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Theme

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in vitro and *in vivo* control of Fusarium spp. of durum wheat (*Triticum durum*) using fungal endophytes isolated from algae

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Abstract

We tested the antagonistic capacities of 15 isolates of endophytic fungi collected from marine algae from the Stidia region of Mostaganem against 3 isolates of Fusarium sp. pathogen of durum wheat. These isolates were isolated from infected samples of wheat collected from El-Hadjaj El-Menea.

In this experiment we did a test *in vitro* to identify the antagonistic endophytes between the 15 isolates of our collection against 3 Fusarium spp. The tests are carried out using an antibiosis test. Endophytes that have shown an antagonistic effect in vitro are selected for an in vivo test by testing their behaviour on wheat plants inoculated with one of the 3 parasites.

Test in vivo includes biopriming on seeds and immersing method for leaves and plants using sporal suspension 10^6 spores/ml of the Fusarium isolates and antagonistic isolate.
The results obtained in vivo negative results of using endophytic antagonist on plant material including decrease in germination rate and increase of disease severity on leaves and plants

Key words: Fusaruim spp. *in vivo*, *in vitro*, endophytes.

Résumé

Nous avons testé les capacités antagonistes de 15 isolats de champignons endophytes collectés à partir d'algues marines dans la région de Stidia, Mostaganem, contre 3 isolats de Fusarium spp., pathogènes du blé dur, isolés à partir d'échantillons de feuilles et d'épis infectés et récoltés de la région d'El-Hadjaj El-Menea.

Un test de screening a été réalisé *in vitro* afin identifier les endophytes antagonistes parmi les 15 isolats de notre collection. Le travail consiste à réaliser un test d'antibiose, en confrontant en boîte de Pétri, sur milieu de culture PDA, deux explants mycéliens, l'un appartenant au parasite (Fusarium sp.), l'autre à l'antagoniste potentiel. Les champignons endophytes ayant provoqué l'inhibition de la croissance mycélienne du parasite sont alors dits antagonistes et sont sélectionnés pour le test *in vivo*. Afin d'évaluer *in vivo* le pouvoir antagoniste vis-à-vis de la fusariose, des plantes de blé sont réparties en plusieurs lots, un lot est inoculé avec l'un des 3 parasites (Fusarium sp.), un lot est inoculé avec un mélange antagoniste et parasite. Nous avons utilisé un inoculum de 10^6 spores/ml que ce soit pour l'antagoniste ou le parasite. Deux lots de plantes témoins sont incorporés dans l'essai, un lot inoculé avec l'antagoniste seul, et un lot de non inoculé.

Le test *in vivo* comprend un biopriming des semences et une méthode d'immersion pour les feuilles et les plantes, en utilisant une suspension sporale à 10^6 spores/ml des isolats de Fusarium spp et des isolats antagonistes.

Les antagonistes ayant montré un pouvoir antagoniste *in vitro* se sont avérés toxiques pour les plantules de blé. En effet des symptômes de maladie ont été notés chez les plantes inoculés avec le parasite seul mais également chez les plantes témoins inoculées avec le champignon antagoniste. Malgré leur pouvoir antagoniste *in vitro*, des lésions des tissus sont apparues chez les plantes inoculés avec l'antagoniste, suggérant ainsi que le pouvoir antagoniste vis-à-vis d'un champignon parasite ne doit être validé qu'une fois que des tests sont réalisés sur la plante et noté la réaction de celle-ci.

Mots-clés : Fusarium spp, *in vivo*, *in vitro*, endophytes.

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General introduction

General introduction

Cereals were and still are the most productive and demanded crops due to their nutritional and cultural importance **(Iseli-Trösch, 2019).** They have accompanied human civilization since the Neolithic era, especially during difficult periods and famines due to the ease of cultivation, storage and consumption.

