـ (الأجليكونات وC-جليكوسيدات). ووفقًا لطريقة DPPH، فإن كلا الجزأين من التمر (الثمرة والبذور) لهما إمكانات مثيرة للاهتمام مضادة للجراثيم.

أُجريت دراسة ميكروبيولوجية لتحديد وعزل الفطريات الموجودة على سطح البذور النابتة. وكانت النتائج على النحو التالي: نوعان ينتميان إلى جنسين: *Aspergillus fumigatus*، *Aureobasidium pullulans*, تمت مقارنة هاتين السلالتين الفطريتين، باستخدام طريقة الاستزراع المزدوج لاختبار العداء في المختبر ضد سلالة *Trichoderma sp* المعادية، لتقييم النشاط المضاد للفطريات لهذه الأخيرة ضد السلالات الممرضة للنباتات المعزولة. وقد أظهرت نتائج هذه الدراسة أن سلالة *Trichoderma sp* كان لها تأثير على النمو الشعاعي لسلالتي *Aspergillus fumigatus* و *Aureobasidium pullulans* (تأثير مثبط).

الكلمات المفتاحية: نخيل التمر، إنبات البذور، المركبات الفينولية، اختبار DDPH، الفطريات الممرضة للنباتات.

Abbreviations list

Abbreviation	**Designation**
APG	Angiosperm Phylogeny Group
DO	Optical density
DPPH	2,2-Diphenyl-1-picrylhydrazyl
MVS	Dry Plant Material
NF	Nuclear factor
NSAIDs	Non-steroidal anti-inflammatories
PAL	Phenylalanine ammonia lyase
PDA	Potato Dextrose Agar
PEEP	Phosphoenolpyruvate
UV	Ultraviolet

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INTRODUCTION

The **date palm** (*Phoenix dactylifera L.*) is a tree described as the prince of the plant world by the botanist Linnaeus. It constitutes the framework of the oases, which are real islands of greenery and life in the middle of the desert; in fact, the covering provided by its foliage creates a microclimate favorable to the life of men, their crops and their animals **(Djerbi, 1991).** It is not only appreciated for its fruit, the date, but also for all its by-products **(roots, leaves, seeds, etc.).**

The date is a high energy food; it allows millions of families to survive in regions with difficult climates. An excellent fruit, it finds large markets in various countries. It is rich in **minerals** (potassium, magnesium, etc.), **primary metabolites** (sugar, lipids and proteins, etc.) which constitute a good source of energy. In addition, dates are a source of antioxidant components (**secondary metabolites: polyphenols, terpenes, etc**.) which demonstrate important bioactive properties beneficial for humans, animals and the environment **(Gaceb-Terrak et al., 2010).**

Algeria has an extremely rich heritage in Phoenicicultural resources characterized by a great genetic diversity expressed by the variability of the morphological, biochemical and molecular traits of cultivars and/or clones such as in the oases (palm groves) of the south or Algerian Sahara where a significant number of varieties (cultivars) of the date palm (*Phoenix dactylifera L.)* was recognized and identified by local phoeniciculturists **(Latrech, 2015)**.

Our **work** consists, on the one hand, of the study of the fleshy part of dates most consumed in human food, which is the **fruit**. On the other hand the analysis of a by-product, used particularly in animal feed, which is the **seed**. To this end, we chose to focus on the phytochemical study of four fractions belonging to phenolic compounds (**total polyphenols, anthocyanins, c-glycosides and flavonic aglycones**) and a microbiological study of the **fungi** associated particularly with the seed and which can attack the date palm, as well as a direct confrontation test with an antagonistic strain to combat these fungi.

Part I: Bibliographic research

State of the art on the date palm (Phoenix dactylifera L.) and polyphenols

1 General information and description of the date palm

The date palm was named *Phoenix dactylifera* by Linnaeus in 1753 **(Munier, 1973).** Phoenix derives from "Phoenix" a Greek word attributed to the date palm and dactylifera comes from the Latin "dactylus" deriving from the Greek "typists» which means finger, due to the shape of the fruit, the date.

1.1 Systematics and phylogeny

Cronquist's classification **(1981)** based on criteria of morphological, anatomical and chemical resemblances and which makes it possible to bring together in common groups plants, which present a high number of similarities between them. It made it possible to establish, for the date palm, the following hierarchical order (Tab. I).

Phylogenetic classification or APG (Angiosperm Phylogeny Group) based on molecular criteria makes it possible to establish the sequence between groups from the most primitive to the most evolved. It is essentially based on the analysis of chloroplast gene sequences (**APG I, 1998** and **APG III, 2009:** modification of **APG II, 2003**) respects the order established previously (Tab. I).

Table 1. Classification of the date palm according to **Cronquist (1981)** and **APG III (2009).**

Taxa	**Cronquist (1981)**	**APG III (2009)**
Domain	*Eukarya*: Eukaryotes	*Eukarya*: Eukaryotes
Reign	*Plantae*: Plants	*Plantae*: Plants
Under reign	*Tracheobionta*: Tracheophytes	Rhizophytes
Division	*Magnoliophyta*	
Branch	*Spermatophyta*	
Under branch	Angiosperms	
Class	*Liliopsida*: Monocots	*Commelinideae*
Subclass	*Arecidae*	
Order	Arecales	Arecales
Family	*Arecaceae*or Palmae	*Arecaceae*or Palmae
Subfamily	*Coryphoideae*	*Arecoideae*

Tribe	*Phoeniceae*	
Gender	*Phoenix*	
Species	*Dactylifera*L.	
Binomial name	*Phoenix dactylifera*L.	*Phoenix dactylifera*L.

1.2 Ecology and geographic distribution

1.2.1 In the world

Today, date palm cultivation is concentrated in Mediterranean Africa to the Near East, from southern Iran in the east to the Atlantic coast of North Africa in the west between the altitudes 35°North and 15°South.We also find the date palm in other regions of the world, where it has been introduced and its cultivation is well adapted in Spain, with the famous palm grove of Elche, in the United States of America, particularly in California. The date palm is also cultivated on a smaller scale in Mexico and the West Indies; plantations exist in Argentina, Australia and Niger **(Jahiel, 1996).**

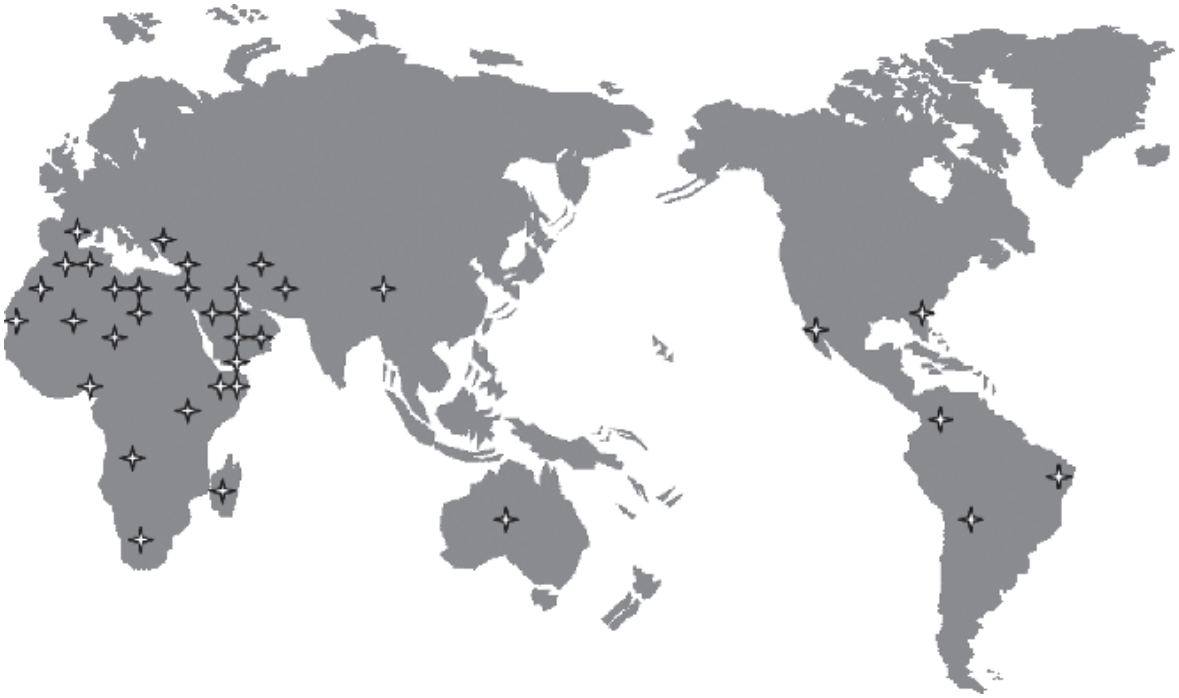


Figure 1. Geographical distribution in the world of the date palm **(Abssi. R., 2013)**

1.2.2 In Algeria

The date palm finds in Algeria a Saharan and pre-Saharan climate favorable for its development and for the ripening of its fruits. Date palm cultivation occupies all regions located under the Saharan Atlas, i.e.60,000 ha from the Moroccan border in the West to the Tunisian-Libyan border in the East. From the north to the south of the country, it extends from the southern limit of the Saharan atlas to Reggane in the west, Tamanrasset in the center and Djanet in the east **(Bouguedoura, 1991).**

Currently, the date palm is cultivated in 17 wilayas, the main producing regions are Biskra, Ouargla, El Oued and Adrar **(Bouguedoura et al., 2010).**

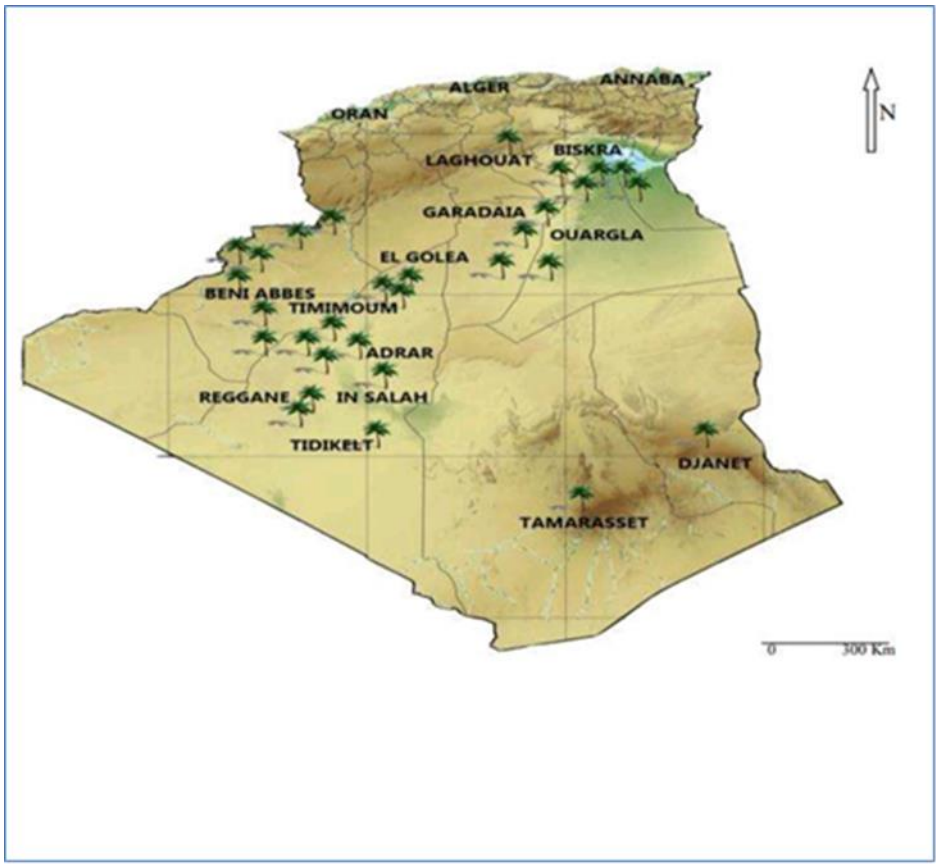


Figure 2. Geographical distribution of the date palm in Algeria. **(Bouguedoura et al., 2010)**

1.3 Botanical Description

1.3.1 Vegetative apparatus

The date palm is made up of three essential parts, which are the roots, the stem, and the aerial part or the crown.

1.3.1.1 The root system

Under normal conditions, the root system of a date palm having a size of 8 to 10 meters, it can extend laterally more than 7m from the trunk, and reach a depth greater than 6m **(EL-HOUMAIZI, 2002).** The roots are arranged in bundles with very little or no branching, they are composed of four types of roots **(MUNIER, 1973; PEYRON, 2000)**, depending on their depth and their function (Figure 3):

1. **Respiratory roots (superficial):** are aerial roots of 0 to 20 cm, they have few rootlets; play an important role in gas exchange with the soil atmosphere **(PEYRON, 2000).**

2. **Nutritious roots (average):** constitute the largest proportion of the roots of the system. They are 20 to 100 cm long, oblique or horizontal. They are provided with numerous rootlets **(PEYRON, 2000).**

3. **The roots of absorption (lower):** from 100 to 200 cm, their function is to fetch water. The zone of these roots is more or less developed depending on the cultivation method and the depth of the water table **(PEYRON, 2000).**

4. **The roots of the pivoting beam:** between 100 and beyond 1500 cm. The absorption root pivot is almost non-existent if the management of the crop allows sufficient absorption at the level of the nutrition and absorption roots. It is reduced if the water table is at a shallow depth, but if necessary, the true taproot of roots can reach the water to a depth of 17 meters **(TOUTAINE, 1967).** Furthermore, the number and density of roots in the soil vary depending on the nature of the soil, cultivation method, depth of the water table, climatic conditions, as well as the cultivars and the origin of the subject **(AL-BAKRE, 1972).**

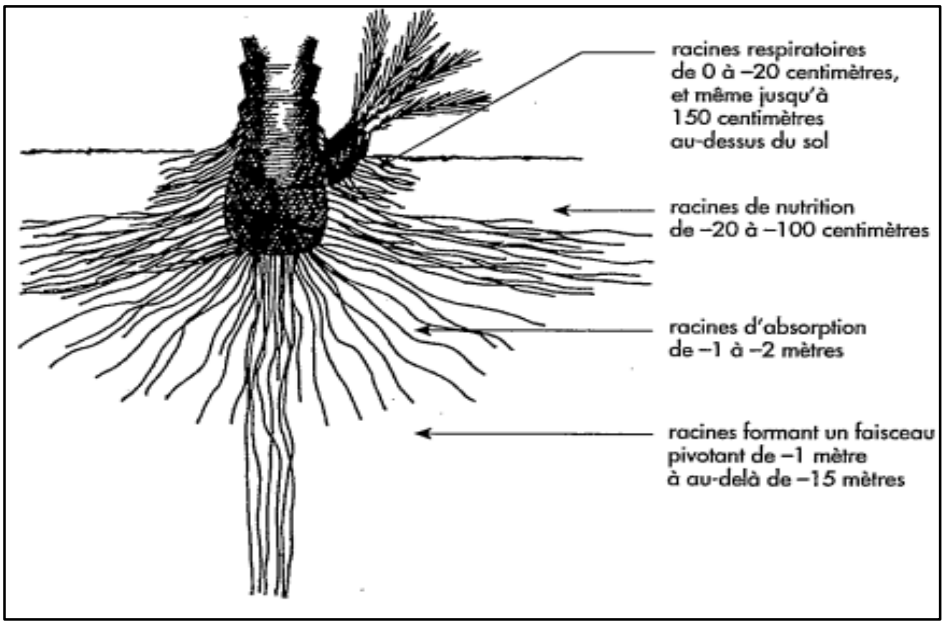


Figure 3. Root types in the date palm **(PEYRON, 2000)**

1.3.1.2 The trunk or stipe

The stem or trunk of the date palm has a slender, unbranched habit called "**stipe**". The stipe is a monopodial, cylindrical, brown-colored, lignified orthotropic axis, covered by the bases of ancient palm fronds. It can reach 30m long **(BEN ABDALLAH, 1990).** Their diameter is the same over the entire height of the date palm (with some exceptions), and the areas of shrinkage can reflect periods of drought or cold, or various accidents **(ZAID and DE WET, 1999).**

Over time, the palms (old dried leaves) fall naturally or artificially (by horticultural and cultural pruning), leaving their petiolar bases or the sheath on the stipe (on which the scars of the leaves remain visible).

