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***Field**: Biological Sciences*

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Theme

Techniques of isolation, identification and occurrence of halotolerant *Fusarium* species in terrestrial saline soils

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Dedication:

I dedicate my work to:

*my heaven **mom**, the one who brought me to this world. there are no words good enough to thank you.*

*To the king **dad**, thanks a lot for raising me.*

*I would also dedicate this work to my **sisters, brothers**, **uncles** and **cousins**. You deserve my thanks.*

*To my besties **Yousra** Laamach and **Yacine** Hadjaj, for standing by my side all the time. Thanks, dear.*

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Abstract

Fusarium is a well-known filamentous fungus that can be found in a variety of environments, including soil. This genus is among the most common mycobiota in hypersaline soils. Identifying fungus as *Fusarium* has generally relied on morphological identification methods. Scientists adopted the morphological technique to address fungal diversity studies issues when more accurate and effective molecular identification technologies became available. The main objective of this work is to present a summary of Isolation, identification and occurrence of halotolerant *Fusarium* species in terrestrial saline soils as well as the effect of the extreme conditions of these habitats on their variability. 13 isolated *fusarium* species from diverse geographic regions in saline soils were identified as fusarium species from the studies analyzed. however, Different physicochemical factors are present in these regions. The occurrence of several *Fusarium* species varies considerably from one location to another. The diversity in species is most likely linked to the physicochemical properties of each analyzed habitat, such as temperature, pH, salinity…etc. most of isolates could tolerant up to 12.5% of NaCl (g/l). Only Acuminatum could tolerant 10% of NaCl, wish optimum of 2.5%. *Fusarium* was the most dominant genera, while *F. equseti* is the most abundant species (37.7%). As a comparison element, four investigations on non-saline soils were considered. Six species are found in these soils that are comparable to those found in saline soils. The isolated species most likely survived and adapted to their extreme environment.

Key words: *Fusarium*, filamentous fungi, saline soils, physicochemical factors, hypersaline soils, mycobiota, isolation techniques, fungal diversity, halotolerant

Résumé

Fusarium est un champignon filamenteux bien connu qui peut être trouvé dans une variété d'environnements, y compris le sol. Ce genre est l'un des mycobiotes les plus courants dans les sols hypersalins. L'identification du champignon Fusarium repose généralement sur des méthodes d'identification morphologique. Les scientifiques ont adopté la technique morphologique pour aborder les questions d'études de diversité fongique lorsque des technologies d'identification moléculaire plus précises et plus efficaces sont devenues disponibles. L'objectif principal de ce travail est de présenter un résumé de l'isolement, de l'identification et de la présence d'espèces de *Fusarium* halotolérantes dans les sols salins terrestres ainsi que l'effet des conditions extrêmes de ces habitats sur leur variabilité. 13 espèces de fusarium isolées provenant de diverses régions géographiques dans des sols salins ont été identifiées comme des espèces de fusarium dans les études analysées. Cependant, différents facteurs physicochimiques sont présents dans ces régions. La présence de plusieurs espèces de *Fusarium* varie considérablement d'un endroit à l'autre. La diversité des espèces est probablement liée aux propriétés physicochimiques de chaque habitat analysé, comme la tentation, le pH, la salinité… etc. la plupart des isolats peuvent tolérer jusqu'à 12,5 % de NaCl (g/l). Seul *F. acuminatum* peut tolérer 10 % de NaCl, souhait optimal de 2,5 %. Fusarium était le genre le plus dominant, tandis que F. equseti est le plus abondant (37,7 %). À titre d'élément de comparaison, quatre études sur des sols non salins ont été envisagées. Six espèces se trouvent dans ces sols qui sont comparables à ceux des sols salins. Les espèces isolées ont fort probablement survécu et se sont adaptées à leur environnement extrême.

Mots clés *: Fusarium,* champignons filamenteux, sols salins, facteurs physicochimiques, sols hypersalins, mycobiota, techniques d'isolation, diversité fongique, halotolérantes

الملخص

الفوساريوم (Fusarium) هي فطريات خيطية معروفة يمكن العثور عليها في مجموعة متنوعة من البيئات ، بما في ذلك التربة. هذا النوع هو من بين الميكوبيوتا الأكثر شيوعا في التربة الملحية المفرطة. وقد اعتمد التعرف على الفطريات باعتبارها فوساريوم بشكل عام على طرق تحديد المورفولوجيا. واعتمد العلماء التقنية المورفولوجية لمعالجة قضايا دراسات التنوع الفطري عندما أصبحت تكنولوجيات التعرف الجزيئي أكثر دقة وفعالية متاحة. والهدف الرئيسي من هذا العمل هو تقديم ملخص لعزل وتحديد وحدوث أنواع الفوساريوم الهالوتيرية في التربة المالحة الأرضية وكذلك تأثير الظروف القاسية لهذه الموائل على تقلباتها. وقد تم تحديد 13 نوعا من أنواع الفوساريوم المعزولة من مناطق جغرافية متنوعة في التربة المالحة على أنها أنواع من الفوساريوم من الدراسات المحللة. ويختلف حدوث العديد من أنواع الفوساريوم اختلافا كبيرا من موقع لآخر. ويرتبط التنوع في الأنواع على الأرجح بالخصائص الفيزيائية الكيميائية لكل موطن محلل ، مثل الترمومتر والأس الهيدروجيني والملوحة... ويمكن لمعظم العزليين أن يتحملوا نسبة تصل إلى 12.5 في المائة من .NaCL (g/l) لا يستطيع سوى F.acuminatum أن يتسامح مع 10% من NaCL ، ويتمنى القدر الأمثل من 2.5%. وكان Fusarium النوع الأكثر هيمنة ، في حين يشكل F.Equsetiالنوع الأكثر وفرة (37.7%). وكعنصر مقارنة ، تم النظر في أربع تحقيقات بشأن التربة غير المالحة. وتوجد ستة أنواع في هذه التربة مماثلة لتلك الموجودة في التربة المالحة. وقد نجت الأنواع المعزولة على الأرجح وتكيفت مع بيئتها المتطرفة.