Wheat was discovered in the Fertile Crescent **(Feldman et Sears, 1981 ; Mouellef, 2010).** As part of the 'Neolithic Revolution', wheat cultivation was the first attempt to create food and was developed by human civilization 10,000 years ago. Domesticating its wild ancestors was the first step toward improving durum wheat **(Shewry, 2009).**

Durum wheat is the 10th most widely grown cereal in the world. The Mediterranean countries are the main consumers and producers, accounting for 75% of overall production **(De Vita and Taranto, 2019; Martınez-Moreno et al., 2022). I**n Algeria, wheat is a vital component of the national diet and economy. From 2010 to 2017, cereals occupied 46% of usable agricultural land, with durum wheat and barley dominating production **.(M.A.D.R, 2022).** Algeria projected a total cereal output of 3.5 million tonnes for 2024, which covers only 22% of the nation's needs. This gap necessitates significant cereal imports, with wheat constituting about 60% of these imports **(FAO, 2024).**

Algerian agriculture cannot supply rising wheat demand due to structural constraints. Yield deficiency can be attributed to three factors: abiotic, biotic, and technological restrictions. Among the biotic factors Plant diseases caused by phytopathogenic fungi are quite common and can lead to yield losses as well as a decline in grain quality **(Hadjout, 2019).**

Fusarium head blight (FHB) poses a significant threat to cereals, especially wheat and barley, as various Fusarium species target different plant organs. This disease can manifest at any growth stage, from sowing to harvest, and is associated with symptoms such as seedling blight and foot rot **(Fernandez and Jefferson, 2004).** FHB negatively impacts both the quantity and quality of grain, raising the risk of mycotoxin contamination **(Demeke et al., 2005).**

The FHB complex consists of over 17 species, mainly from the genera Fusarium and Microdochium **(O'Donnell et al., 2004; Boutigny et al., 2012).** Notable pathogens include Fusarium graminearum, Fusarium culmorum, Fusarium avenaceum, Fusarium poae, and Microdochium nivale **(Parry et al., 1995).** Among these, Fusarium graminearum is the primary causative agent and ranks as the fourth most significant fungal plant pathogen globally **(Dean et al., 2012).**

Farmers often resort to excessive pesticide application to fend off attacks from pathogens. While these chemical products are viewed as the most effective solution,

they have detrimental effects on the environment, disrupt ecological balance, and contribute to the development of resistance mechanisms in pathogens **(Thakore, 2006).**

Given the drawbacks of chemical control, adopting biological protection is a viable alternative for combating phytopathogenic agents while minimizing chemical use.

The introduction of antagonistic microorganisms has shown great promise in managing several soil-borne plant diseases **(Anitha and Andrés, 2011).**

As agricultural practices shift towards more sustainable and eco-friendly approaches, endophytes offer a viable alternative to chemical pesticides. Their application can lead to reduced reliance on synthetic chemicals, improved crop yields, healthier ecosystems and providing resistance against various pathogens and pests **(Bacilio et al., 2009).**

The use of endophytes as biocontrol agents is based on their capacity to produce bioactive compounds, such as antibiotics, antifungals, and phytochemicals, which can effectively inhibit the growth of harmful organisms. Furthermore, endophytes have the ability to induce systemic resistance in plants, thereby boosting their overall resilience to both biotic and abiotic stresses **(Ryan et al., 2009).**

Today, new perspectives are emerging in phytopathology regarding microbiological control of vascular diseases. This approach represents a promising alternative for ensuring effective and sustainable plant protection. Researchers strive to conduct studies and experiments to discover and identify effective strategies for biological control against diseases. These efforts include identifying control factors, improving treatments, and studying environmental impacts on the spread of diseases

There are many examples of successful biological control of plant diseases through the use of antagonistic microorganisms, often isolated from a terrestrial environment. Few examples relate to potential antagonists isolated from a marine environment. Yet the marine environment, a rich and complex ecosystem, is the habitat of an incredible diversity of micro-organisms. Some of these display antagonistic properties towards micro-organisms that are pathogenic to the many algae and plants that inhabit this environment.