The development of the date palm takes place by a single terminal bud with indeterminate growth which does not divide (except in accidents) which implies that the terminal axis does not branch. On the other hand, in each palm there is an axillary bud (adventive) which as it develops can give rise to: an inflorescence (in the coronal part), a gourmand (in the middle part), or a shoot (in the basal part) . The ability to emit suckers is a varietal characteristic. These shoots are true clones (same genome) of the mother plant allowing the clonal reproduction of genotypes of agronomic interest **(PIERRE MUNIER, 1973).**

Therefore, the stipe does not branch, but the development of suckers or aerial shoots (Rekabs) can give rise to ramifications **(DJERBI, 1996).**

Figure 4. Stipe or trunk of the date palm **(DJERBI, 1996)**

1.3.1.3 Leaves or palms

The leaves of the date palm are called "**Palmes** or **Djerids**". The palms are compound leaves, green in color, gathered in a number of 20 to 30 at most and form a sparse apical crown. They have a pinnate shape coming from the terminal bud, inserted in a helix, very close together on the stipe, they have a well-developed petiolar sheath "**cornaf**" buried with dense felting "**Lif**" in a semi-cylindrical petiole spiny towards the base (**cabbage -srab**) hard and leaflet (**zaaf**) **(BELHABIB,1995).**

The leaves have a blade divided into two rows of narrow leaflets, folded lengthwise following their veins, stiff and prickly at the top, the lower leaflets of each leaf are transformed into thorns **(OZENDA, 1983).**

Adult palm fronds can measure up to 6m long and have 3 different segments: the petiole, the spine segment and the leaflet segment. They have a lifespan of up to 7 years. Their structure changes depending on the variety, age and environmental conditions. **(ZAID, 2002).**

The arrangement of the leaflets and spines on the rachis as well as the angles they form between it and with the rachis constitute taxonomic indexes allowing the clones to be differentiated **(MUNIER, 1973).**

In the date palm, three kinds of leaves are produced during its life, if the subject comes from a seed, it produces juvenile leaves, semi-juvenile leaves and adult leaves or palms. In subjects resulting from rejection, we only find the fins **(BOUGUEDOURA, 1982).**

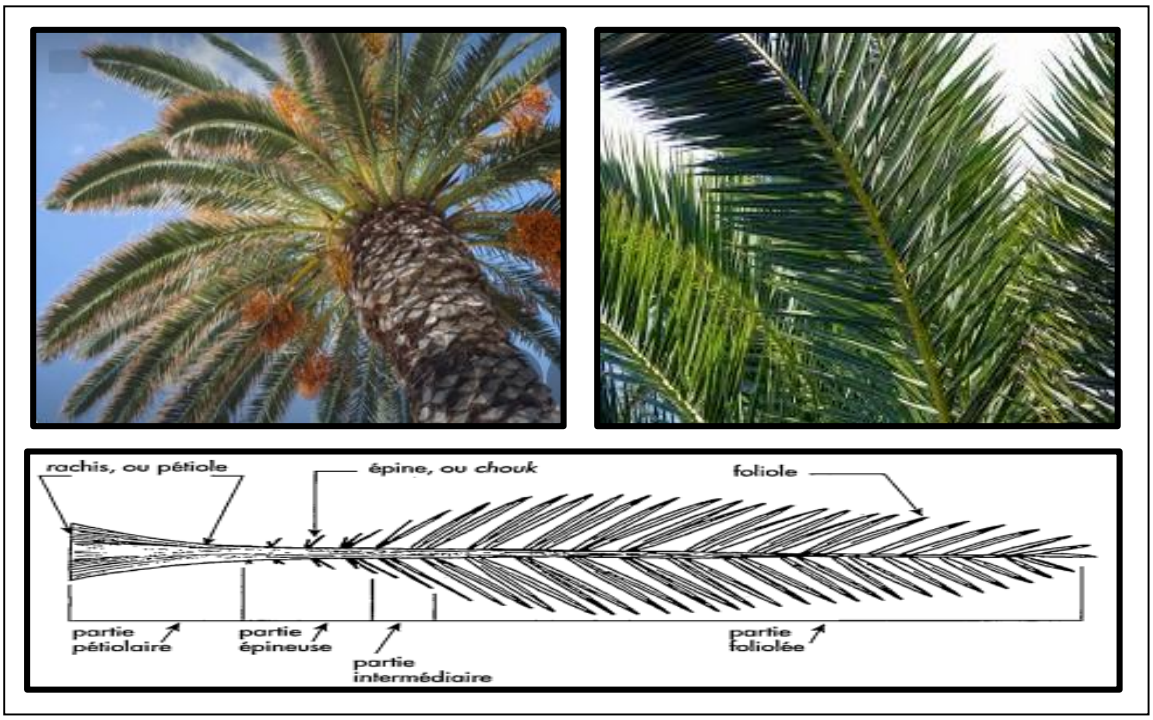


Figure 5. Stipe or trunk of the date palm **(DJERBI, 1996)**

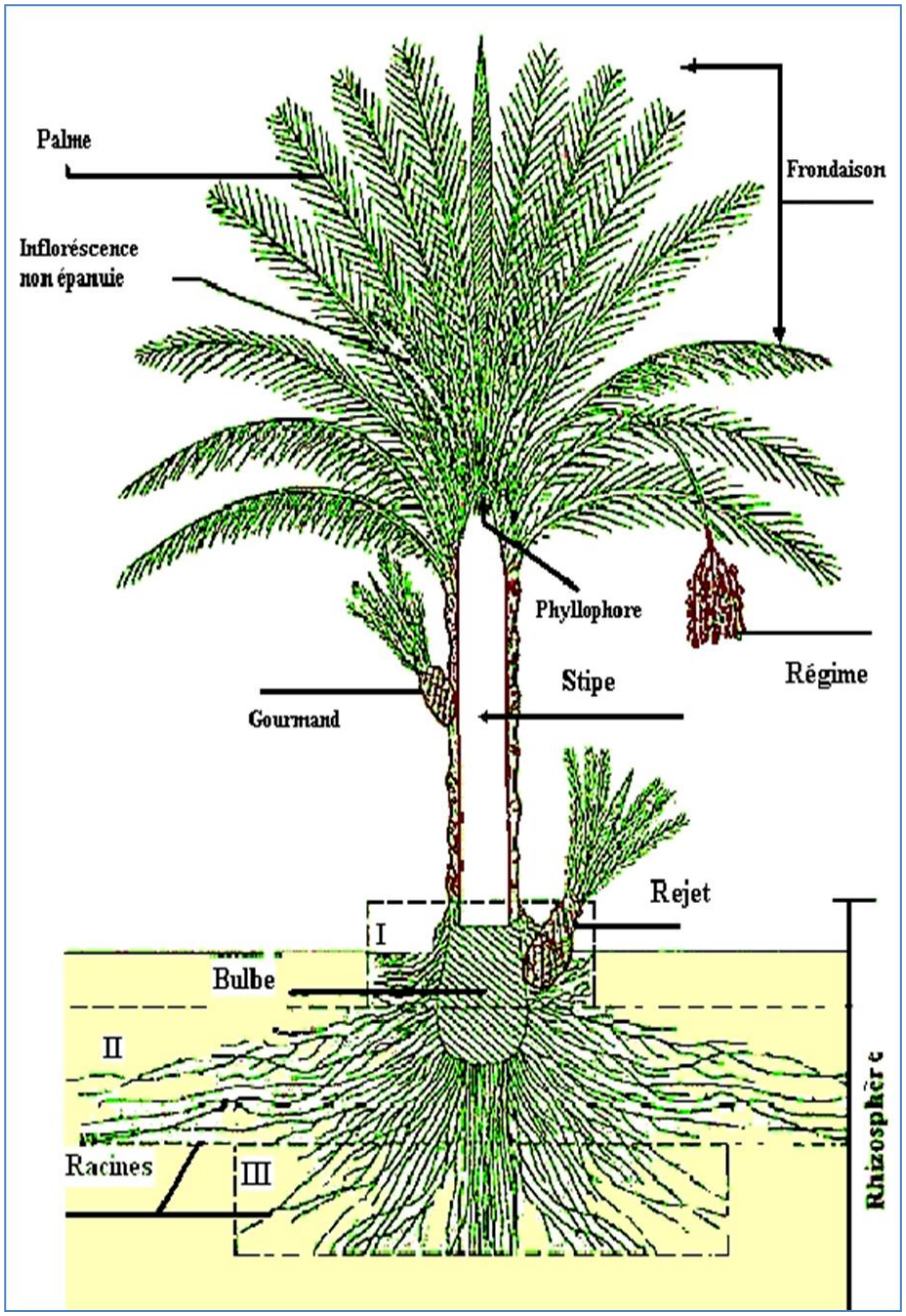


Figure 6. Morphological organization of the date palm (Phoenix dactylifera L.) **(PEYRON, 2000).**

1.3.2 Reproductive system

1.3.2.1 The inflorescences

According to **PEYRON (2000),** the date palm is a **dioecious** species. There are therefore male plants (**Dokkar giving pollen**) carrying male inflorescences which give on average 30 to 40 spathes per year, and female plants carrying female inflorescences (**Nakhla producing fruits or dates**) which gives 12 to 20 husks each year **(AMINE, 1990).**

The date palm begins to flower after a long juvenile phase, between 5 and 8 years after seed germination under favorable growing conditions. Flowering is generally annual and lasts throughout the life of the plant.

Pedicels (or spikelets) which are in turn carried by a fleshy axis (the **scape** or **spadix**) carry the flowers; the whole is enveloped in a large closed membranous bract called the spathe **(PEYRON, 2000)**

Only female date trees bear fruit, so they are the origin of the multiple varieties of dates. In general, before opening, the shape of the spathe makes it possible to recognize the sex of the inflorescence. The female spathe is long and slender, on the other hand, the male spathe is short, wide and swollen **(BOUGUEDOURA, 1991).** Both are composed of an axis called the rachis on which are inserted numerous spikelets bearing sessile flowers depending on the male or female sex.

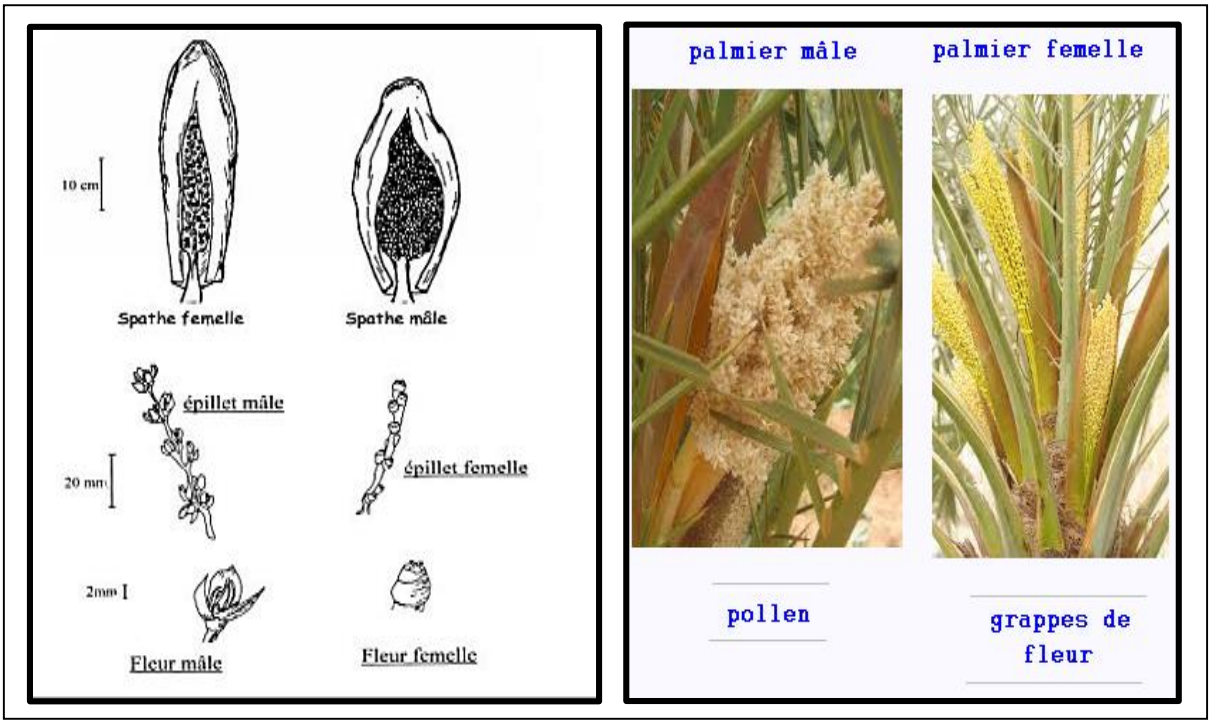


Figure 7. Inflorescence (male and female) of the date palm **(ELHOUMAIZI, 2002)**

The inflorescence has a large bract base (b) at its base, which envelops it and protects it from the heat of the sun **(MASON, 1915).** Its lanceolate shape allows it to easily emerge from the sheath, which compresses it between the petiole and the stipe. The inflorescence consists of a main axis (IA), which includes a sterile part (IC) (the peduncle) and a fertile part (CA) (the rachis) with numerous ramifications (branches) or spikelets.