الكلمات المفتاحية: الفوساريوم ، الفطريات الخيطية ، التربة المالحة ، العوامل الفيزيائية الكيميائية ، التربة الفائقة السمية ، الميكوبيوتا ، تقنيات العزل ، تنوع الفطريات. الهالوتيرية

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List of Abbreviations

C° Degree Celsius

18S Ribosomal RNA 18 (S: Svedberg)

aw Water activity

CzA Czapek's medium

F. *Fusarium*

EC Electrical Conductivity

ETS External Transcribed Spacer

IGS Inter-Genic Spacer

ITS Internal Transcribed Spacer

LSUrRNA Large subunit ribosomal ribonucleic acid

NTS Non-Transcribed Spacer

PDA Potato dextrose agar

rDNA Ribosomal DNA gene

rRNA Ribosomal RNA

SA Starvation Agar

SSU Small subnuit

TEF-1α Translation Elongation Factor

Glossary

Chestnut soil: is made up of lighter-colored top soils with high calcium carbonate or gypsum concentrations, and it is found mostly in the driest part of the steppe zone. (Nachtergaele,2017)

Electrical conductivity: is a measurement of a material's ability to carry electrical current. It is represented by the symbol alpha and is measured in siemens per meter (S/m) in SI units. (Helmenstine,2020)

Filamentous fungus: In filamentous fungi, the hypha is the basic structural unit, and when put together, the hyphae form a mycelium. Individual hyphae are tube-like structures with polarized development and the ability to branch at sub-apical locations. (Pebeerdy,1980)

Halophiles: microorganisms that can adapt to and survive in hypersaline environments. (Niknejad et al., 2013)

Halotolerant: microorganisms that can survive in the presence as well as the absence of salt. (Niknejad et al.,2013)

Mycobiota: are a collection of all the fungi found in a given geographical or geographic location. (Chander,2018)

Mycotoxins: are a wide range of toxic secondary metabolites produced by fungi that are present in the environment. (Sariaslani and Gadd,2014)

Phylogenetic analysis: It is the study of diverse species evolutionary relationships. The similarity in the structure and function of molecules like DNA and proteins between individuals and groups of organisms is attributed to their derivation from a common ancestor, according to evolutionary theory. (Choudhuri,2014)

Secondary metabolites: Compounds that are not required for the growth and reproduction of the producing organisms. These secondary metabolites enable organisms to thrive in their respective ecological niches (Gupta et al., 2014).

Solonchaks: Are soils distinguished by their high concentration of soluble salts (Nachtergaele, 2017)

Sporodochia: Sporodochia consist of masses of branched conidiophores. They grow in culture and appear macroscopically as light-colored elevated bodies on the plectenchymatic culture mat's surface (Refai et al.,2015).

Plectenchyma: A type of 'tissue' found in higher fungi that is made up of a mass of interwoven anastomosing hyphae. When it is created from long fused hyphae, it is called prosenchyma, and when it has a cellular appearance due to regular hyphae divisions, it is called pseudoparenchyma.

Ubiquitous: living organisms that can be found in a wide variety of habitats and are present in a wide range of settings. (Yadav et al.,2018; Visagie et al.,2014)

Mutagens: are agents that damage DNA and can, depending on the ability of an organism to repair the damage, lead to permanent changes (mutations) in the DNA sequence. (LJ Reha-Krantz,2013)

Teratogen: is a substance that can harm a developing fetus if it is exposed to it. The consequences are determined by the teratogen's type, the timing of exposure, and, most likely, the mother's and/or fetus' genetic vulnerability. Teratogenic substances include chemicals in the environment, maternal metabolic variables, medications, and infections. (D. Donnai,2001).

Introduction

Extremophilic microorganisms are microbes living in extreme conditions, in habitats that display unusual physicochemical characteristics (salinity, pH, temperature, pressure… etc.) (Niknejad et al., 2013). These microorganisms present a great interest for microbiologists (Cimerman et al., 2005).

Hypersaline environments are typical extreme habitats, in which the high salt concentration is not the only environmental factor that may limit the growth of organisms. These biotopes are characterized by low oxygen concentrations, and, depending on the geographical area, high or low temperatures. (Rodriguez-Valera, 1988).

Several studies have shown that some extremophiles can grow under saline stress conditions, thus populating saline habitats. These organisms include bacteria, archaea, algae, and fungi (Cimerman et al., 2005).

The world of halophilic microorganisms is wide, and understanding life at high salt concentrations requires more than just looking at one individual or a group of organisms. Halophilic organisms are as diverse as the conditions in which they thrive (Cimerman et al., 2005).

There are two types of microorganisms living in hypersaline environments, the ones who are well adapted to the ecological constraints of these habitats, called halophiles; and others, capable of surviving with and without salts. These are considered halotolerant (Niknejad et al., 2013).

A filamentous fungus called fusarium is found in large quantities in soils and plants. The majority of Fusarium species can be found in temperate zones, however some are also abundant in tropical and subtropical areas.

In the present study, we will be exploring, in two chapters:

- ✓ The biological features of the member of the genus *Fusarium*. Then, taking into account these features, we will present the techniques usually employed for its isolation from saline habitats, and its identification.
- ✓ The second chapter will present an overview and a comparison of the occurrence or Fusarium species in some saline and non-saline terrestrial habitats.

Chapter I:

Bibliographical synthesis

I.1 A Brief history of taxonomy of the *Fusarium* genus

Link proposed the general concept of Fusarium in 1809, and Fries approved the name "*Fusarium*" for banana-shaped conidia-forming fungus in 1821 (Summerell ,2019). Since then, the taxonomy of Fusarium has remained a matter of controversy among taxonomists.

Several authors have attempted to classify the genus using different factors and researchers have published multiple articles using various classification systems and concepts for this genus. Between 1809 and 1935, approximately 1000 species were described (Refai et al.,2015).

In the book ''Die *Fusarium* Wollenweber and Reinking'', published in 1935, members of the genus *Fusarium* were defined based on the fungus characteristics. These include the presence/absence and shape of microconidia, the presence or absence of chlamydospores, their location and their shapes (Refai et al.,2015). Macroconidia, as well as the form of macroconidia's basal foot cell.

Sixteen sections and sixty-five species of *Fusarium* were introduced. These groups were distinguished based on morphological differences (Refai et al.,2015).