The aim of our study is to test, by antibiosis, the antagonistic abilities of 15 algal endophytes against Fusarium spp. agents of Fusarium diseases of durum wheat. The antagonists selected for their in vitro performance will be tested for their antagonistic capacities in vivo on plants inoculated with Fusarium spp.

Bibliography Part

Chapter 1 :General Information on durum wheat

I. Introduction

Cereals were and still the most productive and demanded crops due to their nutritional and cultural importance **(Iseli-Trösch, 2019).** They have accompanied human civilization since the Neolithic era, especially during difficult periods and famines due to the ease of cultivation, storage and consumption. Cereals are the basis of food for humans and animals because they contain proteins and the energy value necessary to carry out muscular effort **(Moule, 1971) (Rahal-Bouziane, 2015).** Nowadays, many processed foods such as bread, pastries, couscous, and even homemade desserts are derived from the raw material, which is cereals **(Ranieri, 2015; Abecassis et Vermeersch,2006)**, and the first attempt to cultivate cereals was the experience of growing durum wheat **(Özkan et al., 2002; Wang et al., 2022).** In the world, corn, durum wheat, common wheat, rice, barley, and sorghum are the five cereals that are grown most extensively.

1. Origin and history of durum wheat

As part of the 'Neolithic Revolution', wheat cultivation was the first attempt to create food and was developed by human civilization 10,000 years ago. Domesticating its wild ancestors was the first step toward improving durum wheat **(Shewry, 2009)** Wheat was discovered in the Fertile Crescent, which included Palestine, Jordan, Iraq, and western Iran **(Feldman and Sears, 1981 ; Mouellef, 2010).** Named Triticum in Latin by the Romans, it traveled by land via the Balkans and by sea to the Mediterranean regions of southern Italy, France, Spain, and Greece and traveled to South Africa by Egypt and Ethiopia (Figure 1).