The spikelets are in turn made up of a sterile part (s) and a fertile part (f). The latter bears flowers, then fruits. The flowers (e) are unisexual, practically sessile **(DAHER et al. 2010).** The male and female flowers carried by different individuals are morphologically different, only the carpels are well developed in female flowers, while it is the stamens that develop in male flowers **(DAHER et al. 2010).**

1.3.2.2 The fruit or date

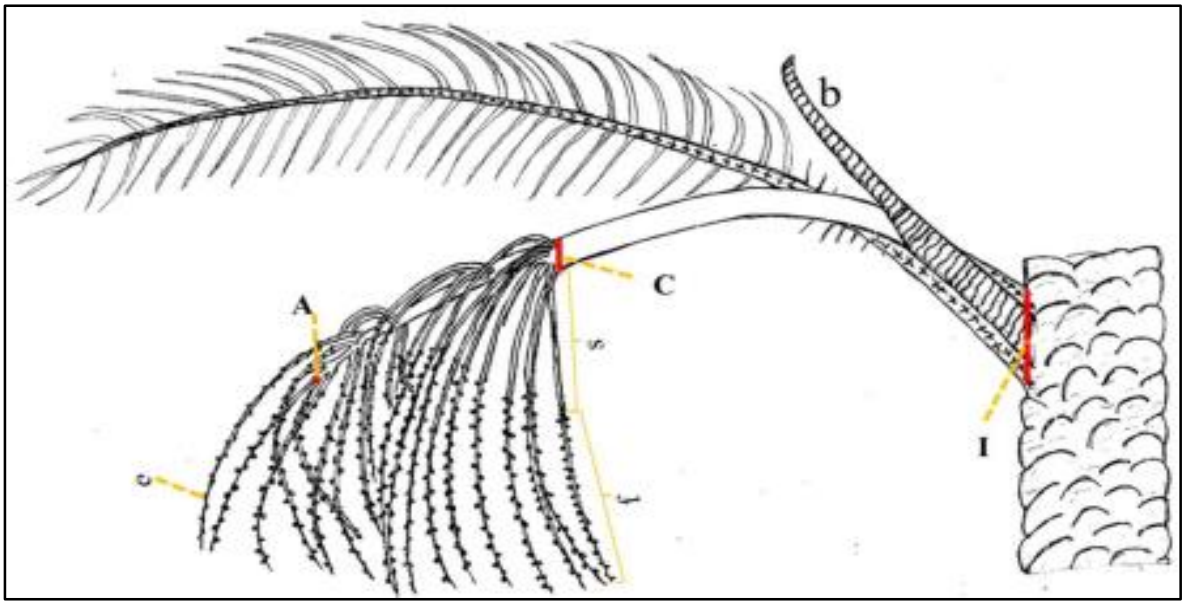


Figure 8. Morphological and architectural description of female inflorescence of the date palm **(ZANGO et al, 2013)**

The date is a single-sperm berry 2 to 8 cm long; it comes from the development of a carpel after fertilization of the ovule. The date consists of a waxy epicarp (skin), a fleshy mesocarp, and a thin, parchment-like endocarp surrounding the seed. **(ESPIARD and ZAID, 2002).**

The seed (nucleus) having a hard consistency, has on one side a longitudinal slit or furrow, opposite this slit is generally the embryo in the middle position. A cross section of the seed shows that it consists of a seed coat and endosperm.

In the absence of pollination, parthenocarpic fruits (**coreless**) sometimes develop, but these rarely reach full maturity.

Dates are generally elongated or rounded depending on the variety, their color varies depending on the cultivars and stage of maturity.

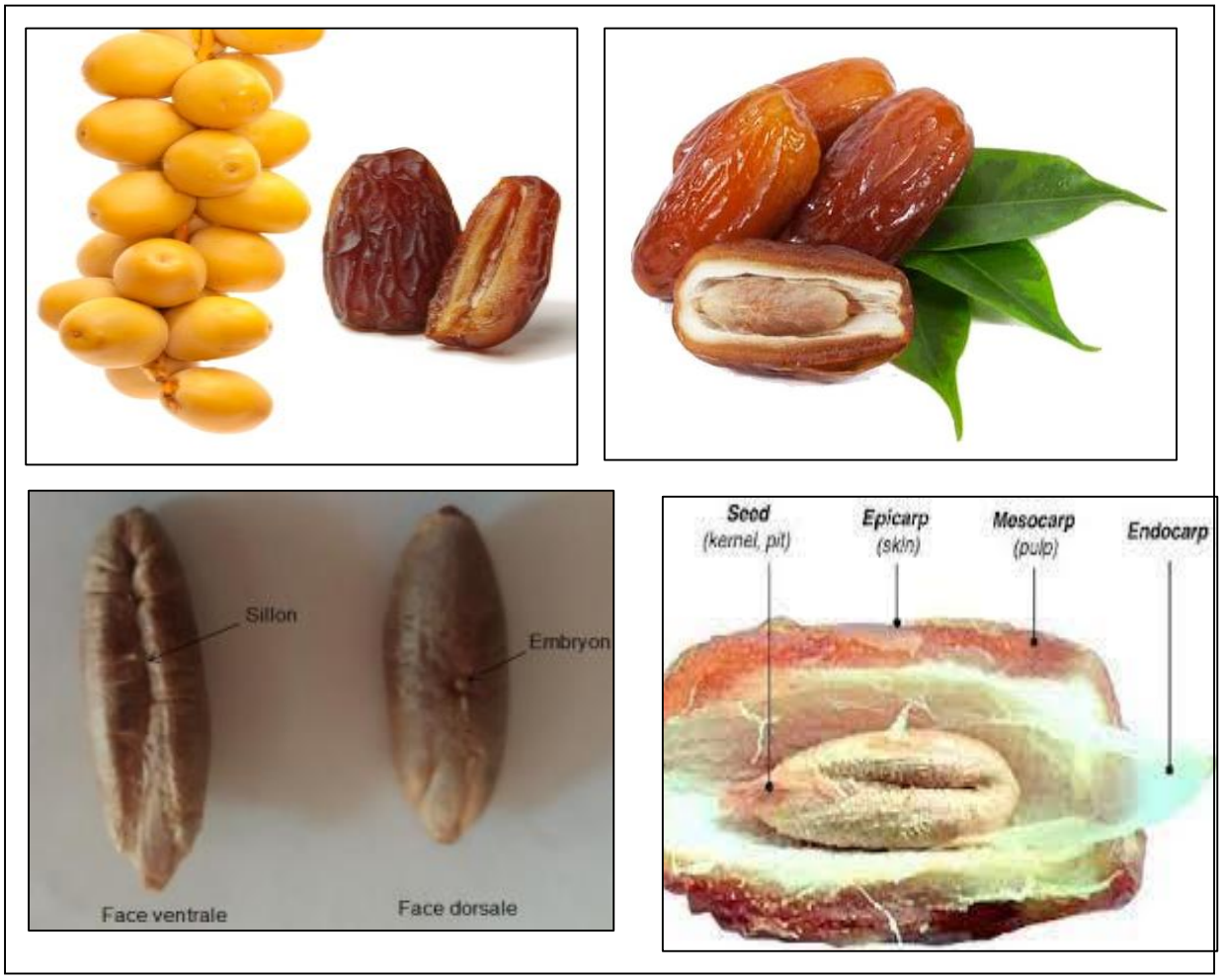


Figure 9. Morphological and anatomical description of the fruit and seed of the date palm **(Original source, 2024).**

1.4 Use and Ethnobotany of the date palm

The date constitutes an excellent food, of great nutritional and energy value described according to **GILLES (2000)** due to their high sugar content, which gives them a great energy value. They also have an interesting content of reducing sugars easily assimilated by the body and qualitatively balanced proteins.

In addition, dates are rich in minerals (plastics such as Ca, Mg, P, S or catalytic such as Fe and Mn). They are remineralizing and significantly strengthen the immune system **(ALBERT, 1998).** But many other uses have been identified among local or oasis populations.

Table 2. Use of date palm by-products **(AL-SHAHIB et al., 2003; MARTIN SANCHEZ et al., 2012).**

Organs	Uses
Trunk	Provides firewood and materials for construction.
Fins	The palm fronds (green or dry) are used for basketry (making baskets, hats, fans and mats). They are also found on fences, under roofs.
Diets	As traditional brooms and as fuels.
Lif	For making sandal soles.
Sap	The sap that flows from the incised stem is used for the preparation of a drink called wine or palm juice or Lagmi which can be drunk fresh or fermented.
Pulp	Is used as a sweetener in the preparation of beer as well as in other industrial processes such as syrup and confectionery production
Seeds	Used for fattening livestock. The crushed kernels are used as a restorative for emaciated camels. This diet is invigorating and very rich in carbohydrates. Roasted seeds can provide a coffee substitute.
Inflorescences	The inflorescence is used as a broom and the spathe as firewood.

The date palm is considered a medicinal plant, it is traditionally used for several treatments, it is recommended alone or combined with other plants.

The fruit helps fight anemia and demineralization. It is therefore recommended for breastfeeding women. Regulators of intestinal function, dates piled in water treat hemorrhoids and constipation, but also green dates treat intestinal disorders such as diarrhea. Calming in the form of a very concentrated syrup (the rob), this preparation soothes and puts children to sleep.

It is also used for nervous diseases and in bronchopulmonary conditions. In decoction or infusion, dates treat colds, and gargled, they treat sore throats **(BENCHALAH and MAKA, 2006).** Foucauld's father, in his famous dictionary, cites the use of poultices

composed of dates, millet and the fruit of an Asclepiadaceae, Solen ostemma argel, to calm rheumatic pain.

From a symbolic point of view too, the date plays an important role. The ethnologist *Saskia Walentowitz* reports that among "the *Tuareg Kel Ewey of the Air*", the custom is for an old woman to rub the palate of the newborn with the pulp, this sweet substance, but also with the bitter fruit of an acacia tree, so that he can have a taste of life, sometimes sweet and sometimes bitter.

Table 3. Use of date palm in traditional medicine.

Author	Traditional Pharmacopoeia
BELLAKHDAR (1997)	• Esophago-gastrointestinal pathology • Pathology of the brancopulmonary system • Pathology of the oral sphere • Eye conditions
BELGUEDJ et al (2008)	• Recovery of the uterus after childbirth and simulation of lactation. • Regulation of high blood pressure. • Fortifying for babies. • Treatment of anemia. • Skin hydration.

2 Phenolic compounds or polyphenols

2.1 Chemical structure

Polyphenols, or phenolic compounds, constitute a set of more than 8,000 molecules divided into around ten chemical classes. Their common characteristic is the presence of at least one 6-carbon aromatic ring in their structure, carrying a variable number of hydroxyl functions (OH). Among them, flavonoids are the most numerous, with more than 5,000 isolated molecules, and also the best known. **(LAOUINI, 2014)** Polyphenols are generally classified into simple phenols, phenolic acids (derived from benzoic or cinnamic acid), coumarins, naphthoquinones, stilbenoids (two C6 rings linked by 2C), flavonoids, isoflavonoids and anthocyanins (C6-C3-C6 structure), as well as in polymerized forms such as lignans, lignins and condensed tannins. These basic carbon structures come from the secondary metabolism of plants, created by the shikimate pathway. **(Jain et al., 2011)**

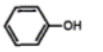
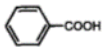
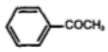
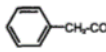
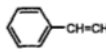
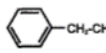
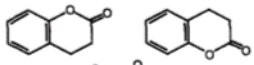
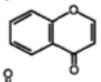
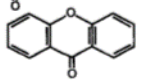
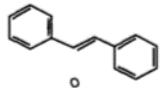
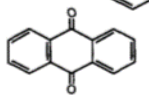
Class	Basic Skeleton	Basic Structure
Simple phenols	C_6	
Benzoquinones	C_6	
Phenolic acids	C_6-C_1	
Acetophenones	C_6-C_2	
Phenylacetic acids	C_6-C_2	
Hydroxycinnamic acids	C_6-C_3	
Phenylpropenes	C_6-C_3	
Coumarins, isocoumarins	C_6-C_3	
Chromones	C_6-C_3	
Naftoquinones	C_6-C_4	
Xanthones	C_6-C_1-C_6	
Stilbenes	C_6-C_2-C_6	
Anthraquinones	C_6-C_2-C_6	
Flavonoids	C_6-C_3-C_6	
Lignans, neolignans	$(C_6$-$C_3)_2$	
Lignins	$(C_6$-$C_3)_n$	

Figure 10. Main classes of polyphenolic compounds. **(Bravo. 1998)**

2.2 Biosynthesis

Phenolic compounds constitute the largest group of secondary metabolites found in plants, ranging from the simplest aromatic rings to more complex structures like lignins. Derived from phenylalanine, these compounds are also called phenylpropanoids. Polyphenols are distinguished by the presence of numerous large structural units. The physical, chemical and biological properties of each class vary depending on the number and characteristics of these phenolic structures. In reality, phenols are classified into different groups, such as phenolic acids, flavonoids, stilbenes and lignans, each with specific properties. The biogenetic synthesis of plant phenolic compounds occurs via the shikimate/phenylpropanoid pathway. **(Sharma et al., 2019)**

The deamination of phenylalanine leads to the formation of immediate precursors of phenols. The following biosynthetic sequence, called the phenylpropanoid sequence, promotes the synthesis of the main hydroxycinnamic acids. Concurrently, a mevalonate pathway produces aromatic systems such as chromones, isocoumarins, and quinones through repeated condensations. This biosynthetic origin further increases the structural diversity of phenolic compounds, due to the frequent presence of shikimate and acetate in the formation of mixed compounds such as flavonoids, stilbenes and xanthones. **(TRIFA et al., 2020)** These two secondary pathways generate numerous monomeric and polymeric structures that play a very varied role in plant physiology and biochemistry. Secondary metabolites are phytochemicals synthesized by secondary metabolism. Phenylalanine is formed from erythrose 4-phosphate and phosphoenolpyruvate (PEP) during the biosynthesis of phenolic compounds. The conversion of phenylalanine to trans-cinnamic acid is then catalyzed by phenylalanine ammonia lyase (PAL). This pathway also produces many other phenolic compounds, such as flavonoids, coumarins, hydrolyzable tannins, monolignols, lignans, and lignins, and is officially called the phenylpropanoid pathway. **(Sharma et al., 2019)**

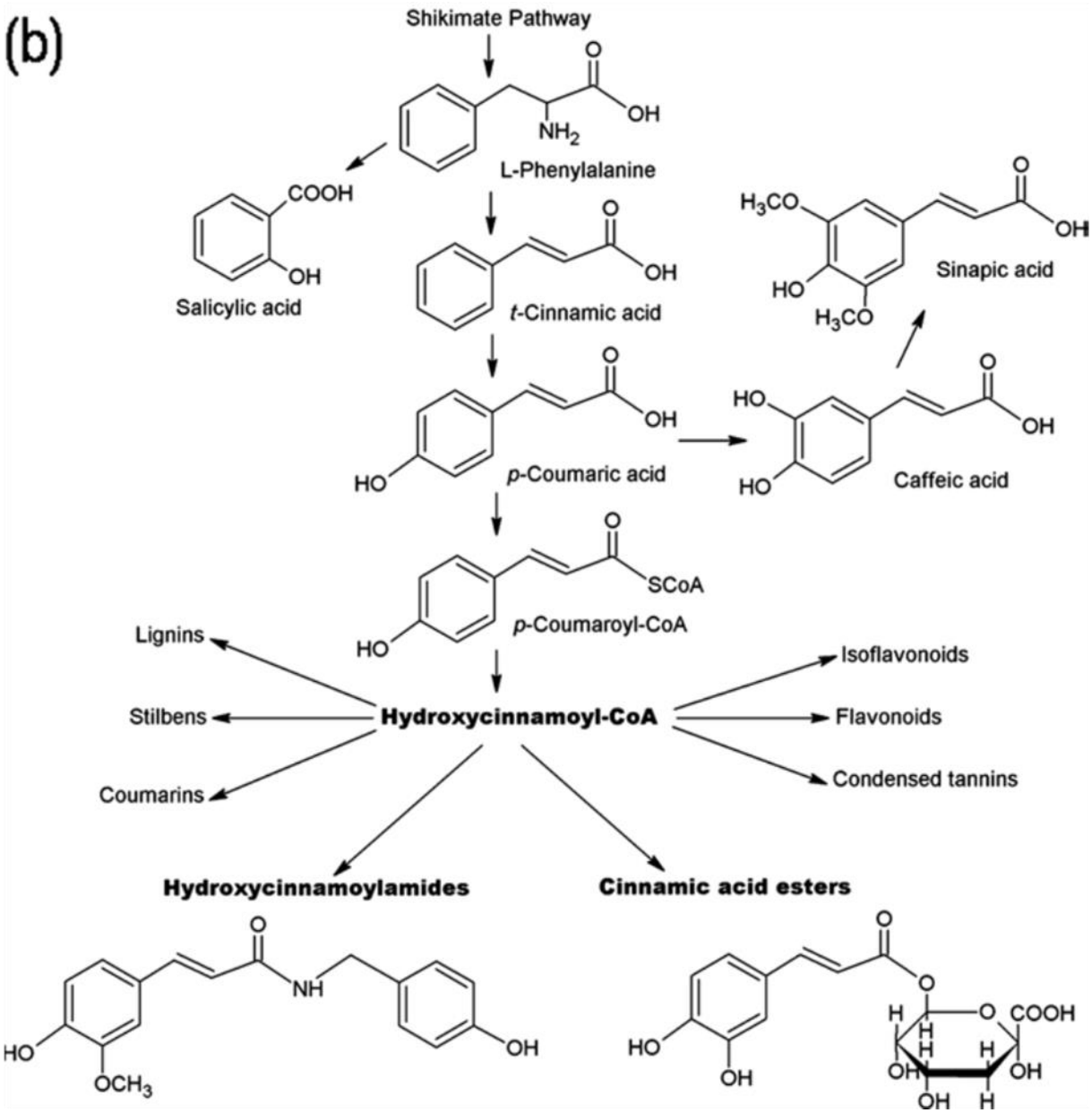


Figure 11. A simplified diagram of the metabolic pathway leading to phenolic compounds **(Jain et al., 2011)**