Snyder and Hansen created a new taxonomy system and reduced the number of *Fusarium* species to nine throughout the 1940s and 1950s in the US, and the species in section Elegans into a single species, *F. oxysporum*. Their identification was based on the use of single spore cultures and any *Fusarium* isolate might be identified to species level using the Snyder and Hansen species taxonomy (Refai et al.,2015).

Leslie and Summerell released ''The *Fusarium* laboratory Manual'' in 2006 where seventy *Fusarium* species were included. This manual introduced the morphological, biological and phylogenic identification concepts (Leslie And Summerel, 2006).

One of the main reasons that taxonomy of *Fusarium* genus is still complex is that several species belonging to this genus are characterized by various ecological, morphological and physiological characteristics (Edel-Hermann et al.,2015).

I.2 Characteristics of the genus *Fusarium*

Fusarium is a genus of filamentous Ascomycota fungi (Ma et al.,2013), belonging to the Nectriaceae family. It is commonly found in the soil and in association with plants. The species of this genus have the ability to survive as saprotrophs in the soil (Srivastava et al., 2018).

Furthermore, many *Fusarium* species are clinically important, causing serious corneal infections (Chang et al. 2006), and invasive infections in immunocompromised patients (Guarro 2013).

The filamentous fungal genus *Fusarium* has a worldwide distribution and contains more than 300 species, most of which are plant pathogens (O'Donnell et al., 2015).

I.2.1 Habitat of the genus *Fusarium*

The members of the *Fusarium* genus are well-known ubiquitous fungi that can be found in a variety of habitats: air, aquatic and terrestrial habitat, on and with association with plants and insects (Al-Hatmi et al., 2016 in Sharma 2018).

Fusarium species can be isolated from aquatic habitats, both fresh and marine waters, such as seawater or river water (Palmero et al.,2009). They can also be found in drinking water sources (Oliveira et al., 2013). Some populations seem to be particularly adapted to complex water distribution systems (Steinberg et al., 2015).

Members of this genus are commonly present in tropical and temperate regions. Moreover, they can be found in extreme habitats like deserts, alpine and arctic regions. (Sharma et al., 2018).

I.2.2 *Fusarium* water and temperature requirements

Most *Fusarium* species encountered in the indoor environment are slightly xerophilic and have a minimum aw in the 0.86 to 0.91 range. Its species can grow in contaminated stagnant water such as humidifier pans (Flannigan et al., 2002).

Fusarium species grow well at temperatures ranging from 0C° to 37 C° (Thrane, 2014).

I.3 Morphologic characteristics of *Fusarium* species

Fusarium species are named after their anamorphic (asexual) and teleomorphic (sexual) forms. However, the correct name in such a case is the teleomorph name, according to Article 59.1 of the International Code of Botanical Names (ICBN). Nevertheless, under certain conditions, the use of the anamorph name is acceptable (Leslie and Summerell 2007).The table below show some *Fusarium* species and their teleomorph name.

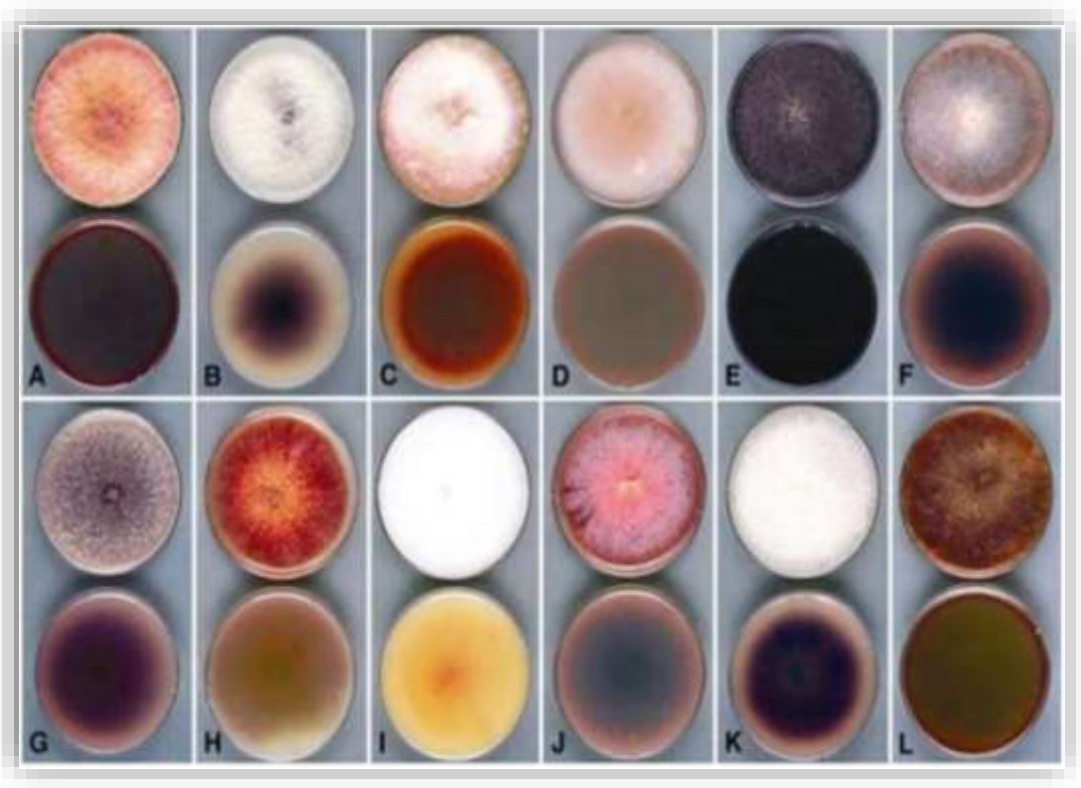
Table 1: Some of the anamorph and teleomorph names of *Fusarium*

Species number	*Fusarium* species	Teleomorph name
1	*F. decemcellulare*	*Albonectria rigidiuscula*
2	*F. acuminatum*	*Gibberella acuminata*
3	*F. avenaceum*	*Gibberella avenacea*
4	*F. lateritium*	*Gibberella baccata*
5	*F. fujikuroi*	*. Gibberella fujikuroi*
6	*F. verticillioides*	*Gibberella moniliformis*
7	*F. solani*	*Haematonectria haematococca*

Source: (Leslie and Summerell 2007).

a. Macromorphology

The majority of Fusarium species create flat, spreading colonies that are fuzzy to cottony in appearance. The colors of the surface of the colonies can vary: white, cream, tan, salmon, cinnamon, yellow, red, violet, pink, or purple. The colony reverse can appear colorless. However, in some species, and in certain growth conditions, the reverse may be colored: tan, red, dark purple, or brown (Fig. 02)(Refai et al.,2015).