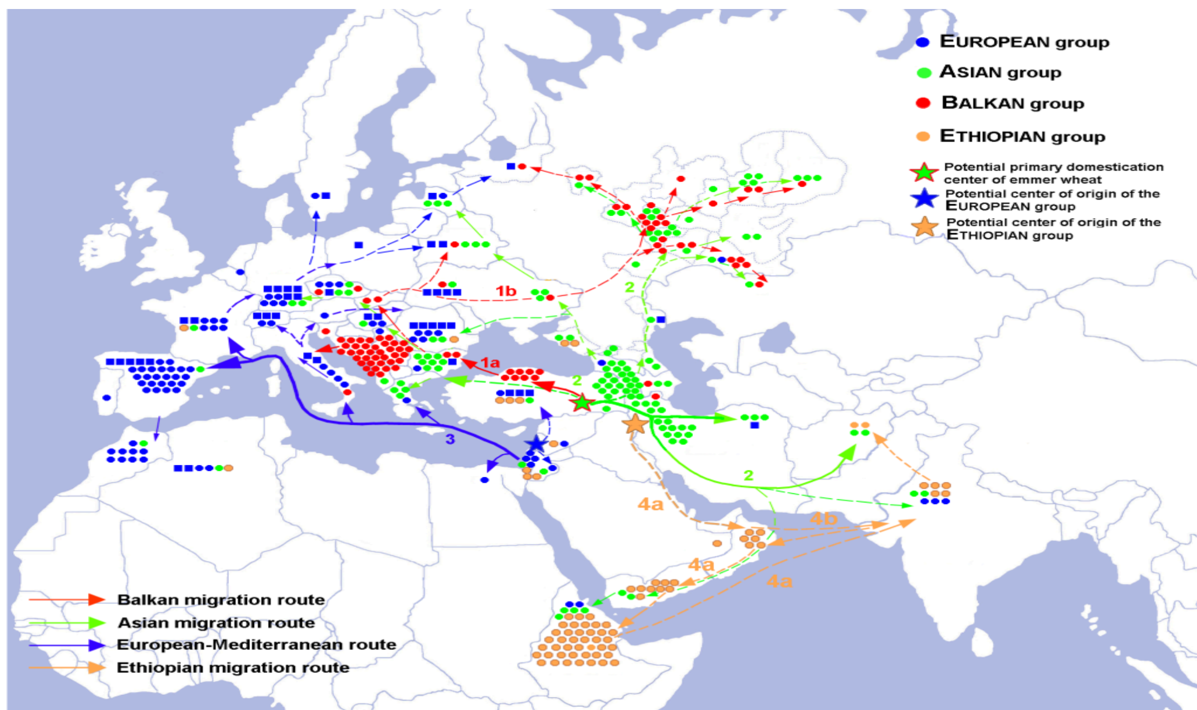

Figure 1: Map of the domestication sites and migrations of cultivated durum wheat (Source: Badaeva et al., 2015).

2. Importance in world

With an estimated 38 million tons produced annually, durum wheat is the 10th most widely grown cereal in the world and represents about 7% of total worldwide wheat area **(Xynias et al., 2020)**.The culture is grown across around 17 million hectares globally in 2019 (**AAFC.2019, July 19)**.In 2018, the EU produced 9 million tonnes, making it the top producer. Canada, Turkey, the United States, Algeria, Mexico, Kazakhstan, Syria, and India came next **(EUROSTAT,.2019;AAFC,.2019; USDA.2019; Sall et al., 2019;Tedone et al., 2018) (Table 2)** . The Mediterranean countries are the main consumers and producers, accounting for 75% of overall production **(De Vita and Taranto, 2019; Martınez-Moreno et al., 2022).**

Table 1:The world's leading durum wheat producing countries (2010–2019)

Country	**Average Production (Millions of Tonnes**)
Canada	5.2
Italy	4.3
Turkey	3.7
USA	2.3
Kazakhstan	2.2
Syria	2.2
Algeria	2.2
France	1.9
Morocco	1.8
Greece	1.1
Spain	1.0
Tunisia	1.0

(EUROSTAT,.2019; AAFC,2019; USDA,2019).

3. In algeria

Cereal products play a strategic role in the national diet and economy. Over the period 2010–2017, cereal acreage accounted for an annual average of 46% of

usable agricultural land. The area sown to cereals is estimated at around 33,855,560 hectares, with durum wheat and barley accounting for most of this, or around 80% of the total cereal area.

The cereal production rate for the period 2010–2017 is estimated at 41.2 million quintals, an increase of 26% compared with the decade 2000–2009, when the production rate was estimated at 32.6 million quintals. Production is mainly made up of durum wheat and barley, which account for 51% and 29%, respectively, of the total cereal production rate between 2010 and 2017**(M.A.D.R, 2022)**.

The projected total production of cereals in 2024 is 3.5 million tonnes, it is still unchanged from 2023. It only meets 22% of the needs of the nation. The nation imports a significant amount of cereal grains. Approximately 60% of all cereal imports are made up of wheat. As one of the nation's primary suppliers of wheat, the Russian Federation has increased its exports of the grain**(FAO, 2024) (Figure 2).**