2.3 Classification

The human diet contains a large amount of antioxidants, of which flavonoids are the most important and best-studied class. The structure of these compounds allows polyphenols to be classified into four main categories: flavonoids, phenolic acids, lignans and stilbenes. Flavonoids have a characteristic structure composed of two aromatic rings linked by a group of 3 carbons, thus forming an oxygenated heterocycle. Based on their hydroxylation pattern of the

oxygenated heterocycle and the arrangement of hydroxyl groups, polyphenols are divided into four subclasses: flavonols, chalcones, flavones, and anthocyanins. **(Kumar et al., 2019)**

2.3.1 Non-flavonoids

Despite the presence of multiple hydroxyl groups on the aromatic rings of the structural backbone of polyphenols, the basic structure of non-flavonoids consists of a single aromatic ring. Non-flavonoid compounds include phenolic acids, stilbenes, lignans and lignins. **(Singla et al., 2019)**

- **Phenolic acids**

Phenolic acid is one of the major aromatic secondary metabolites of plants. It is characterized by the presence of a hydroxyl group on an aromatic benzene ring, accompanied by one or more carboxylic acid groups. Phenolic acids are divided into two broad categories: those derived from benzoic acid, having seven carbon atoms, and those derived from cinnamic acid, having nine carbon atoms. **(Al-Alawi et al., 2017)**

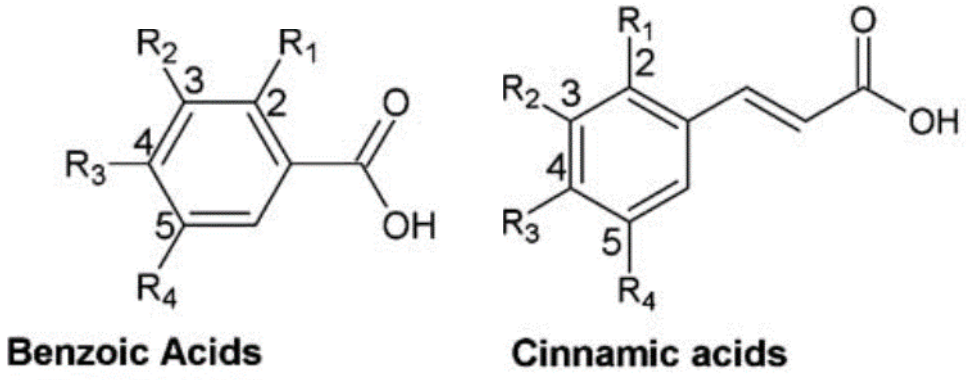


Figure 12. Benzoic acid and cinnamic acid derivatives general formula **(Vayalil, 2012)**

- **The stilbenes**

The plant kingdom naturally contains more than 30 stilbenes and stilbene glycosides. The basic chemical structure of stilbenes consists of two aromatic rings connected by a methylene bridge. The chemical and biological properties of the two stilbene isomers (cis and trans) differ. Inter-conversions between trans and cis forms are observed under the influence of heat or UV radiation. **(Sonia et al., 2011)**

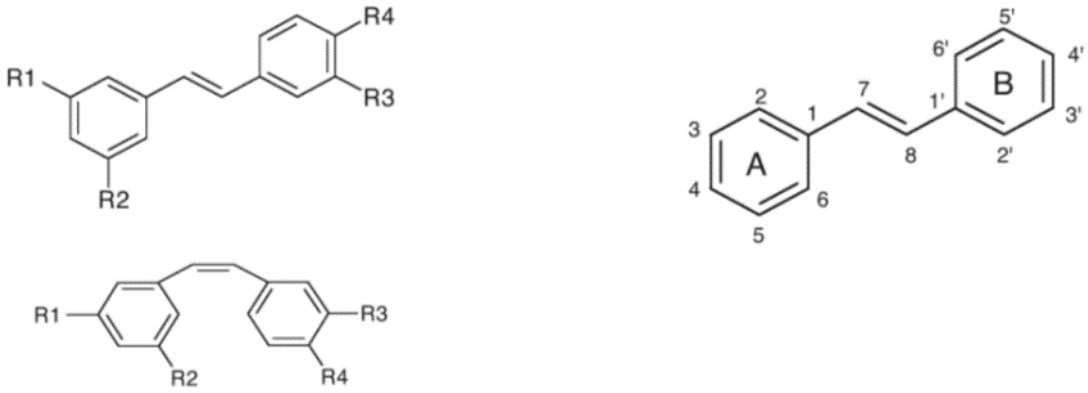


Figure 13. Basic structure of stilbenes (trans or cis) **(Sonia et al., 2011)**

❖ **Lignans and lignins**

Cinnamic acid derivatives, called monolignols, serve as precursors for phenylpropanoid compounds such as lignans and lignins. Lignans come in the form of (C6-C3), where the unit (C6-C3) corresponds to a propylbenzene. Plants produce these lignans by oxidative dimerization of two coniferous alcohol units. When this dimerization results in an oxidative bond between the 8-carbons of the propenyl side chains of two coniferous alcohol units, thereby forming a (C8-C8) bond, the metabolites produced are called lignans. All other bond forms are referred to as neolignans. When the units (C6-C3) are not directly bonded, but are bonded through an ether oxygen atom, the compound is known as an oxyneolignan. Lignans can be subdivided into sesquineolignans (three units of C6-C3) and dineolignans (four units of C6-C3). **(Muanda, 2010).**

In legumes, seeds and vegetable oils, lignans are mainly found in their free forms, while the glycosylated form is rarely present. (Singla et al., 2019) Lignins constitute an essential category of natural products present in the plant kingdom, and they are believed to be produced by oxidative polymerization of monolignols (monomers) such as p-coumaric, coniferic and sinapic alcohols. **(Muanda, 2010)**

2.3.2 Flavonoids

Albert Szent-Gyorgyi discovered flavonoids. They constitute a set of 6000 natural compounds classified according to their phenylbenzopyrone composition. These pigments play a crucial role in plant coloring and are known for their antioxidant activities **(Macheix et al., 2005).** The general structural skeleton of flavonoids is C6–C3–C6, with the two C6 units (ring A and ring B) phenolic in nature. Flavonoids can be subdivided into subgroups according to the

hydroxylation pattern and chroman ring (C-ring) variations, such as anthocyanidins and anthocyanins, isoflavonoids, flavanones, and flavonols. **(Tsao., 2010)**

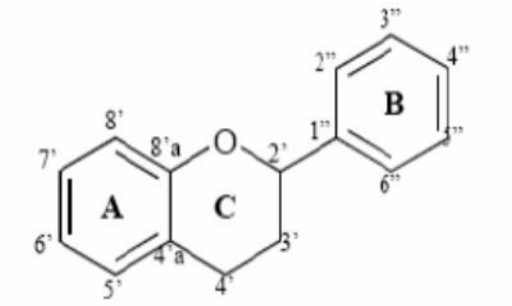


Figure 14. General structure of flavonoids. **(ZIBOUCHE, 2014)**

- **Flavanones**

The absence of the double bond between C2 and C3 and the presence of a chirality center in C2 are the characteristics of flavanones **(Achat, 2013).**

- **Flavonols**

Flavonols are characterized by the presence of an OH group in the C3 position and a C2-C3 double bond. They can be in the form of aglycones or glycosides. Aldoses (L-rhamnose and D-glucose) are the sugars most frequently involved. **(Purchase, 2013)**

- **Anthocyanidins and anthocyanins**

Different from other flavonoids, anthocyanidins and anthocyanins have two double bonds in their heterocycles. Anthocyanins are glycosylated anthocyanidins, distinguished by their hydroxylation and methoxylation patterns on the B ring. Anthocyanins vary depending on the number of hydroxy groups and the nature as well as the number of sugar units associated with their structure. Typically, the associated sugar units are monosaccharides such as glucose, galactose, and arabinose. **(Singla, 2019)**

2.3.3 Tannins

Tannins are intermediate to high molecular weight compounds, distinct from other previously described groups of plant phenolic compounds. Tannins are highly hydroxylated molecules, capable of forming insoluble complexes with carbohydrates and proteins **(Bravo, 1998).** In higher plants, there are two categories of tannins: hydrolyzable and condensed **(Benouamane, 2023**). Hydrolyzable tannins are basic molecules composed of polyols like

sugars and phenolic compounds like catechin, which can be hydrolyzed. Tannic acid is the model compound of this group, represented by ß-penta-O-galloyl-D-glucose. Enzymatic and non-enzymatic hydrolysis of hydrolyzable tannins is more sensitive, and they are generally more soluble in water. Hydrolysable tannins are distinguished according to the hydrolysis products: gallotannins produce gallic acid and glucose, while ellagitannins produce ellagic acid and glucose **(Reed, 1995).** Condensed tannins, or proanthocyanidins, are high molecular weight polymers. A flavan-3-ol (such as catechin or epicatechin) constitutes the monomeric unit, with the flavan-3,4-diol or leucoanthocyanidin molecule as the precursor. The C-4 carbons of the heterocycle are oxidatively condensed with the C-6 or C-8 carbons of neighboring units **(Bravo, 1998).**

2.4 Role and Biological Activities

2.4.1 Role of polyphenols within a plant

In plants, phenolic compounds are ubiquitous and play essential roles in various metabolic and physiological processes. These compounds influence many physiological processes related to plant growth and development, such as seed germination, cell division, and photosynthetic pigment production. Phenolic compounds are used in applications such as bioremediation, allelochemistry, plant growth stimulation and as antioxidant food additives. The accumulation of phenolic compounds is often a permanent feature of plants exposed to stress, serving as a defense mechanism to cope with various abiotic stresses. Plants play a crucial role in various physiological processes to improve their tolerance and adaptability under suboptimal conditions. In particular, many secondary metabolites possess antioxidant properties and can improve plant performance under stress. **(Sharma, 2019)**

2.4.2 Role in biological activity

2.4.2.1 Antifungal and antibacterial agent

- **Antibacterial agent**

Polyphenols present in plant foods and medicinal plants have been widely studied for their antibacterial activity against various microorganisms. Among polyphenols, flavan-3-ols, flavonols and tannins have been the most studied due to their broad spectrum and superior antibacterial activity compared to other polyphenols, and the fact that most of them are capable of suppressing a number of microbial virulence factors (inhibition of biofilm formation, reduction of host ligand adhesion, neutralization of bacterial toxins) and exhibit synergy with

antibiotics. Depending on certain classes of polyphenols, the antibacterial properties have been suggested either to create new food preservatives, due to increasing consumer pressure on the food industry to avoid synthetic preservatives, or to develop novel therapies to treat different microbial infections , due to the increase in bacterial resistance to traditional antibiotic therapy. **(Daglia, 2012)**

Table 4. polyphenol classes and the microorganisms that are sensitive to them. **(Daglia, 2012)**

Flavan-3-ol Flavonol	*V.cholerae - S.mutans -C.jejuni - C.perfringes - E.coli - B.Cereus - H.pylori - S.aureus - L.acidophilus - A.naeslundii - P.oralis - P.gingivalis - P.melaninogenica - F.nucleatum - C.pneumonia*
Condensed tannin	*S. mutans* *E.coli* *S. aureus*
Hydrolyzable tannin	Different strains of: *Salmonella - Staphylococcus - Helicobacter - E.coli - Bacillus - Clostridium - Campylobacter - Lysteria*
Phenolic acid	*S.aureus - L.monocytogenes* *E.coli - P.aeruginosa*
Néolignan	Different strains of: *Mycobacterium tuberculosis*

- **Antifungal agent**

The structural diversity of secondary metabolites from plants may make them effective as antifungal agents, due to their continuous exposure to fungal attack. In addition to their antibacterial characteristics, polyphenols have antifungal properties, particularly against phytopathogenic fungi, and can therefore be considered as metabolites that play a role in the natural defense of plants against fungi. It appears that their relative acidity and the high number

of hydroxyl groups play a crucial role in their antifungal activity. Polyphenols play a role in deteriorating the integrity of the membrane structure of fungi. The increase in membrane permeability is due to functional groups such as phenols which react with membrane enzymes. The membrane is destroyed, leading to leakage of the cytoplasmic contents and death of the strain. **(Allab, 2019)**

2.4.2.2 Antioxidant agent

Oxidative stress is characterized by an imbalance between the generation of free radicals and reactive metabolites, known as oxidants or reactive oxygen species, and their removal by protective mechanisms, known as antioxidants. This imbalance causes the deterioration of large biomolecules and cells, with the potential to impact the entire body. Oxidative stress can cause damage to lipids, proteins, carbohydrates and DNA in cells and tissues. This can lead to membrane damage, random fragmentation or cross-linking of molecules such as DNA, enzymes and structural proteins, and even cell death caused by DNA fragmentation and lipid peroxidation. **(Knežević et al., 2012)** By acting directly on reactive oxygen species or by stimulating endogenous defense systems, antioxidants present in foods can help reduce this damage. The presence of an electron in the phenolic groups of polyphenols allows the formation of relatively stable phenoxyl radicals, which disrupts the chain oxidation reactions in cellular elements. The antioxidant power of polyphenols was evaluated in vitro by studying their ability to capture free radicals and reduce other chemical substances. They are compared to the activity of a reference substance, Trolox (a water-soluble derivative of vitamin E), gallic acid or catechin. In all situations, the reaction examined involves reducing an oxidant using polyphenols. Polyphenols containing catechol groups (aromatic rings containing two hydroxyl groups in the ortho position) exhibit higher antioxidant capacity than polyphenols containing single phenol groups (aromatic rings containing only one hydroxyl group). **(Scalbert et al., 2005)**

2.4.2.3 Anti-inflammatory agent

Inflammation is the body's immune response to infections or injuries caused by bacteria, viruses and other pathogens. The key clinical features of inflammation are called rubor (redness), calor (heat), tumor (swelling) and dolor (pain) in Latin **(Pérez et al., 2016)**. Anti-inflammatory properties have been demonstrated for certain phenolic compounds, which act similarly to NSAIDs (non-steroidal anti-inflammatory drugs). In addition to inhibiting COX (Cyclooxygenase), some of these compounds inhibit other pro-inflammatory mediators by

reducing their activity or gene expression. Additionally, certain phenolic substances can up or down modulate transcription factors such as nuclear factor kB (NFkB) or Nrf-2 in inflammatory and antioxidant pathways. The anti-inflammatory mechanisms of action of phenolic compounds strongly depend on their structure. For example, unsaturation in the C ring ensures the stability of intermediate radical species through resonance. The presence of a C2-C3 double bond results in coplanarity between the A and C rings, facilitating the interaction of the flavonoid with the enzymatic active site. On ring B, the catechol group participates in enzymatic oxidation, creating electrophilic species favoring nucleophilic addition. Finally, the ligands of phenolic compounds play a role in the formation of covalent bonds between flavonoids and macromolecules. Although the precise mechanisms of this anti-inflammatory activity are not fully understood, high consumption of foods containing these compounds is associated with down regulation of the inflammatory response. Phenolic compounds are thought to exert anti-inflammatory action by inhibiting the production of pro-inflammatory mediators, altering the production of eicosanoids, inhibiting activated immune cells, or inhibiting nitric oxide synthase and cyclooxygenase-2 by inhibitory effects on nuclear factor NF-kB. **(Yoon et al., 2005)**

Material and methods

I. Materials and Methods

I.1 Plant material

The samples or plant material were offered to me by Mr. BAIK Nourdine. It consists of:

- Seeds of the Deglet-Nour Variety (reference).