Source: Refai et al., 2015

Fig 01: the variation of the colony morphology of some *Fusarium* species grown on potato dextrose agar. Each photo displays the surface and the reverse of the colony.

***A**, F. poae. **B**, F.oxysporum. **C**, F. acuminatum. **D**, F. nelsonii. **E**, F. subglutinans. **F**, F. nygamai. **G**, F. pseudonygamai. **H**, F. lateritium. **I**, F. thapsinum. **J**, F. decemcellulare. **K**, F. verticillioides. **L**, F. culmorum.*

b. Micromorphology of *Fusarium* species

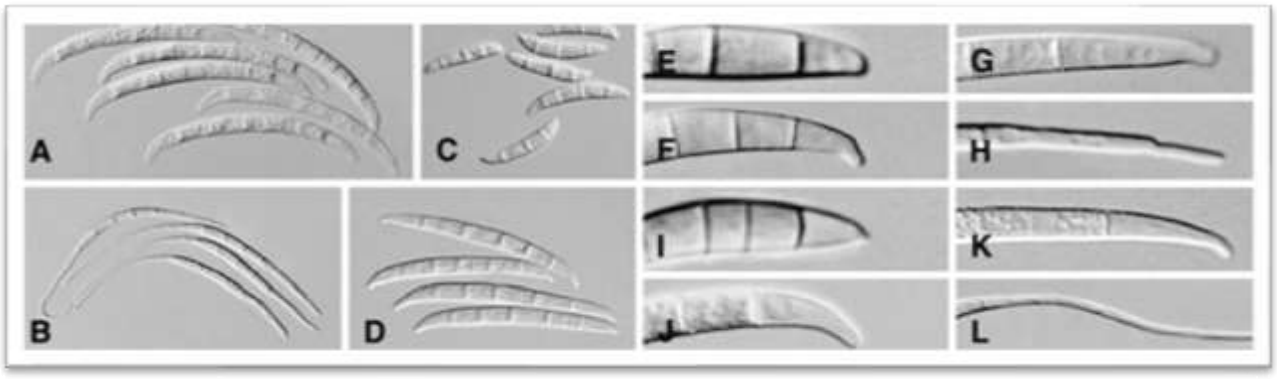
The development of hyaline, fusiform (banana-shaped), multicellular macroconidia with a foot cell at the base characterizes the genus *Fusarium*. Species identification can be challenging in some circumstances, and molecular approaches may be required (Nucci and Anaissie, 2007).

Microscopically, hyaline septate hyphae, conidiophores, phialides, macroconidia, and microconidia are the core constituents of *Fusarium spp*. Some species additionally create chlamydospores in addition to these fundamental structures (Refai et al., 2015).

i.Macroconidia

Macroconidia are transmitted through sporodochia. They are typically long, slender, pointed at both ends, curved dorso-ventrally, sickle-shaped, septated, and have a basal foot cell (that is, the basal cell of the septated spore has a slight notch on the dorsal side near the attachment point to the conidiophore) (Refai et al.,2015).

Macroconidia are phialospores, which means they are generated in a phialide, which is a small aperture at the tip of the conidiophore from which the spores emerge one by one, apex end first, all attached to the conidiophore at first (Fig. 03) (Refai et al.,2015).



Source: Summerell and Leslie, 2003

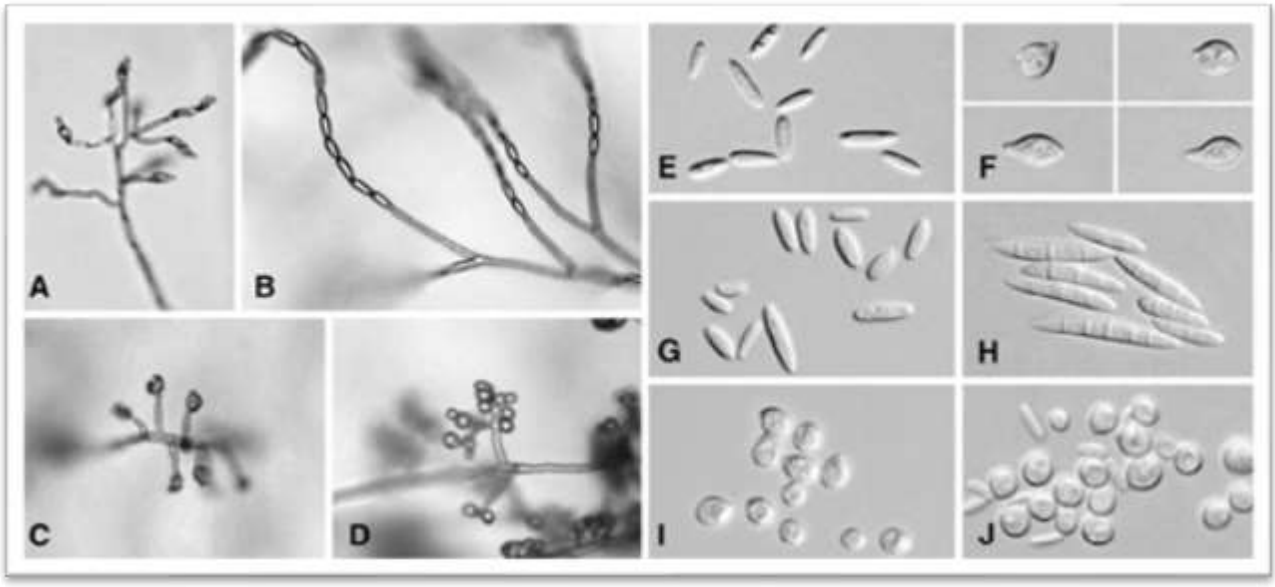
Fig 02: Macroconidia of *Fusarium* species.