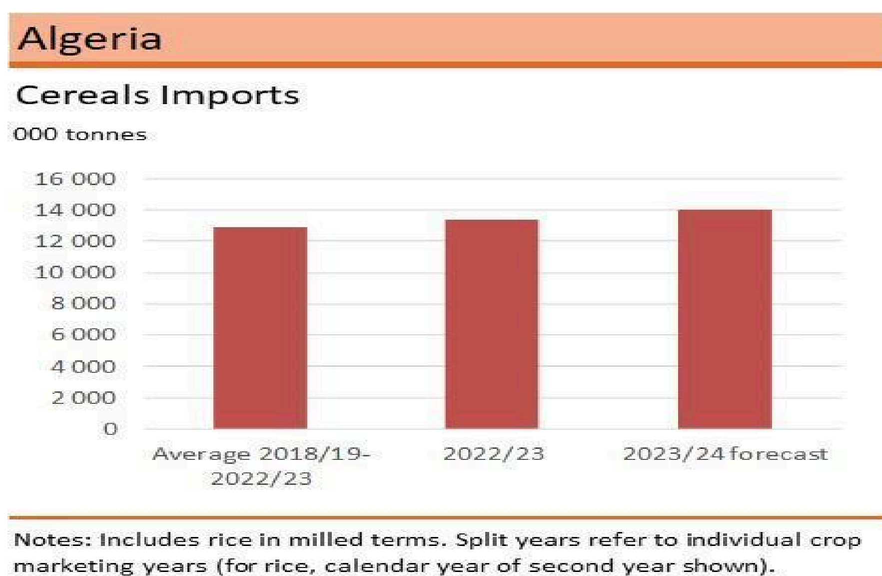

Figure 2: A chart representing the units of grains in Algeria during the period 2018-2023 (FAO,2024).

4. Chemical composition of durum wheat

Durum wheat takes an important place among cereals in the world, as it ranks 10th in terms of productivity **(Xynias et al., 2020).** Its grains are used in human and animal nutrition, so that humans have developed their use of durum wheat to include its uses even in the field of non-food industries, including biofuels and chemical industries (carbohydrates, lipids) **(Abecassis et Vermeersch, 2006)**.

Table 02: Chemical composition of 100g of ripe, raw durum wheat seeds.

Chemical composition	Quantity
Water	10.9
Energy (kcal)	339 kcal
Protein	13.7g
Fats	2.47g
Carbohydrates	71.1g
Dietary Fiber	-
Proline	1.46g
Phosphorus	508 mg
Calcium	34 mg
Magnesium	144mg
Potassium	431mg
Zinc	4.16mg
Sodium	2mg
Manganese	3.01mg

(Source:USDA,2019)

Durum wheat is a monocotyledonous annual herbaceous plant, is a tetraploid species 2n = 28 **(Soltner, 2012)**.**Louali, 2016)** describes this plant as having a spike with a firm rachis and keeled glumes, a lower glume with a colorful beard, a large grain (45-60 mg) with sub-triangular parts, and a vitreous albumen.The wheat kernel is a dry, indehiscent fruit with a single seed. The tegument attaches to the fruit wall, known as the caryopsis **.(Codou–David, 2018)**.

5. **Durum Wheat's biology**

5.1. Taxonomy

According to the classification of **APG III (2009)**, durum wheat is classified as follows:

- **Kingdom:** *Plantea*
- **Sub-kingdom:** *Tracheobionta*
- **Phylum :** *Phanerogamiae*
- **Subphylum:***Magnoliophyta (Angiosperms)*
- **Division:** *Magnoliophyta*
- **Class:** *Liliopsida (Monocotyledons)*
- **Subclass:** *Commelinidae*
- **Order:** *Poales (Glumiflorales)*
- **Family:** *Cyperales*
- **Sub-family:** *Poaceae (Grasses)*
- **Tribe :** *Pooideae (Festucoideae)*
- **Sub-tribe :** *Triticeae*
- **Genus:** *Triticinae (Triticum)*
- **Species:***Triticum durum* Desf.

5.2. Morphological characteristics

5.2.1. Grains

From a morphological perspective, the grain has an oval form, a yellowish to brownish tint, and a ventral surface groove. Having a 6.5–8.5 mm long and 3–4 mm diameter groove on the ventral face, up to 4 mm across. According to histology**,** the durum wheat kernel is composed of three different types of tissue (Figure 3) the albumen (80%), the envelopes (17%), and the germ (3% of the kernel weight)**(Fredot, 2005).**