- Fruits and seeds of the Khalte Variety (3 different individuals) sampled in Taghit in the wilaya of Béchar (Fig.16 and Tab. 5).

- Another batch of 12 seeds of the Deglet-Nour variety (hydropriming seed).

Figure 15. Date palm individuals of the Khalt variety **(original, 2024).**

Table 5. The different varieties of dates offered

Var. Ref	Var. Khalte
3 batches of seeds Lot 1: 10	Individual 1: - 34 dates (fruit) - 34 seeds
Lot 2: 10	Individual 2: - 35 dates (fruit) - 35 seeds
Lot 3: 10	Individuals 3: - 33 dates (fruit) - 33 seeds

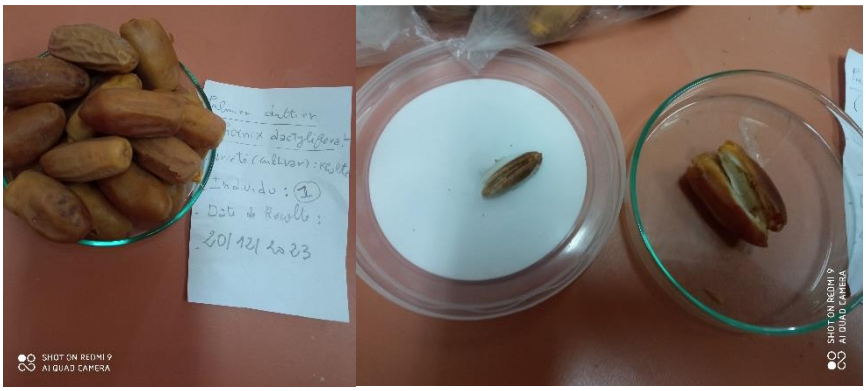


Figure 16. Fruit and seed of the Khalte variety. **(Original, 2024)**

I.2 Microbiological material (fungal)

- **Plant pathogens**

For the study we used two fungal strains isolated from date seeds and identified using the optical microscope and which are Aspergillus fumigatus, Aureobasidium pullulans.

- **Antagonist agent**

The fungal strain used as an antagonist in this study is a strain of Trichoderma sp.

I.3. Experimental method

I.3.1 Germination

All fruits from each individual were first pitted to collect the seeds, then they were cleaned (removed the cellulose husk) and sorted. They were cleaned with tap water to remove all traces of the endocarp.

Germination was carried out on 30 seeds of Deglet Nour dates and 30 seeds of dates of the Khalte variety, collected at the Tmar stage.

Figure 17. The pitted fruits of each individual **(Original, 2024)**

❖ **Germination of seeds**

In order to understand how pretreatment influences the germination of seeds of the Deglet Nour variety and the Khalte variety and the seeds (hydropriming) all batches underwent different pretreatments.

❖ **The control seeds**

The Deglet Nour variety and the Khalte variety are prepared in three separate batches, in Lot 1 (control), 10 seeds of the Deglet Nour variety and 10 seeds of the Khalte variety are soaked in water for 24 hours and placed in containers. Petri dishes, each lined with paper towels to absorb excess water.

Figure 18. The control seeds of the two varieties (DN and Khalte). **(Original, 2024)**

❖ **Chemical treatment**

In Lot 2, another 10 seeds of the Deglet Nour variety and 10 seeds of the Khalte variety are immersed in pure sulfuric acid for 5 minutes, then transferred to running water until a clean water to remove all traces of sulfuric acid. The seeds are then soaked in water for 24 hours and placed in labeled petri dishes, each lined with paper towels to absorb excess water.

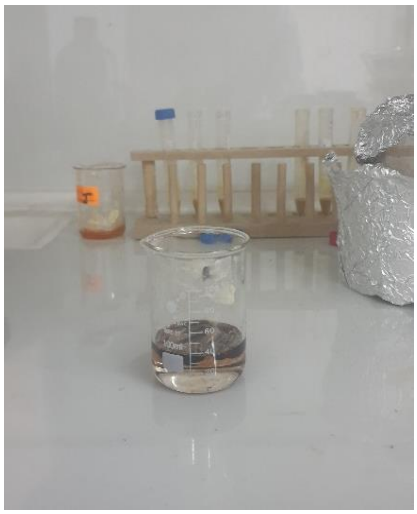


Figure 19. Chemical treatment of seeds with pure sulfuric acid. **(Original, 2024)**

❖ **Mechanical treatment**

Lot 3 consists of scarifying 10 seeds of the Deglet Nour variety and 10 seeds of the Khalte variety with sandpaper, the seeds are soaked in water for 24 hours and placed in Petri dishes, each lined with paper absorbent to absorb excess water.

Figure 20. Mechanical processing of seeds (DN and Khalte). **(Original, 2024)**

The other 12 seeds of the Deglet NOUR variety (hydropriming) are soaked in water for 24 hours and then placed directly in Petri dishes.

Finally, the Petri dishes containing the treated seeds are placed in a culture chamber set at the temperature (25°C) until the dates germinate.

Figure 21. Incubation of the seeds obtained by the different treatments. **(Original, 2024)**

➔ **Formulas selected for the study**

The following formulas are used to calculate the germination rate, speed and germination index of each batch and each individual.

- **Germination rate (%)** = $\frac{\text{number of grains germinated}}{\text{Total number of grains}} 100\times$
- **Germination speed**= $\frac{(N1\times T1)+\ldots\ldots\ldots+(Nn\times Tn)}{N1+\ldots\ldots\ldots+Nn}$
- **Germination index**=$N1\times 1+\frac{(N2-N1)}{2}+ +\ldots+\frac{(N3-N2)}{3}\frac{(Nn-Nn-1)1}{n}$

N1:is the number of grains germinated at time T1.

N2:is the number of grains germinated at times T1 and T2.

N1, N2, N3,…, Nn-1and **Nn** are the percentages of germination obtained depending on the days.

I.3.2. Study of total polyphenols by hydromethanolic extraction

- **Drying and grinding**

The seeds of the 3 individuals of the Khalte variety are first cleaned, starting by removing the skin of the fruit (date) then washing in tap water to remove all remains of the skin. Finally dried them for a period of 10 days at room temperature.

At the same time, the fruits (dates) of the 3 individuals were subjected to a three-week drying period in an oven maintained at a constant temperature of 40°C.

Figure 22. Drying of fruits (dates) in the oven. **(Original, 2024)**

Once dried, the samples (fruits and seeds) were ground thoroughly using a mortar and pestle and a mixer grinder until a fine and homogeneous consistency (powder) was obtained and were then stored in glass jars.

- **Hydromethanolic extraction**

For each individual (seeds and fruits), 5 g of dried plant material (powder) are precisely weighed and placed in separate Erlenmeyer flasks, to which 35 ml of methanol and 15 ml of

distilled water are added. Each Erlenmeyer flask containing the plant material is then covered with aluminum foil to avoid any external contamination. The Erlenmeyer flasks are then placed on a magnetic stirrer and set to an appropriate speed, then left to stir for 48 hours cold. After 48 hours, the Erlenmeyer flasks are removed from the magnetic stirrer and the aluminum foil is removed. The resulting extracts are filtered using Whatman No. 1 filter paper, into clean vials, then each vial is labeled with the appropriate information, including the source of the plant material (seeds and fruits) and any other relevant data.

Figure 23. Agitation and filtration of extracts (fruits and seeds). **(Original, 2024)**

Then the filtrate was dried under vacuum at 50ºC in a rotary evaporator to eliminate the solvent until an extract was obtained, which would then be freeze-dried and stored under appropriate conditions, protected from light andat room temperature until analysis.

Figure 24. Evaporation of extracts (fruits and seeds) using a rotary evaporator. **(Original, 2024)**

Determination of total polyphenols

➔ **Principle**

The estimation of the content of total phenolic compounds was carried out by the Folin-Ciocalteu colorimetric method. Phosphotungstic acid (H3PW12O40) and phosphomolybdic acid (H3PMo12O40) form the reagent. Oxidation of polyphenols transforms it into a mixture

of blue oxides of tungsten and molybdenum. The coloring produced is linked to the quantity of polyphenols in the plant extracts. **(Boizot et al, 2006).**

Operating mode

From the gallic acid solution (0.2 mg / ml) we carry out a dilution series (range point) in order to draw a calibration curve,In order to determine the absorbance (OD) at 760 nm, 1 ml of Folin-Ciocalteu reagent (diluted 10 times) is added to 200 µL of range point. We then add 800µL of Na2CO3 (75g/L).

The reaction mixtures, corresponding to each range point and sample, are shaken and incubated for 5 min at 40°C or 30 min at room temperature. The results obtained are expressed in mg gallic acid equivalent per gram of dry extract (mg EGA/g ES).

In parallel and under the same operating conditions, the operation is repeated for 200 µL of each extract (sample) diluted (1 mg/ml). The blank test or the standard not containing polyphenols (0 µl/ml) is made by adding 0.2 ml of distilled water, 1 ml of Folin-Ciocalteu reagent (diluted 10 times) and 800µL of Na2CO3 (75g/L)

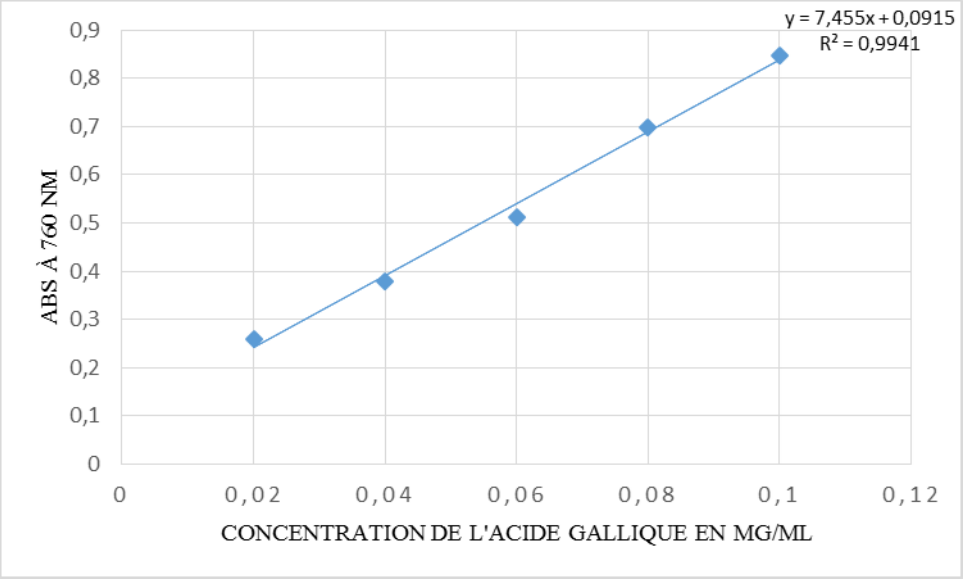


Figure 25. Calibration curve of the gallic acid standard used in the determination of total polyphenols.

I.3.3 Study of 3 classes of flavonoids by acid hydrolysis

In a boiling water bath (95°C), 0.5 g of dry plant material (MVS) is hydrolyzed with 40 ml of HCl (2N) for 40 minutes, blowing in air every 10 minutes. Oxygen allows the oxidation

of proanthocyanidins to the corresponding anthocyanidins. At each extraction two phases appear, one upper called epiphase and the other lower called hypophase:

- Ethereal Epiphase

It is greenish yellow and contains aglycones (flavones and flavonols) and phenolic acids. After each extraction, it is collected in a beaker and evaporated in the open air. The dry residue is taken up in 5 mL of ethanol, then stored in a cool place before being quantitatively analyzed by UV-visible spectrophotometry.

- Acid hypophase

Reddish in color, it is composed of anthocyanins, C-glycosides and simple oses. It is collected and mixed with 35 ml of n-butanol, then placed in a separatory funnel. In the hypophase, n-butanol creates a reddish butanolic epiphase, which will be collected in a Petri dish and evaporated, in the open air. The dry residue is taken up in 5mL of ethanol, and then kept in a cool place before having it quantitatively analyzed by UV-visible spectrophotometry.

✓ **Quantitative dosage**

A colorimetric method was employed based on the property of certain compounds that absorb more light at UV-visible spectrophotometric wavelengths. This method makes it possible to carry out dosages using Beer Lambert's law, which is based on the following principle:

Passing through a solution, part of the light of intensity I0 is absorbed by the solute(s). The transmitted light therefore has an intensity I less than I0.

According to Beer Lambert the law of absorbance of the solution is defined as follows:

$$A=\log_{10}(I°/I)$$

➢ **Dosage of proanthocyanins**

The extracts are analyzed spectrophotometrically at 520 nm immediately after extraction, as they degrade rapidly in light.