***A** to **D**, Variation in macroconidial shapes and lengths. **A, F**. decemcellulare. **B, F**. longipes. **C,** F. culmorum.**D**, F. chlamydosporum. **E** to **H**, Variation in basal cells of macroconidia. **E,** F. culmorum.*

***F**, F. crookwellense. **G**, F. avenaceum. **H**, F. longipes. **I** to **L**, Variation in apical cells of macroconidia. **I**, F. culmorum. **J**, F. decemcellulare. **K**, F. verticillioides. **L**, F. longip*

ii.Microconidia

Microconidia can be commonly seen on the aerial mycelium as little, one-celled spores that are oval in shape. However, they can also be apiculate, tear-drop or pear-shaped, even spherical in some species. Microconidia are either phialospores or blastospores, which are dry spores formed by budding at the conidiophore's tip (Refai et al., 2015).



Source: Summerell and Leslie, 2003

Fig 03: Formation and types of microconidia produced by *Fusarium* species.

A**,** Microconidia produced in short chains (F. brevicatenulatum). **B**, Microconidia produced in long chains (F. decemcellulare). **C**, Microconidia produced in false heads (F. circinatum). **D**, Napiform microconidia in false heads (F. konzum). **E**, Oval microconidia (F. babinda). **F**, Pyriform microconidia (F. anthophilum). **G**, Clavate microconidia (F. anthophilum). **H**, Fusiform microconidia (F. semitectum). **I**, Napiform microconidia (F. poae). **J**, Globose microconidia (F. anthophilum)

Taxonomists use these sporogenesis characteristics to classify taxa, however they are typically difficult to determine, and some isolates produce both spore forms. Occasionally, phialide microconidia remain linked to one another in a sequence to produce chains. This character is also useful in taxonomy. Microconidia are primarily spread by wetness, although they can also be spread by air, though only for short distances (Fig. 04) (Refai et al., 2015).

iii.Chlamydospores

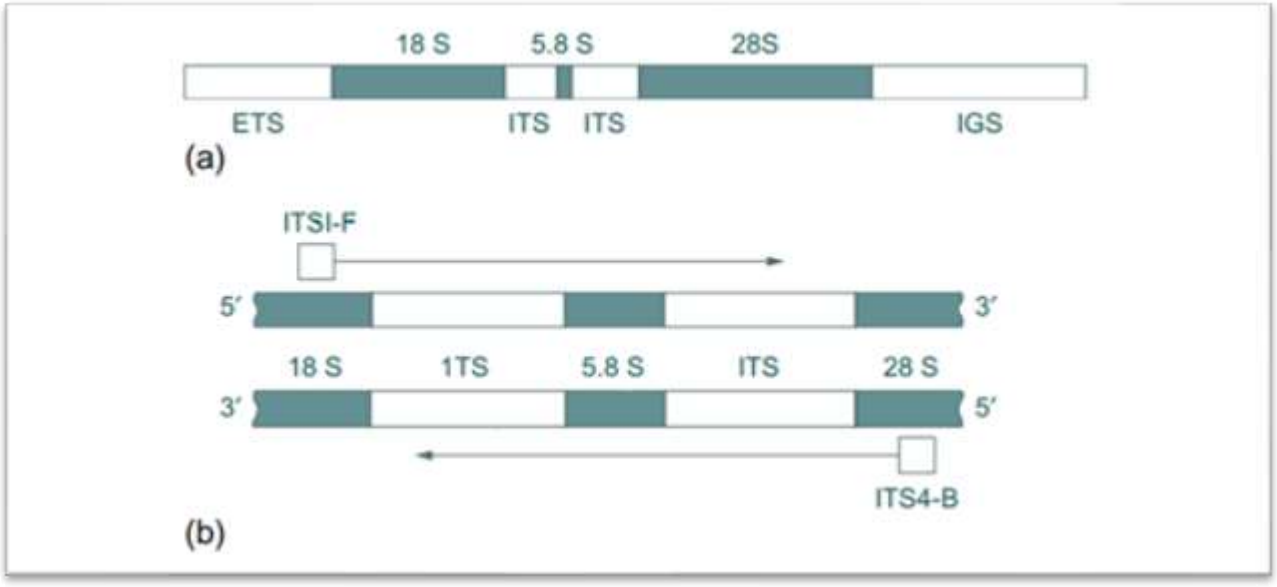
Chlamydospores are found in some *Fusarium* species, but not all. These spores are more or less sphaerical, with a diameter of 7-16 mm. They are usually found alone, although in some species, they can also be found in pairs, chains, or large clusters. Their cell walls are thick, double, and often very rough, and their cytoplasm is full of nutrients, as evidenced by greasy globules. (Refai et al.,2015).

The walls seem light yellowish in color when seen microscopically, however they are brown when viewed macroscopically. As a result, big clumps in culture can show as brown clumps beneath the agar surface. They appear when the available nutrients are reduced and the culture is already old, and they form in conidia or hyphae, either terminally or intercalary. (Refai et al.,2015).

I.4 Molecular and phylogenetic identification

i.rDNA Sequences (Fig.05) : The rRNAs needed in the creation of ribosomes, the sites of cellular protein synthesis, are coded for by ribosomal DNA (rDNA). (Gregory,2005). In prokaryotes and eukaryotes, RNA-specific genes are frequently similar. These genes are mostly conserved, however there are a few introns that must be spliced away before they may become functional RNA molecules. (Waikagul and Thaekham,. 2014).

i.The ITS1 and ITS2 clusters encode three ribosomal RNA subunits (18S [small subunit (SSU)], 5.8S and 28S [large subunit (LSU)] rRNA) plus intervening regions, called internal transcribed spacers. The ITS region is made up of two spacers and the gene that codes for the ribosome's 5.8S subunit. External transcribed spacer sequences (ETS) are found at the cluster's 5′ and 3′ ends. When the functional rRNA is transcribed, the non-functional RNA molecules encoded by ETS, ITS1, and ITS2 are destroyed. (Watkinson et al.,2016) .



Source: Watkinson et al.,2016

Fig 04: Ribosomal genes sequence organization.