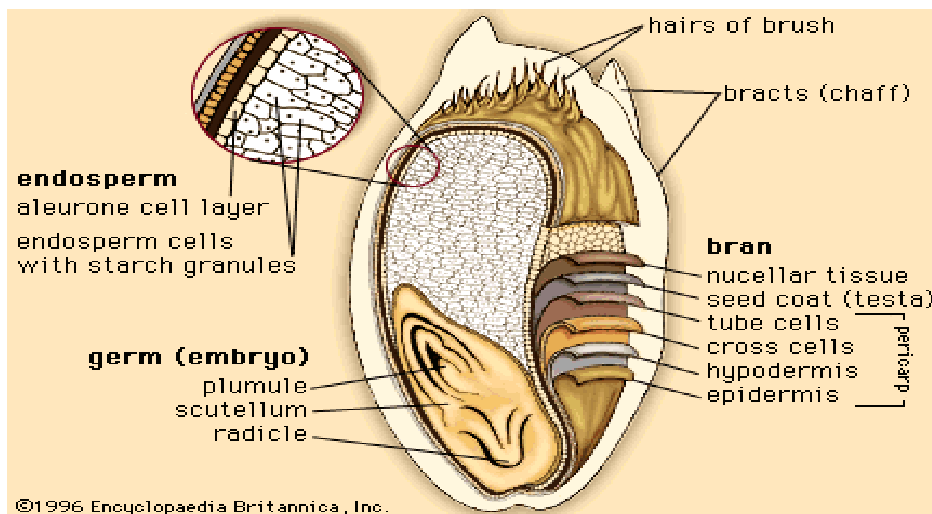

Figure 3: Internal anatomic of durum wheat seed.

5.2.2. Vegetative system

5.2.2.1. Aerial system

It is made up of a number of tillers, each of which is made up of:

5.2.2.2. stem

Cereal plants have a primary stem known as the master stalk and smaller stems called tillers that sprout at the base. Each stem is made up of many nodes that connect the base and the top **(Boulal et al., 2007).**

5.2.2.3. The leaf

The leaf is the primary source of assimilates produced during photosynthesis and exported to the seeds. The leaves are positioned on opposing sides of the stem, with

each beginning in the axil of a node. The plant is made up of four parts: sheath, stipules or auricles, ligule, and leaf blade **(Boulal et al., 2007).**

5.2.3. Root System

This fascicle type is fairly well developed, with a depth of 1.5 to 2 **(Clement et al., 1971)**. Throughout its development, every cereal plant has two root systems.

- The primary or seminal root system is active from germination until tillering. Durum wheat contains six seminal roots **(Monneveux, 1992).**
- A secondary or adventitious root system of the fasciculated kind that forms during tillering and gradually replaces the prior one. Increased tillering leads to more roots, while longer tillering times result in more roots. The adventitious roots enter senescence after flowering **(Boulal et al., 2007)**.

5.2.4. Productive system

- **Ear (spike)**
  From the terminal bud of the tillering tray. After stem development, the ear is covered in the last leaf and can be studied in detail after a few days. This is the stage where the main idea is presented. The ear is made up of a bent and strangled stem with spikelets on both sides.spikelets **(Hacini, 2014).**
- **Spikelets**
  lack a stalk and are directly linked to the rachis. Spikelets, which can number up to 25, are little groupings of flowers inserted along the spike's axis. The spike has two glumes (bracts) at the base and glumellae to protect the flowers**(Hacini, 2014)**.

5.3. Development cycle

The wheat growing cycle is divided into three main stages:

5.3.1. Vegetative period

5.3.1.1. Germination is characterized by seed imbibition, enzyme reactivation, and destruction of reserves ingested by the embryo.The coleoptile, which serves as a protective sheath for the initial leaf, then punctures the soil **(Boufenar et al., 2006)**.The quantity of leaves on a young plant and their stage of development are indicators of emergence **(Giban et al., 2003)**.

5.3.1.2. The tillering phase

Tillering starts when the plant has three to four leaves and a new stem appears in the axil of the oldest leaf. Primary tillers grow from buds in the axils of the main stem's leaves, following that, secondary tillers emerge in a similar manner on the buds of the primary tillers' leaves. Finally, tertiaries can appear using the same method **(Gate, 1995)**.

spikelets are deposited on the plant's top section through a simple constriction. They distinguish through repeated constrictions of the spike-forming cone. Herbaceous tillers develop dynamically. This phase is called tillering-mounting **(Clément-Grandcourt et al., 1970).**
The vegetative phase ends after tillering ends. With the elongation of the internodes, it signals the beginning of the reproductive phase, which is conditioned by photoperiod and vernalization **(Steinfort et al., 2017)**.