The following formula makes it possible to determine the total quantity of anthocyanins, with a correction coefficient of 6.

$$T(mg/g)=5.2\ 10\text{-}2.D0\ .V.\ d/p$$

DO: optical density

V: volume of the methanoic phase

d: dilution factor

p: dry weight of hydrolyzed plant material

- **Dosage of C-glycosyl-flavones**

It is also possible to read the extracts using spectrophotometry at 340 nm, and the absolute content is expressed as Orientin and calculated according to the following formula.

$$T(mg/g)=2.37\ 10\text{-}2\ .DO\ .Vd/p$$

DO: optical density

V: volume of the methanoic phase

d: dilution factor

p: dry weight of hydrolyzed plant material

- **Dosage of flavonic aglycones**

Flavonoids with a free hydroxyl (-OH) in position 3 and 5 can react with metals. Aluminum chloride (AlCl3) binds to flavones and/or flavonols to form a complex. The differential assay method was used to evaluate the quantity of aglycones. And carried out two dilutions: the first dilution in ethanol (ethereal extract + 95° ethanol) and the second dilution in the AlCl3 solution (ethereal extract + AlCl3 in 95° ethanol). The AlCl3 solution is prepared by mixing 1 g of aluminum chloride in 100 ml of 95° ethanol. After reacting for 15 minutes, the dry ethereal residue is taken up in 95° ethanol by adding 1% aluminum chloride. Aglycones are measured between 400 and 435 nm using the typical spectrophotometer. The formula used to measure flavonic aglycones (420 nm) and flavonols (435 nm) is as follows:

$$T(in\%°\text{ or }mg/g)=1.3\ 10\text{-}2.\ \Delta DO.v\ .d/p$$

ΔDO: optical density at the differential peak equal to DO(AlCl3) – DO (ethanol 95°)

ε: quercetin absorption coefficient equal to 302 g

V: volume of the ethanolic solution

d: dilution factor

p: dry weight of hydrolyzed plant material

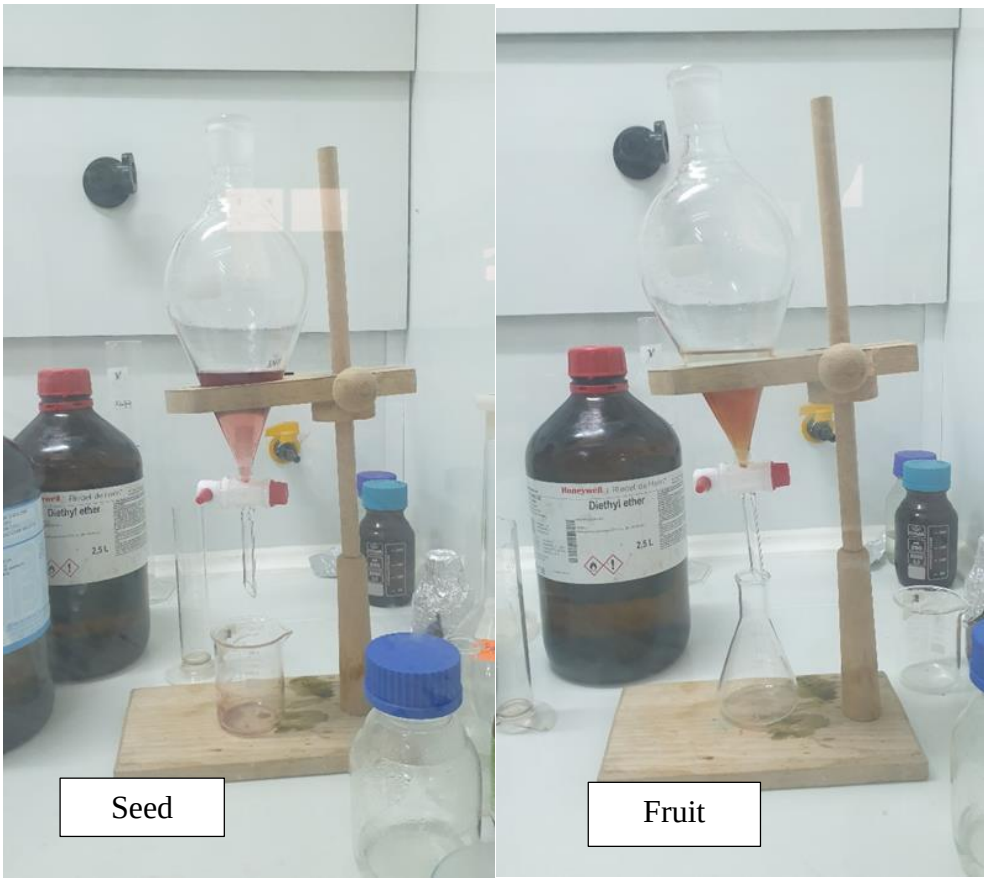


Figure 26. Acid Hydrolysis (seed and fruit). **(Original, 2024)**

I.3.4 Antioxidant activity at DPPH

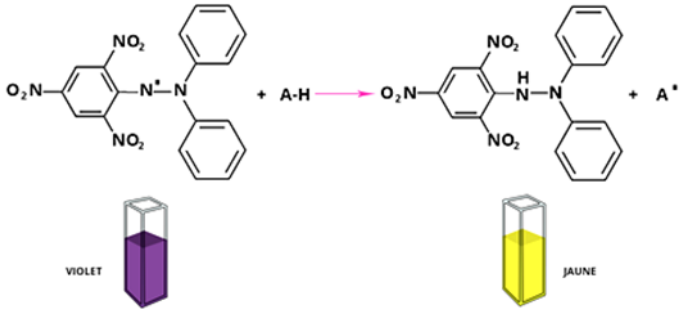
We used the DPPH (diphenyl picryl hydrazyl) method as a relatively stable radical to study the antiradical activity of total polyphenols extracted from the seed and fruit.

➔ **Principle**

According to Marinova et al (2011) the DPPH test is used to evaluate the antiradical capacity of pure molecules or plant extracts in a model system comprising an organic solvent and a controlled temperature.

It evaluates the ability of an antioxidant (AH), generally phenolic compounds, to reduce the chemical radical DPPH° (2,2-diphenyl-1-picrylhydrazyl) by transferring a hydrogen. DPPH°, initially purple, metamorphoses into DPPH-H, pale yellow.

The reaction rate varies depending on the nature of the antioxidant, and the amount of DPPH-H produced will be influenced by the antioxidant concentration.



Operating mode

To evaluate this activity, we prepared a stock solution of 1 mg/ml from each seed and fruit extract. Then, a range of dilutions was carried out, going from 0 to 200 µg/ml.

The initial DPPH° solution is obtained by dissolving 0.0024g of DPPH in 100ml of ethanol.

The anti-radical reaction is triggered by adding 1.5 ml of DPPH to 0.5 ml of each dilution of the extract. The mixture is left in the dark for 30 minutes, then the OD is measured at 517 nm.

The anti-radical activity is estimated according to the following equation:

$$I\ (\%) = [(A.0 - A.1) / A.0]$$

I: anti-radical activity of the sample.

A.0: DO control (containing only DPPH).

A.1: OD of the sample.

Calibration (the negative or blank control) is carried out using 96° ethanol (OD = 0.00)

The negative control (control) is formed by 1.5 ml of the initial DPPH° solution + 0.5 ml 96° ethanol (control OD).

I.3.5 Microbiological study

- **Preparation of PDA medium (Potato Dextrose Agar)**

In this study, we used PDA culture medium, which is a favorable medium for the growth and sporulation of a wide range of fungi, especially phytopathogenic fungi.

To prepare one liter of PDA medium, 39 g of dehydrated PDA medium was weighed and mixed with 1 liter of distilled water. Then, the medium was stirred on a hot plate. After homogenizing, it must be placed in bottles, then autoclaved at 120°C for 20 minutes. Then, the agar medium is distributed into 9 cm diameter petri dishes under aseptic conditions under the laminar flow hood.

- **Isolation and identification of fungal strains present on the surface of date seeds**

In order to isolate the fungi present on the surface of the seeds, the date palm seeds are placed in Petri dishes containing PDA medium. These plates are incubated at a temperature of 25°C for a period of 3 days. At the end of this period, the Petri dishes are examined for any fungal growth. Then, subculture is carried out using sterile Pasteur pipettes to collect fragments of mycelium. These fragments are moved to new Petri dishes containing PDA. This subculturing operation is repeated several times until pure cultures are obtained. After obtaining the pure cultures, the growth of the mycelia is observed. A graduated ruler is used to measure growth, with diameter measurements taken at regular intervals (five days per week), to determine the rate of growth, followed by a microscopic identification.

- **Evaluation of the direct confrontation test of Trichoderma sp against target strains**

The method used by Benhamou and Chet (1996) involves placing two explants of the antagonist agent (Trichoderma sp) and the target agent (Aspergillus fumigatus and Aureobasidium pullulans) in the same petri dish (8.5 cm in diameter) at a distance of 3 cm from each other. Incubation lasts 5 days at a temperature of 25°C. Under the same conditions, control plates containing the target agent and the antagonist agent are incubated separately. Colony diameter measurements were taken, the inhibition rate is then calculated according to the formula:

Inhibition rate = (R control - R test) / R control × 100

- Rate of inhibition of mycelium growth in percentage (%).
- R control: diameter in cm of the control phytopathogenic fungus.
- R test: diameter in cm of the phytopathogenic fungus in the antagonism test.

Results and discussion

II.1 Results

The results of our work will be illustrated and interpreted according to the graphs produced on the basis of the raw data from the following analyses: germination, total polyphenol contents, flavonoid contents, antioxidant power of DPPH and microbiological analysis.

II.1 Germination

a- Germination rates

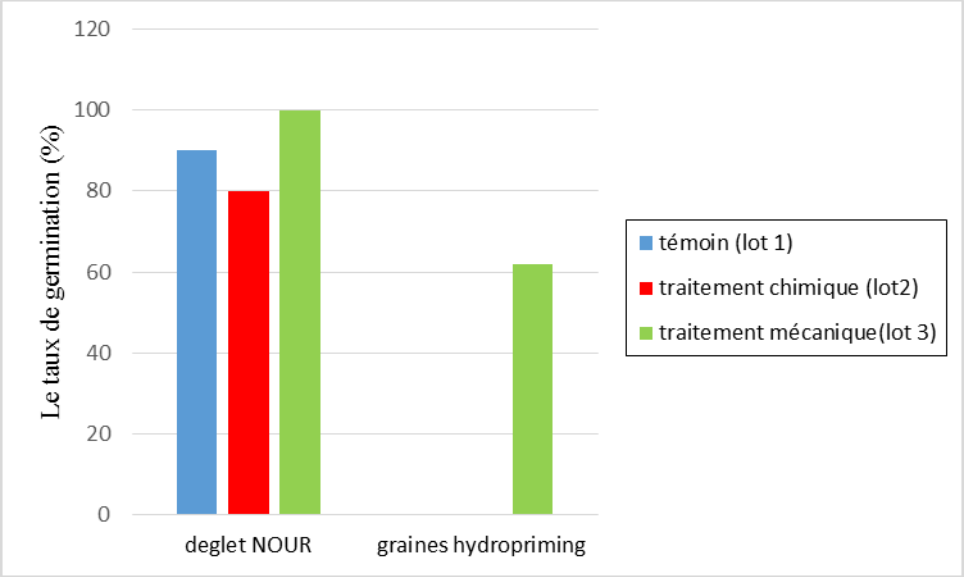


Figure 27. Germination rate obtained depending on the pretreatments applied to theseeds of the Deglet NOUR variety.

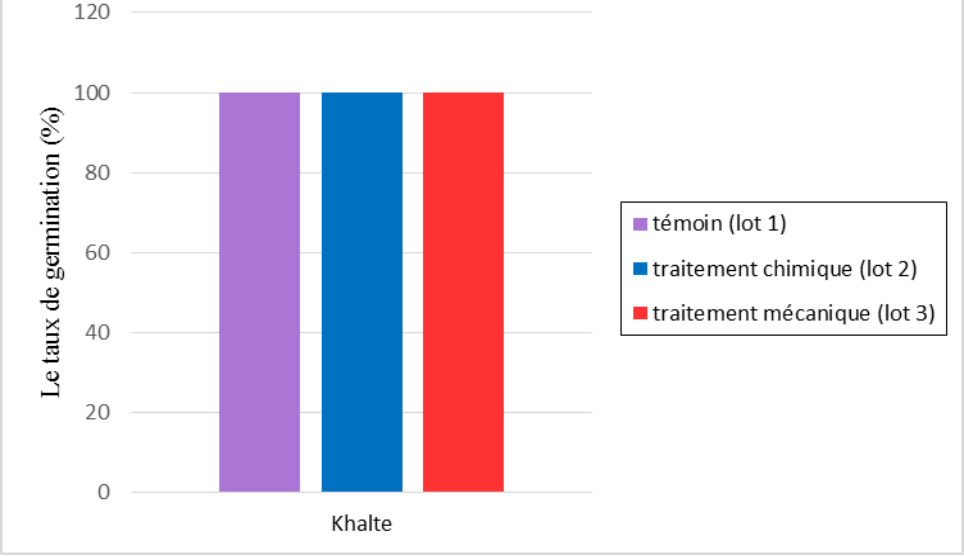


Figure 28. Germination rate obtained according to the pretreatments applied to the seeds of the Khalte variety.

b- Germination speed

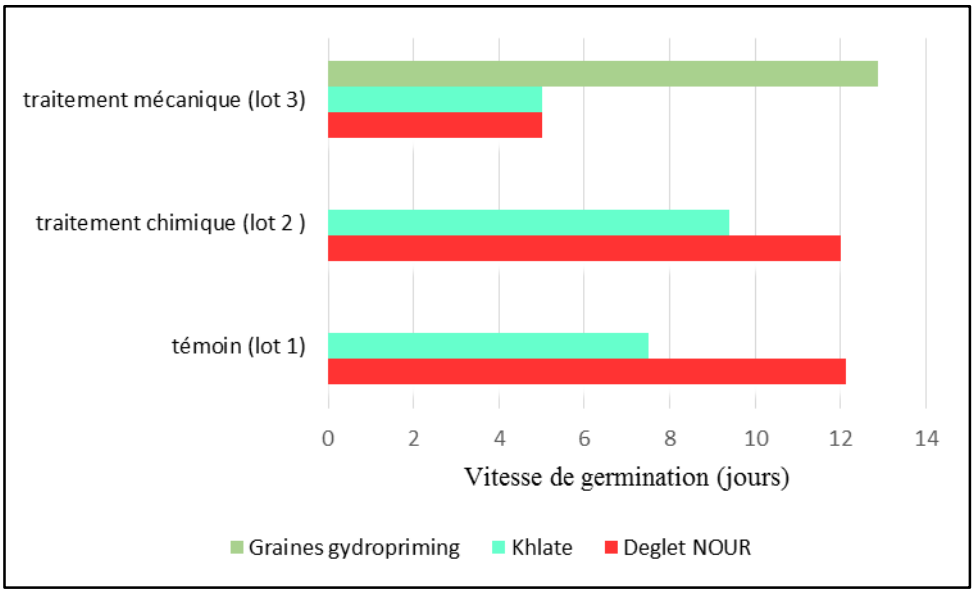


Figure 29. Germination speeds obtained according to the pretreatments applied to the seeds of the Deglet NOUR variety and the Hydropriming seeds as well as the Khalte variety.

c- Germination Index

Variety	Lot 1	Batch 2	Batch 3
Deglet NOUR	0.94	0.88	2
Khalte	1.38	1.56	2
Hydropriming	/	/	0.72

The results obtained show that the mechanical scarification technique accelerates and improves the germination rate compared to other techniques.

Figure 30. Germination results (Khalte variety) **(Original, 2024)**

Figure 31. Germination result (Deglet NOUR variety). **(Original, 2024)**

II.2 Determination of total polyphenols

The contents of total phenols were determined from the linear regression equation of the calibration curve (**Fig. 32**), plotted using gallic acid as a standard.

The values obtained are expressed in **mg EAG/g ES** (**Fig. 32**).

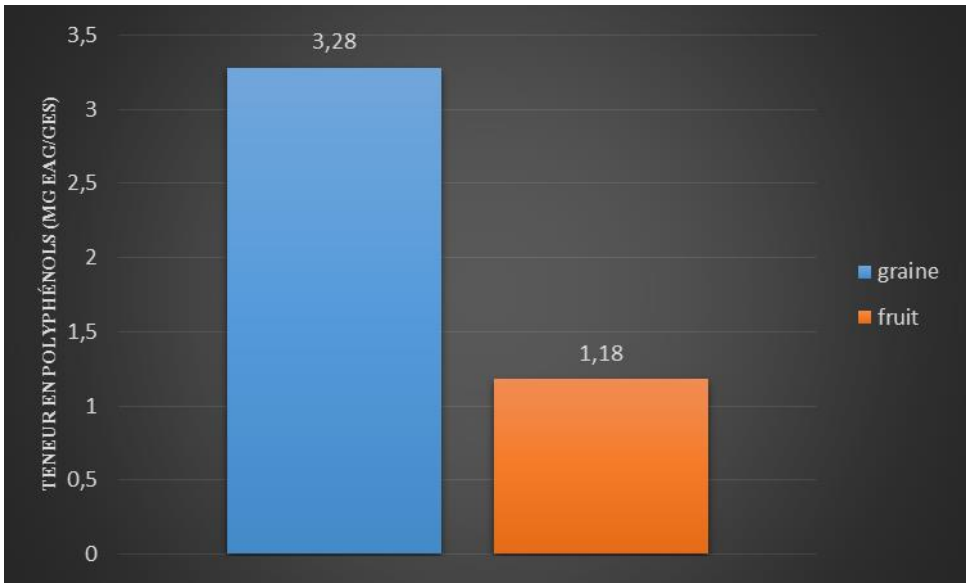


Figure 32. Comparison of total polyphenol contents of the 3 individuals (seed and fruit).