(a) single copy of ribosomal **DNA (rDNA),** with coding and non-coding regions. An internally transcribed spacer (**ITS**) lies between the genes coding for **18S** and **28S RNA,** with that coding for **5.8S RNA** embedded within it. Beyond the **RNA** genes are the externally transcribed spacer (**ETS**) and the intergenic spacer (**IGS**). (**b)** The complementary strands in the **ITS** region of **DNA**, with adjacent coding regions, showing the points at which the two primers**, ITS1-F** and **ITS4-B**, bind, and the direction of **DNA** replication towards the 3′ end of each strand.

I.5 Taxonomy of the genus *Fusarium*

according to Sharma et al., 2018 these are the taxonomy of the genus *Fusarium*

- Domaine: Eucaryota
- Kingdom: Fungi
- Phylum: Ascomycota
- Class: Sordariomycetes
- Order: Hypocreales
- Family: Nectriaceae
- Genus: *Fusarium*

The teleomorphs of *Fusarium* species are mostly classified in the genus Gibberella. A certain number of the sexual forms are classified within the genera Hemanectria and Albonectria (Moretti, 2009).

I.6 Mycotoxin and Secondary metabolism of *fusarium*

Fungi, often found in food, can produce toxins known as mycotoxins. These toxins have caused large epidemics in people and animals, such as the ergotism. Mycotoxins are secondary metabolites. These compounds do not trigger immune responses and can cause acute, chronic, mutagenic, and teratogenic damage. Aflatoxin A, fumonisins, trichothecans, and zearalenone are the most significant mycotoxins. *Fusarium* species can only produce trichothecans, and zearalenone, in varying amounts depending on the species (Pitt,.2000).

Some fusarium species can be used in many applications such as industry, agriculture, medicine, and environment (Al-Daamy et al., 2018). Secondary metabolites, such as mycotoxins, has a deleterious impact on agricultural and human health. (Shweta et al., 2010).

However,some species of fusarium are deleterious to other microorganisms and can inhibit or destroy them completely. Thus, they are used to treat infections caused by bacteria and other organisms that can cause illness to both humans and animals by different modes of action (Al-Daamy et al., 2018).

Chapter II: A preview on halotolerant Fusarium species in some saline terrestrial habitat

II.1 Results of a few studies on the isolation of halotolerant *Fusaria*

In an attempt to assess the diversity and occurrence of *Fusarium* species in saline and non-saline soils, five studies on the isolation and identification of filamentous fungi, including *Fusarium* species, were selected for results analysis. These studies were conducted on soils located in different regions of the globe. To this purpose, a summary of each study is presented in this section.

A study of **Georgieva et al. (2012)** investigated the variety of mycelial fungi in saline soils from soda solonchaks, sulfate solonchaks, and solonchakous chestnut soil in the Western Transbaikal Region of Russia. These locations have varying degrees and types of salinization (0.01-13.94 percent), as well as humus content and pH values, ranging from 6 to 10. Fungi belonging to 24 genera were isolated, totaling 40 species. *Fusarium*, represented by with seven species, was the most abundant. *F.oxysporum,F.solani* and *F.sulphureum* were isolated from the solanchakous chestnut soil; *F.anthophilum* , *F.Chlamydosporum* , *F.dimerum, F.incarnatun, F.solani* and *F.sulphureum* were obtained from the sulfate solonchak area. The halo-alkali-tolerant and alkalophilic micromycetes were dominant in the soda solonchak area were only *F.chlamydosporum* was isolated.

F. chlamydosporum were found in both Soda and sulfate solonchak area, while *F.solani* and *F. sulphureum* were found in solonchakous chestnut soil and sulfate solonchak area (Georgieva et al., 2012).

Another study of **Evans and colleagues** was conducted an investigation on the isolation and characterization of halotolerant soil fungi from the Great Salt Plains of Oklahoma, in the United States (2013). The 18S rRNA gene sequence analysis was used to identify the twenty-five isolated fungi. Six families were found in Great Salt Plains of Oklahoma, including two isolates clustered with *Fusarium* in the Nectriaceae A. *Fusarium*, grew best above pH 7 and did not grew across the entire PH range. The isolates were halotolerant in general. (Evans and colleagues, 2013).

In order to examine the effect of salinity on fungal occurrence, **Al Tamie (2014)** isolated and identified 17 halotolerant fungi in Al Shega Area Al Qasim (Saudi Arabia). *Fusarium*. *spp, F.*

chlamydosporum, and *F.oxysporum*, are able to grow and survive in a 5% NaCl concentration. *F.chlamydosporum* and *F.oxysporum* was able to grow moderately at PH ranging (6.5-8). *F.chlamydosporum* and *F.oxysporum* are weak halotolerant isolates, according to this investigation (Al Tamie, 2014).

Chamekh and her colleagues (2019) accessed the diversity of halotolerant and halophilic mycroflora in two different regions of the Great Sabkha of Oran, northwestern Algeria. The first sampled location was neutral, where cereal crops were grown. The second site exhibited an alkaline pH where no vegetation grew. Both locations were extremely saline. The obtained fungal isolates, from each site, were identified using morphological and molecular analysis (TEF-1α, and ITS genes sequencing).

According to the results of this study, Fusarium was one of the most common filamentous fungi found in this Sebkha, with a frequency of 32.5 % in the first location. 34 fungal species have been isolated from this site, with five belonging to the genus *Fusarium*: *F. oxysporum, F. equiseti, F. brachygibbosum, F. acuminatum* and one unidentified species. The species *F.equiseti* was the most frequently isolated representing 18% of all isolates and 55.5% of *Fusarium* isolates, while there were a total absence of *fusarium* species in the second site.

All *Fusarium* isolates could grow in the absence of NaCl and tolerated a salinity up to 12.5%. However, *F. acuminatum* was the only one who could grow at 10% of NaCl with an optimum of 2.5%. It is safe to say that these isolates are halotolerant (Chamekh et al., 2019).

Alotaibi and colleagues (2020) performed a study on microbial diversity. Soil samples were collected from 9 different geographical regions (Al-Aushazia lake, AlQasab, AlKasar, Tabuk, Al-Kharj, Al-Madina, Jubail, Taif and Abqaiq). Molecular methods of identification were used to identify the isolated fungi, based on 18S rDNA sequencing. This technique allowed the identification of 203 fungal species, belonging to 33 genera.