5.3.2. Breeding period

It extends from bolting to fertilization

5.3.2.1. Climbing phase

During this period, some herbaceous tillers develop spike-crowned stems while others regress. The growth of size and dry matter is then active. This phase concludes with the differentiation of stigmas and lasts 29-30 days **(Clément-Grandcourt et al., 1970)**.

5.3.2.2. The heading

Heading begins when the ear emerges from the flag leaf sheath. This signals the conclusion of bloom creation. Flowering occurs four to five days later and lasts three to six days, depending on the weather. At this stage, we speak of swelling, the total number of spikes, as well as the total number of flowers per spike **(Bebba, 2011).**
Once fertilized, the ovary rapidly expands. Within two weeks of fertilization, the embryo becomes physiologically functioning and can produce a new seedling **(Bozzini, 1988)**.

5.3.3. Maturation period

According to **(Gate, 1995)**, this phase signals a transition in the plant's function, with an emphasis on grain filling from the biomass produced. Grain volume and weight increase.This phase consists of three sequential stages:

- **The milky grain stage:** is when the envelopes of future grains are produced.
- **The doughy grain stage:** corresponds to cell filling via the transfer of assimilates from photosynthesis.
- **The physiological maturity stage:** occurs when there is no longer any migration of dry matter into the kernel and the kernel has reached its final dry weight.

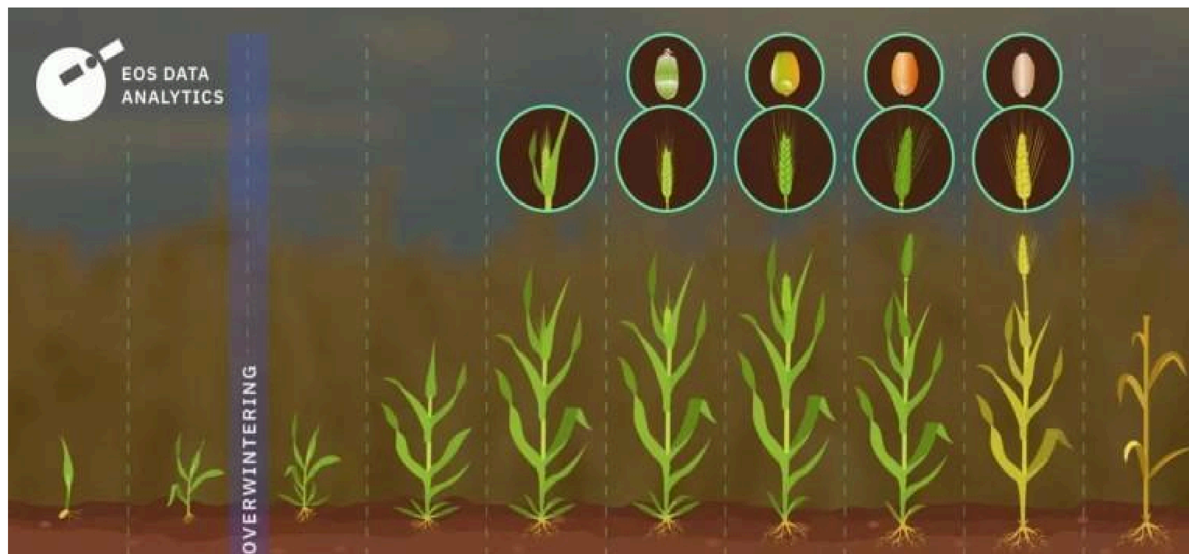

Figure 4:life cycle of durum wheat

5.4. Pedo-climatic requirements for cereals

5.4.1. Water

For high-yield crops, water requirements (Table 3) range from 450 to 650 mm, depending on the climate and the duration of the growing season **(FAO,2024).**

Table 3: the variation of the wheat crop's water requirements .