The results obtained show that the seed is richer in total polyphenols than the fruit. We note a highly significant difference at the 99% security threshold (P<0.01)

II.3 Flavonoids

The results obtained after determinations of anthocyanins, C-glycosides and Aglycones are represented by a histogram for each of the samples analyzed from the Fruit and the Seed (**Fig. 33**).

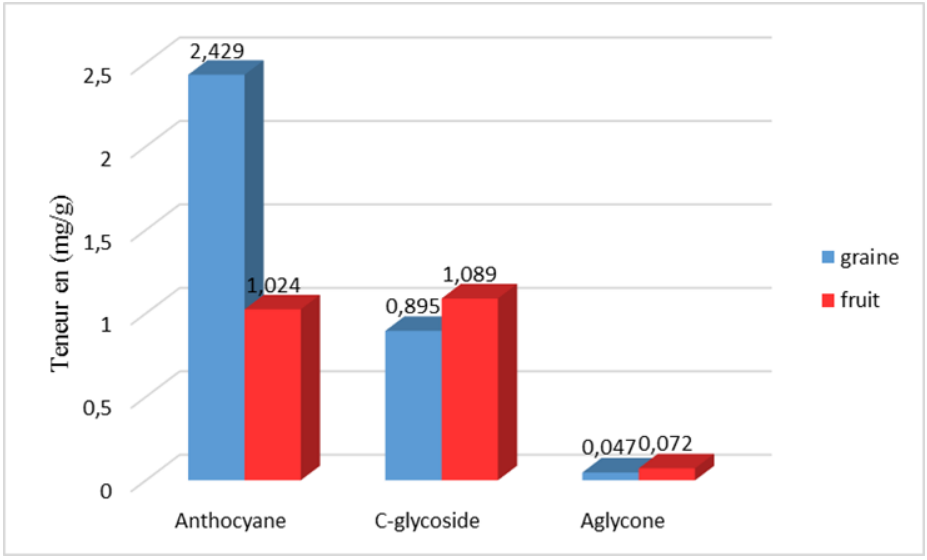


Figure 33. Content (mg/g) of anthocyanins, c-glycosides and aglycones in fruit and seed of 3 individuals of date palm Var. Khalte.

The histograms show that the **anthocyanin** levels at the **seed** level are higher than the **fruit**. On the other hand, the contents of **C-glycosides** show that the **fruit** is **richer** in this molecule than the **seed**. While, those of **Aglycones** do not show **significant differences** between the **two organs** studied.

II.4 Activity antioxidant (DPPH test)

The anti-radical activity (I%) or antioxidant power by the DPPH test is estimated by measuring the inhibitory concentration at 50% or (half maximum inhibitory concentration) (IC.50): this is the concentration tested necessary to reduce 50% of the activity of the DPPH.

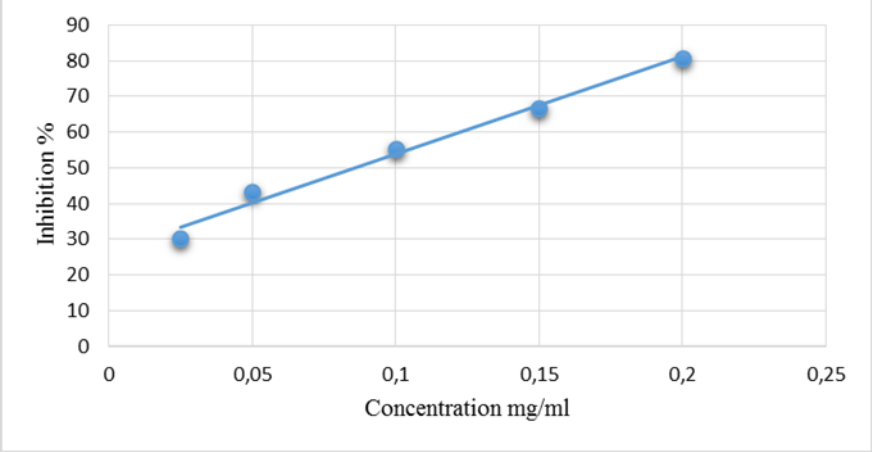


Figure 34. Extract curve for 3 individuals (seed) of the Khalte variety as a function of inhibition percent

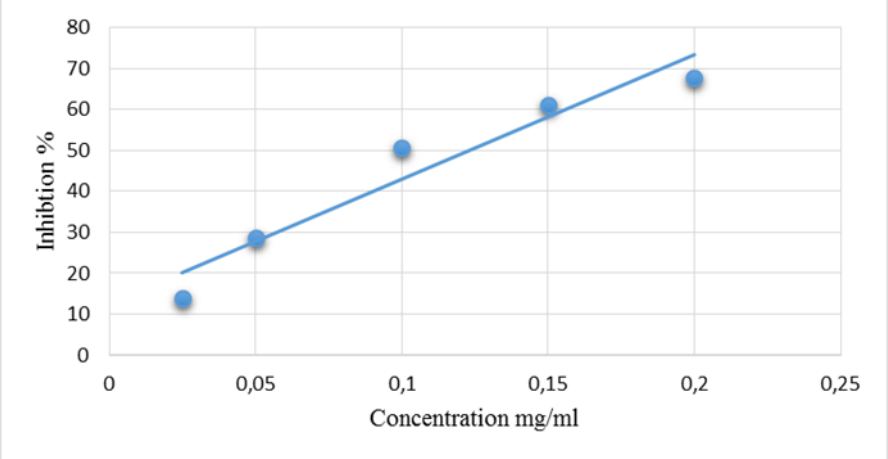


Figure 35. Extract curve for 3 individuals (fruit) of the Khalte variety as a function of inhibition percentage.

EC50 value calculations for individuals:

Individual	Equation	R^2	EC 50 (mg/g)
Ind 1/2/3 (graine)	272,55x + 26,618	0,9846	0,0857897633
Ind 1/2/3 (fruit)	303,17x + 12,592	0,9307	0,1233895174

The graphs of the anti-radical activity or tested at DPPH (**IC%)** show that the **seed** has a **lower minimum inhibitory** value than that expressed by the **fruit**.

II.5 Microbiological analysis(mushrooms)

- **Isolation and identification of fungal strains present on the surface of date seeds**

Once the petri dishes with the mycelia have been incubated at a temperature of 25°C for 3 days. Fungal growth has been observed including:

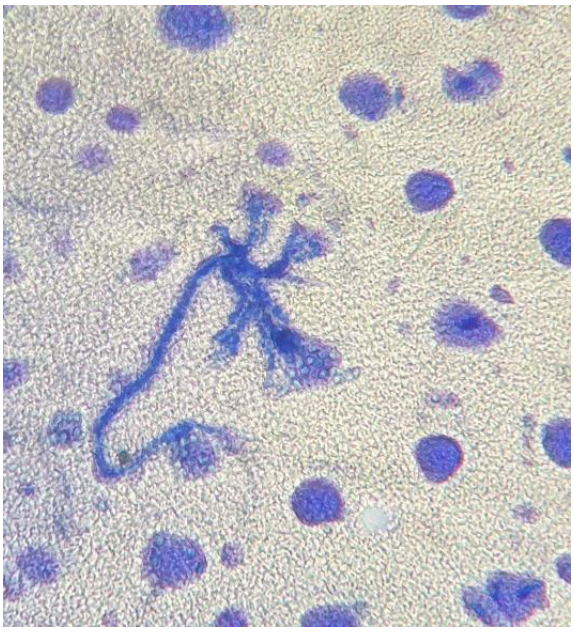


Figure 36. Microscopic view of *Aspergillus fumigatus*. **(Original, 2024)**

Date variety: Deglet NOUR (hydropriming seeds).

Contaminant :

- Genus: *Aspergillus.*
- Species: *Aspergillus fumigatus*.

These are filamentous fungi, whose colony is powdery. This mushroom has echinulate conidia of a gray-green color. *Aspergillus fumigatus* allows rapid growth; the colony can reach 4.6 cm in one week when grown on Czapek-Dox culture medium at a temperature of 25°C **(Latgé, 1999)**.

Microscopic observation shows conidiophores generally columnar erect with vesicular heads and radiating phialides which is characteristic of the genus *Aspergillus*. **(Schmidt et al, 1997).**

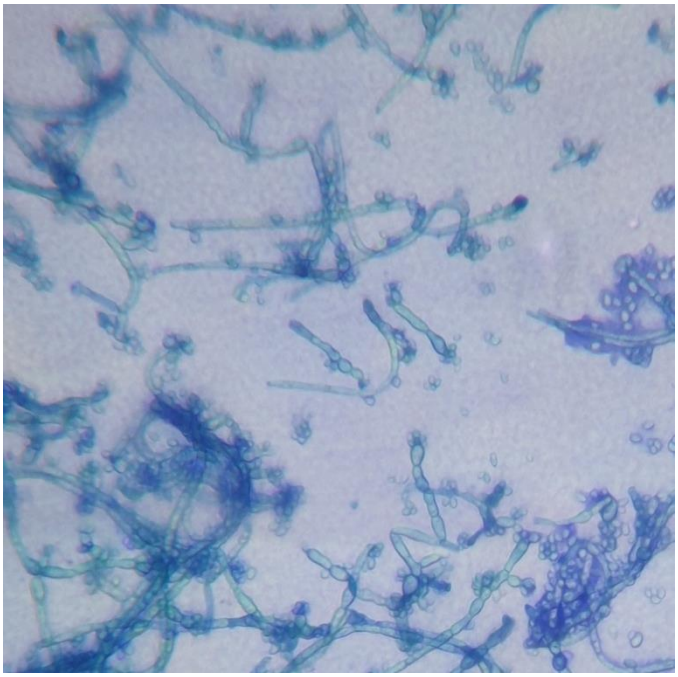


Figure 37. Microscopic view of *Aureobasidium pullulans*. **(Original, 2024)**

Date variety : Deglet NOUR (hydropriming seeds).

Contaminant :

- Genus: *Aureobasidium*.
- Species: *Aureobasidium pullulans*.

The colonies appear smooth, moist, and yeast-like. With age, colonies become more filamentous and may develop a velvety texture. The color changes to dark brown or black as the colony matures due to melanin production. **(Barnett et al, 1972).**

Microscopic observation shows that the hyphae are septate (divided by transverse walls) and initially hyaline (clear), becoming pigmented with age. Blastospore-like cells are frequently observed, Of the Arthroconidia are formed by fragmentation of hyphae and can appear rectangular or barrel-shaped. Pigmentation is a key element in differentiating *A. pullulans*. Younger cells and hyphae are generally hyaline, while older cells and structures become darkly pigmented due to melanin. **(Barnett et al, 1972).**

- **Test results of the direct confrontation test of *Trichoderma sp* against *Aspergillus* and *Aureobasidium* strains**

✓ *A. fumigatus* :

During the direct confrontation test, in the absence of *Trichoderma sp*, the average diameter of *A.fumigatus* increased from 0.7 cm on the third day to 3.6 cm on the tenth day (figure). However, in the presence of *Trichoderma sp*, the growth speed of *A.fumigatus* is altered, as well as the average diameter which goes from 0.1 cm on the first day to 0.3 cm on the tenth day, which represents a rate of inhibition of 91.66%.

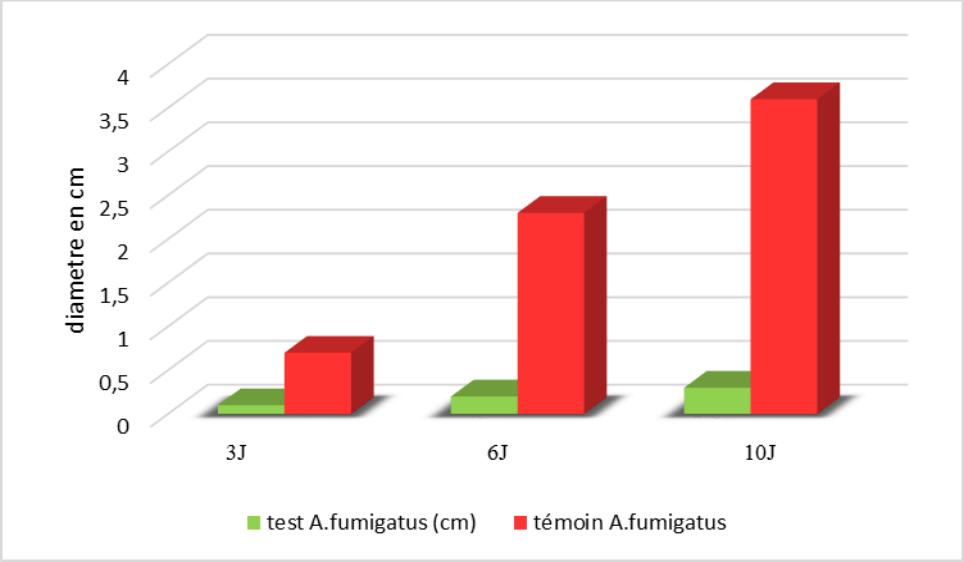


Figure 38. Effect of *Trichoderma sp* on the radial growth of *A.fumigatus*.

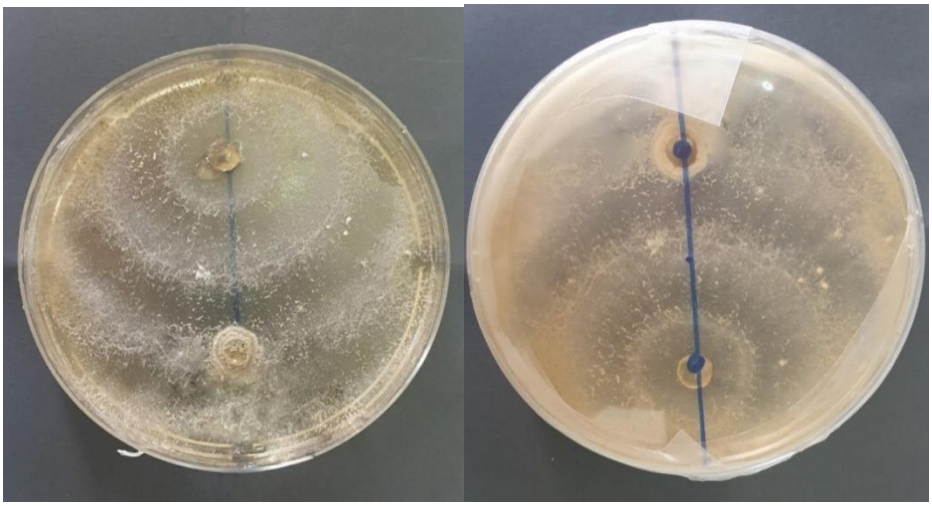


Figure 39. Confrontation *Trichoderma sp – A.fumigatus* on PDA medium (left: front, right: reverse). **(Original, 2024)**

- ✓ *A.pullulans :*

In the absence of *Trichoderma sp*, the average diameter of *Aureobasidium pullulans* colonies is 0.1 cm after one day of incubation and increases to 1.8 cm on the tenth day (figure). On the other hand, when *Trichoderma sp* is present, the growth speed is modified (becomes slower) and the average diameter of *A.pullulans* increases from 0.1 cm on the first day to 0.2 cm on the tenth day, with a rate of inhibition of 88.88%.