Fusarium species were found in 5 out of the 9 screened samples. Al-Aushazia area showed 14.2% fungal isolates. *Fusarium* spp. Were the most abundant fungal with 21.9%. *Fusarium proliferatum* was isolated only from the sandy loam soil of Al-Aushazia Lake. Knowing that

this site exhibits an alkaline pH (8.5), the identified isolates were considered halotolerant and alkalitolerant.

Al-Taif is the second highest location for microbial abundance that contains 34 fungal isolates with the core microbiome Fusarium spp. (24.1%). *Fusarium* spp are moderate alkalitolerants with a pH of 7.2. Electric conductivity 1.96 is an acceptable range for low salinity at Al-Taif.

17 *F. oxysporum* were isolated from Jubail with a percentage of (12.9), which consists of sandy soil with a pH of 7.2 and EC 4.4.

Moreover, 5.3% of fungal were isolated from Al-kharj including *F.Oxysporum* With 50% as the core fungal strain with a PH 7.5.

Fusarium species presented in Al-Taif and Al-kharj are moderate alkalitolerants (Alotaibi et al., 2020)

II.2 Analysis of the results of the studies

Five research studies on the subject, from various geographic regions, were analyzed in order to present and overview of the diversity of *Fusarium* species in diverse terrestrial saline environments, as well as the effect of the extreme conditions of these habitats on their variability.

- Soil samples were collected from various saline environments in each region and at various depths. Different methods were used for the isolation of halophilic and halotolerant fungi after from the sampled soils:

- Al Tamie (2014) and Chamekh et al. (2019) used two methods: the soil plate method which is a direct inoculation method; and an indirect method.

- The remaining three studies used only the soils plate method (Geogieva et al., 2012; Evans and colleagues.2013; Alotaibi et al.,2020).

- The following media were used to isolate and identify halophilic and halotolerant fungi in the presented studies:

- ✓ Sabouraud Dextrose Agar
- ✓ Dox's agar
- ✓ CzA and SA
- ✓ PDA (potato dextrose agar)

- The temperature of incubation ranged from 22 to 37°C. Twenty-five Celsius degrees 25°C was the most frequently used incubation temperature.

- The pH values used for the isolation of halotolerant fungi in these studies ranged from 6.5 to 8.2.

- The incubation period ranged from one to four weeks.

- The isolated fungi were identified according to their cultural characteristics. Isolates were identified using molecular methods using the following molecular markers: the rDNA gene cluster (ITS, SSU,5.8S, LSU, and 18S RNA); and the TEF-1α and β-Tubulin genes.

- The genus *Fusarium* has been isolated from the studies of saline soils. However, 13 isolates were identified as fusarium species. Moreover, five isolates were identified only to the genus level as *Fusarium*, the respective species were not characterized.

- These regions all have high salinity, according to a study of physicochemical factors. Depending on the field of study among the research, an area in the Great Sebkha of Oran (Algeria) presented the highest salinity, with 46% of NaCl g/l.

- In most of the analyzed studies, the temperatures in these regions were not mentioned. Although, some stated that temperatures changed seasonally. In the region of Al Shega Area at Al Qasim, in Saudi Arabia, it is extremely warm, where the temperatures can reach 50C°.

Among the analyzed investigations, Mycelial Fungi in Saline Soils of the Western Transbaikal Region had the most *Fusarium* species. Georgieva et al (2012) were able to isolate seven species. Chamekh et al. (2019) found four species in the Great Sebkha of Oran in Northwestern Algeria. Different *Fusarium* species are present. Although the physicochemical parameters of each soil differed, salinity and temperatures were nearly same.

Al Shega Area Al Qasim (Saudi Arabia) by Al Tamie (2014) and the Great Salt Plains of Oklahoma, in the United States, by Evans and colleagues (2013) had the lowest levels of *Fusarium* species among the analyzed studies, with two species. It could be owing to the 30°C and 40°C temperatures.

The percentage of genera present in each of the five investigated is shown in the circle chart below (Fig.06). Many of the fungal isolates were distributed to various genera.

In which the genus *Fusarium* is the most present in those saline soils habitats with a percentage of (28.12%). Followed by *Aspergillus* (25.62%) and *Pencicillium* with (15%).

The other percentages represent 16 different genera that found in different regions.

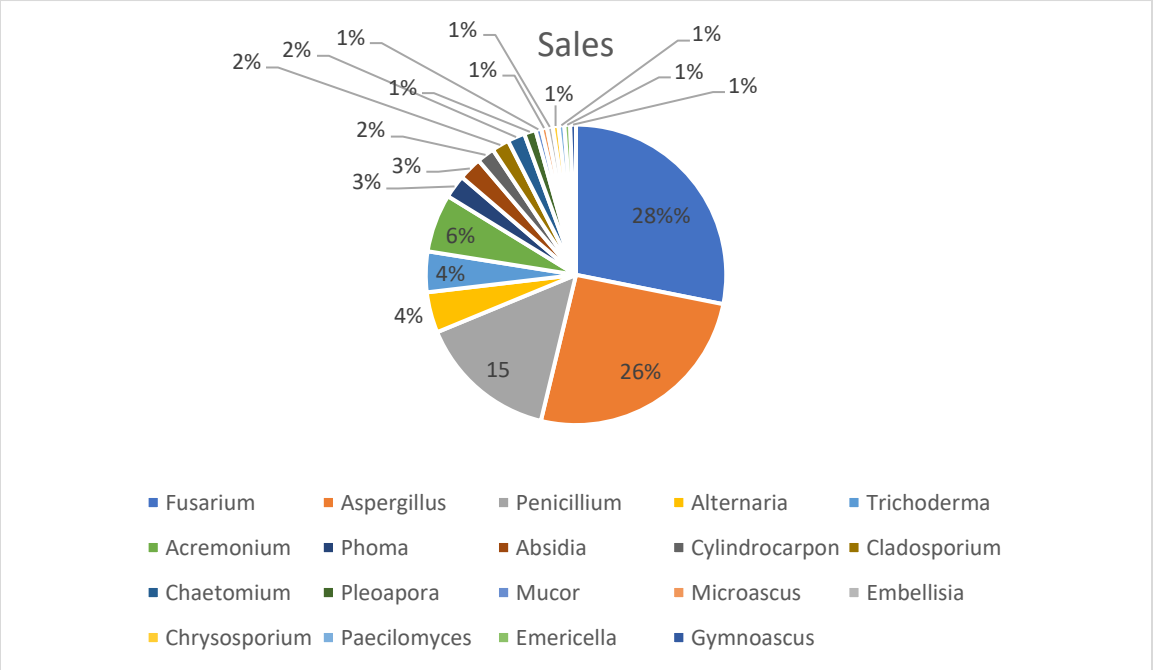


Fig. 05: Percentages of fungal genera present in 05 different saline soils habitats.

The distribution of *Fusarium* species varies from one place to another, possibly due to differences in pH, salinity, and temperature.

According to the studies, each species has its own growth optimum and can tolerate different values of these parameters:

Concerning the response of the *Fusarium* isolates to the salinity, is has been recorded that most of isolates could tolerate up to 12.5% of NaCl (g/l). Only *F.acuminatum* could tolerant 10% of NaCl, with optimum of 2.5%.

Regarding the pH optima, most of the isolated Fusarium species were halotolerant, some were moderate alkalitolerants .

According to the circle chart (Fig.07), *F.equseti* is the most abundant species (37.7%).

Followed by *F.oxysporum* (24.4%), it has been found in four regions.

The third was *F.brachygibbosum* with (13.3%) which found in one region.

Three other species were found in two regions with a percentage of 4.4% each.

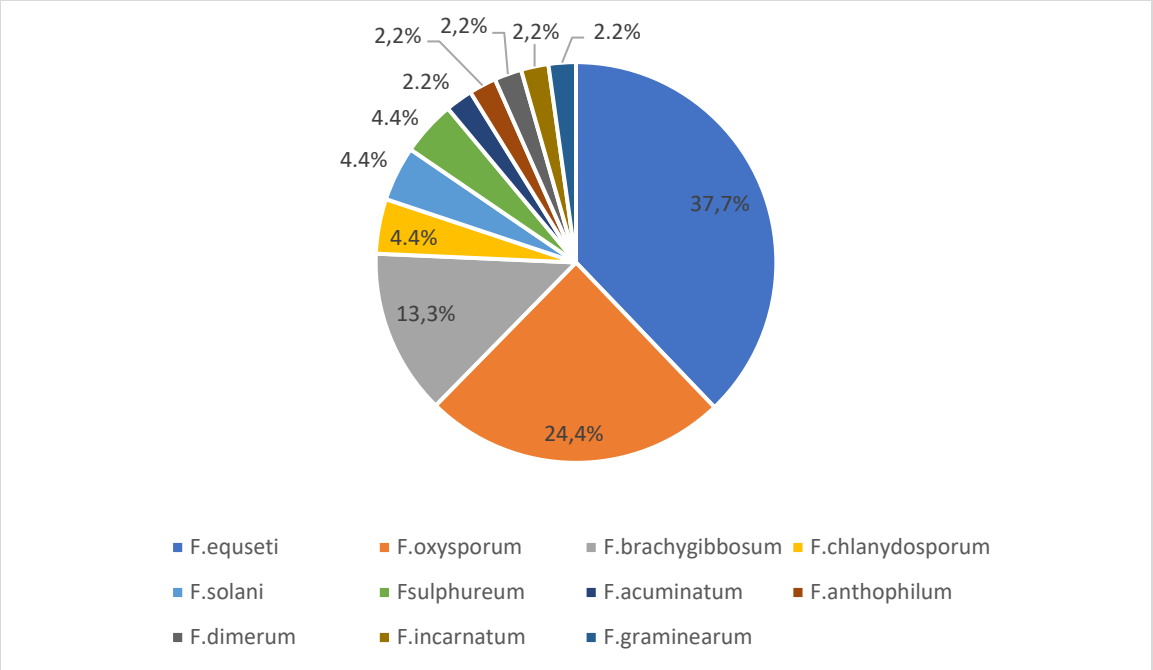


Fig. 06: Percentages of *Fusarium* species present in 05 different saline soils.

In order to compare between *Fusarium* species present in the studied saline soils with those found in non-saline soil, two studies of non-saline soils were analyzed.

The first study was conducted by Zohri et al., 2014, in four new reclaimed sites in the Assiut Governorate, Egypt, to study the diversity of soil fungi, in which 8 *Fusarium* species were isolated. The second was by Latiffa et al. (2009), who carried out a study on *Fusarium* species isolated from peat soil in Pondok Tanjung and Sungai Beriah in Perak, resulting isolation of four *Fusarium* species.

In the two studies 12 species has been identified in which *F.Solani* and *F.oxysporum* were found both in the two regions studied. However, these two studies shared six species with the one who found in saline soils.

the soil type in the Sebkha environment is unusual for its neutral pH, high calcium carbonate accumulation, and calcium sulfate, and is highly susceptible to water. However, Low salt concentrations, variable degrees of temperature, and low to moderate NaCl concentrations were all reported to enhance fungal growth. (Alotaibi et al., 2020).

The Study of Al Tamie (2014) showed that the community of fungal in saline environment varies from site to site and from season to season dependent on the physichochemical parameters.

Conclusion And Perspective

Conclusion And Perspective

By reviewing previous studies on the isolation, identification, and occurrence of halotolerant *Fusarium* species in terrestrial saline soils, we can conclude that the variety of physicochemical conditions influenced the significantly different *Fusarium* species isolated from soils of different locations. This study demonstrates the variety of the *Fusarium* genus in saline soils. when the isolated species are not halophilic fungus.

The isolated species were halotolerant in general, and some of them can also be found in non-saline soils. Changes in habitat parameters are most likely influencing their variability.

The species detected in non-saline soils in the analysed studies were also found in saline soils. The isolated species most likely survived and adapted to their extreme environment.

More research is needed to investigate and test the promising potential of *Fusarium* species for producing novel bioactive compounds. It will be interesting to study more about their biogeochemical functions. High and multifaceted influences of soil variables, particularly salinity elevation, can be used to understand soil ecosystems.

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