This suggests that the *Trichoderma sp* strain affected the radial growth of the two strains of *Aspergillus fumigatus* and *Aureobasidium pullulans* (inhibitory effect).

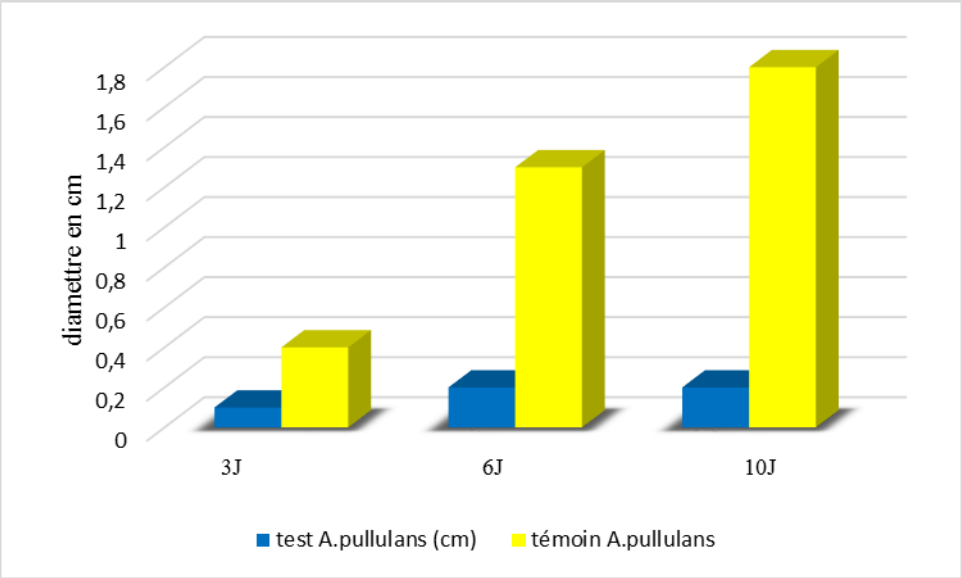


Figure 40. Effect of *Trichoderma sp* on the radial growth of *Aureobasidium pullulans*.

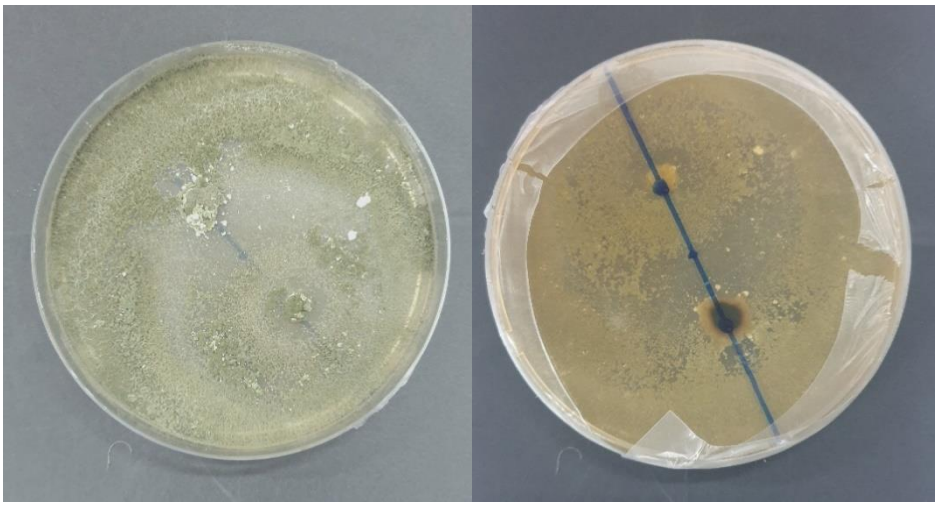


Figure 41. Confrontation *Trichoderma sp – A.pullulans* on PDA medium (left: front, right: reverse). **(Original, 2024)**

III.1 Discussion

The work carried out began with the in-vitro germination of the seeds of the date palm (*Phoenix dactylifera L.*) var. Khalte using some techniques such as: mechanical scarification, chemical treatment with sulfuric acid and hot water in order to know their impact on the germination rate. The results obtained show that the three types of treatment improve germination performance (by increasing the speed and rate of germination). This allows us to deduce that the treatments allowed the dormancy of the seeds to be lifted. On the other hand, the results indicate that mechanical scarification of seeds is more effective on the germination rate compared to chemical treatment and water, which allows us to deduce that the inhibition of germination in the seeds of the Date palm (*Phoenix dactylifera L.*), is integumentary. **(Mazliak, 1982).**

The results of the phytochemical analysis of polyphenols showed that the contents of total polyphenols as well as the class of flavonoids (anthocyanin) are higher in the seed than in the fruit and the opposite for the classes (c-glycoside and aglycone). However, the DPPH TEST revealed strong anti-radical activity in both organs studied. These results corroborate with those of **BOSILA et al. (2001)** on varieties from Egypt and **GACEB-TERRAK (2010)** and **OUAFI (2007)** on Algerian date varieties.

The head-to-head experiment demonstrated that all *Trichoderma* isolates were capable of inhibiting mycelial growth of *Aspergillus* and *Aureobasidium* strains, although inhibition rates varied among strains **(Dabire et al., 2016 Boughalleb-M'Hamdi et al., 2018).**

Conclusion

Our work consists of a phytochemical study and a microbiological study of a traditional date variety (*Phoenix dactylifera L.* var. Khalte) by the analysis of polyphenols, flavonoids and fungi associated mainly with the seed during its germination.

We came to the conclusion that mechanical scarification is more reliable and faster for obtaining germinations compared to pure sulfuric acid and water treatment. The polyphenol contents (total and flavonoids) as well as their anti-radical activity are important in the two organs studied (the seed and the fruit) which suggests that the date palm in addition to its use in human food is a source bioactive molecules (anti-oxidants).

At the end of our study, we can say that fungal contamination (*Aspergillus* and *Aureobasidium* type molds) is a frequent problem in germination studies of plants that reproduce by seed. The variety most exposed to contamination is the Deglet NOUR variety (hydropriming seeds). However, it allows us to better understand the enemies of our variety. The direct confrontation test revealed the inhibitory effectiveness of the *Trichoderma sp* strain used in our study. With a high inhibition rate for the two strains studied (*A.fumigatus* and *A.pullulans*).

Through this study, we obtained exciting results that allow us to explore new research perspectives, such as:

- The study of other varieties or cultivars of the date palm, particularly those grown in the southwest region.
- Other organs such as roots, leaves, sap, etc.
- Other molecules such as : essential oils, lipids, etc.
- Other microbiological activities such as antifungal, antibacterial activities, etc.
- Other biochemical analysis techniques: GC-MS and HPLC for better identification of the synthesized substances.
- Molecular analysis (PCR, sequencing, etc.) in order to identify and isolate the genes, which code for these substances.

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Annexes

Annex 1. Non-biological material

Glassware	**Accessories**	**Reagents**	**Culture centre**
- Beaker - Funnel -Erlenmeyer - Volumetric flask - Bottles - Separating funnel - Test tubes - Test tube	- Magnetic bar - Sterile petri dish - Scissors - Micropipette - Aluminum foil - Filter paper - Wattman paper N.1 - Parafilm - Pissette - Tube rack - Spatula - pliers	- Methanol - Ethanol - Diethyl ether - N-butanol - Sulfuric acid - Hydrochloric acid - Folin ciocalteu -DPPH - Gallic acid - Na2Co3	-PDA

Annex 2. Equipment

- Oven

- Bain marie

- Precision scale

- Hot plate stirrer

- Spectrophotometer

- Rotary evaporator

- freeze dryer

Annex 3. Flavonoid calculations

- Individual 1 (seed)

1) Anthocyanin

V	Acid hypophase (Hcl)	50ml
P	MVS	0.5g
d	Dilution factor	/
DO	520nm	0.326
The absolute content	mg/g	1,627

2) C-glycoside

V	Butanolic phase	40ml
P	MVS	0.5g
d	Dilution factor	/
DO	340nm	0.379
The absolute content	mg/g	0.718

3) Aglycone

V	Ethanolic Phase	5ml
P	MVS	0.5g
d	Dilution factor	/
DO	420nm	0.260
The absolute content	mg/g	0.034

- Individual 1 (fruit)

1) Anthocyanin

V	Acid hypophase (Hcl)	48ml
P	MVS	0.5g
d	Dilution factor	/
DO	520nm	0.169
The absolute content	mg/g	0.878

2) C-glycoside

V	Butanolic phase	41ml
P	MVS	0.5g
d	Dilution factor	/
DO	340nm	0.919
The absolute content	mg/g	1,785

3) Aglycone

V	Ethanolic Phase	5ml
P	MVS	0.5g
d	Dilution factor	/
DO	420nm	0.874
The absolute content	mg/g	0.114

- Individual 2 (seed)

1) Anthocyanin

V	Acid hypophase (Hcl)	49ml
P	MVS	0.5g
d	Dilution factor	/
DO	520nm	0.570
The absolute content	mg/g	2,845

2) C-glycoside

V	Butanolic phase	42ml
P	MVS	0.5g
d	Dilution factor	2/10
DO	340nm	0.489
The absolute content	mg/g	0.486

3) Aglycone

V	Ethanolic Phase	5ml
P	MVS	0.5g
d	Dilution factor	/
DO	420nm	0.307
The absolute content	mg/g	0.039

- Individual 2 (fruit)

1) Anthocyanin

V	Acid hypophase (Hcl)	48ml
P	MVS	0.5g
d	Dilution factor	2/10
DO	520nm	0.416
The absolute content	mg/g	1,059

2) C-glycoside

V	Butanolic phase	40ml
P	MVS	0.5g
d	Dilution factor	2/10
DO	340nm	0.692
The absolute content	mg/g	0.656

3) Aglycone

V	Ethanolic Phase	5ml
P	MVS	0.5g
d	Dilution factor	/
DO	420nm	0.845
The absolute content	mg/g	0.109

- Individual 3 (seed)

1) Anthocyanin

V	Acid hypophase (Hcl)	48ml
P	MVS	0.5g
d	Dilution factor	/
DO	520nm	0.564
The absolute content	mg/g	2,815

2) C-glycoside

V	Butanolic phase	42ml
P	MVS	0.5g
d	Dilution factor	2/10
DO	340nm	0.486
The absolute content	mg/g	0.483

3) Aglycone

V	Ethanolic Phase	5ml
P	MVS	0.5g
d	Dilution factor	/
DO	420nm	0.536
The absolute content	mg/g	0.069

- Individual 3 (fruit)

1) Anthocyanin

V	Acid hypophase (Hcl)	48ml
P	MVS	0.5g
d	Dilution factor	2/10
DO	520nm	0.455
The absolute content	mg/g	1,135

2) C-glycoside

V	Butanolic phase	40ml
P	MVS	0.5g
d	Dilution factor	2/10
DO	340nm	0.868
The absolute content	mg/g	0.828

3) Aglycone

V	Ethanolic Phase	5ml
P	MVS	0.5g
d	Dilution factor	/
DO	420nm	0.725
The absolute content	mg/g	0.094

Annex 4. The total polyphenol content of the 3 individuals

Individual 1 (seed) :

DO 1	0.122
DO 2	0.112
DO 3	0.108
Average	**0.114**
Standard deviation	**0.007**
Extract volume (ml)	1
Mass of extract (g)	0.001
Polyphenol content (mg EAG/gES)	3.01±0.007

Individual 1 (fruit):

DO 1	0.097
DO 2	0.103
DO 3	0.105
Average	**0.101**
Standard deviation	**0.004**
Extract volume (ml)	1
Mass of extract (g)	0.001
Polyphenol content (mg EAG/gES)	1.27±0.004

Individual 2 (seed):

DO 1	0.129
DO 2	0.111
DO 3	0.118
Average	**0.119**
Standard deviation	**0.009**
Extract volume (ml)	1
Mass of extract (g)	0.001
Polyphenol content (mg EAG/gES)	3.68±0.009

Individual 2 (fruit):

DO 1	0.102
DO 2	0.096
DO 3	0.107
Average	**0.101**
Standard deviation	**0.005**
Extract volume (ml)	1
Mass of extract (g)	0.001
Polyphenol content (mg EAG/gES)	1.27±0.005

Individual 3 (seed):

DO 1	0.109
DO 2	0.110
DO 3	0.127
Average	**0.115**
Standard deviation	**0.010**
Extract volume (ml)	1
Mass of extract (g)	0.001
Polyphenol content (mg EAG/gES)	3.15±0.01

Individual 3 (fruit):

DO 1	0.098
DO 2	0.087
DO 3	0.112
Average	**0.099**
Standard deviation	**0.012**
Extract volume (ml)	1
Mass of extract (g)	0.001
Polyphenol content (mg EAG/gES)	1.00±0.012

Annex 5. I% calculations

Individual 1 (seed)

[ ] mg/ml	DO 1	DO 2	DO 3	average	I %
0.025	0.503	0.501	0.516	0.506	31.06
0.05	0.421	0.403	0.426	0.416	43.32
0.1	0.351	0.360	0.356	0.355	51.63
0.15	0.287	0.273	0.291	0.283	61.44
0.2	0.170	0.173	0.158	0.167	77.24

Individual 1 (fruit)

[ ] mg/ml	DO 1	DO 2	DO 3	average	I %
0.025	0.625	0.619	0.637	0.620	14.85
0.05	0.518	0.531	0.516	0.521	29.01
0.1	0.371	0.356	0.372	0.366	50.13
0.15	0.269	0.267	0.278	0.271	63.07
0.2	0.239	0.232	0.230	0.233	68.25

Individual 2 (seed)

[ ] mg/ml	DO 1	DO 2	DO 3	average	I %
0.025	0.519	0.506	0.513	0.512	30.24
0.05	0.418	0.406	0.431	0.418	43.05
0.1	0.312	0.314	0.301	0.309	57.90
0.15	0.250	0.232	0.246	0.242	67.02
0.2	0.113	0.095	0.110	0.106	85.55

Individual 2 (fruit)

[ ] mg/ml	DO 1	DO 2	DO 3	average	I %
0.025	0.636	0.651	0.631	0.628	13.35
0.05	0.512	0.516	0.516	0.514	29.97
0.1	0.351	0.348	0.355	0.351	52.17
0.15	0.286	0.279	0.291	0.285	61.17
0.2	0.219	0.244	0.239	0.234	68.11

Individual 3 (seed)

[ ] mg/ml	DO 1	DO 2	DO 3	average	I %
0.025	0.529	0.515	0.517	0.520	29.15
0.05	0.412	0.406	0.418	0.412	43.86
0.1	0.320	0.315	0.327	0.320	56.40
0.15	0.267	0.265	0.270	0.267	63.62
0.2	0.152	0.147	0.157	0.152	79.29

Individual 3 (fruit)

[ ] mg/ml	DO 1	DO 2	DO 3	average	I %
0.025	0.637	0.621	0.642	0.633	13.76
0.05	0.536	0.541	0.522	0.533	27.38
0.1	0.364	0.368	0.371	0.367	50
0.15	0.299	0.310	0.304	0.304	58.58
0.2	0.241	0.250	0.246	0.245	66.62

Annex 6. Macroscopic view under a stereomicroscope of the fungus grown on the surface of a seed.