During the stage	Duration in days	Quantity mm
ears 1 cm - 2 nodes	20-25 days	60 mm
2 nodes - flowering	30-40 days	160 mm
flowering - milky grain	20-25 days	140 mm
milky grain - maturity	15-20 days	90 mm

(Source: Bonnefoy and Moynier,2014),

In arid zones, water requirements are estimated to be around 800 mm, with the highest demand during flowering (01 cm). The essential water phase occurs 20 to 35 days following flowering **(Ondo, 2014)**.

5.4.2. Soil

Soltner(2000) found that clay-limestone or silty to silty-clay soils are ideal for fasciculated wheat roots due to their high contact surface. However, light-textured and acidic soils are not suggested for durum wheat **(Novak et al., 2006).** Wheat grows best in well-drained deep soils. Avoid foods high in sodium, magnesium, or iron. The optimal pH is in the range **(Novak et al., 2006)**.

5.4.3. Light

Light plays a crucial role in photosynthesis and wheat activity **(Feillet, 2000).** According to **(Soltner, 2000)**, early growth necessitates low light intensity and a photoperiod of 12 to 16 hours of light.

5.4.4. Fertilization

Nitrogen is essential for the development and composition of vegetative organs. Nitrogenous ammonium nitrate solutions are used to supply it **(Gouasmi, 2008)**.

5.4.5. Temperature

Germination begins when the temperature climbs above 0°C **(Soltner, 2000)**.The optimal temperature for wheat development is between 21 and 25 °C **(Boukensous, 2014)**.Resistance to cold and sensitivity to frost or excessive heat are changeable characteristics that vary with age and developmental stage of the plant.

5.5. Wheat production constraints

Algerian agriculture cannot supply rising wheat demand due to structural constraints. Yield deficiency can be attributed to three factors: abiotic, biotic, and technological restrictions.

5.5.1. Abiotic stress

Abiotic stress is caused by a significant increase or decrease in one of these factors: temperature, drought, cold, heat, salinity and light.
Major climate dangers that affect cereal growing in Algeria include:

- inadequate and erratic rainfall during the crop's vegetative cycle (also known as water stress).
- The cycle's conclusion and grain filling are characterized by high temperatures and water deficits.
- The crop is impacted at crucial stages of growth (flowering and grain production) by late frosts and early siroccos.

5.5.2. Biotic stress

The term "biotic stress" describes the detrimental effects that living things have on plants. These are represented by “Insects, nematodes, bacteria ,fungi, virus and weeds.

5.5.3. Cryptogamic diseases of durum wheat

Fungal diseases are among the many that can flourish in the biotope that durum wheat creates. According to **Aouali and Douici-Khalfi (2009),** based on the symptoms that they produce, that diseases can be classified into 03 categories

- Diseases that affect foliage and produce localized symptoms.
- Root-rot-causing diseases.
- Conditions resulting in ear problems.

Table 04: The main fungal diseases affecting wheat production.

Disease / Organs attacked	Disease name	Causal agents	Transmission by seed	Sources
Damping off	Damping-off	*Stagonospora nodorum (Berk.) E. Castell. & Germano*,	Yes	**(Aouali et Douici-Khalfi, (2009) (Zillinsky, (1983)**
		Fusarium spp.	yes	
		Microdochium nivale	yes	
The foot of wheat	Eyespot	Oculimacula yalundae (Wallwork & Spooner) Crous & W. Gams,	_	**(Zillinsky, (1983)** **(Aouali et Douici-Khalfi , (2009)**
	Take-All	Gaeumannomyces graminis (Sacc.) Arx & Oliver var. tritici Walker	_	
	Common foot rot	Fusarium spp. Cochliobolus	Yes Yes	

		sativus (Si.Ito & Kurib.) Drechsler ex Dastur Microdochium nival	Yes	
The ear	Common Bunt	Tilletia caries (DC.) Tul. et C. Tul. Tilletia laevis J.K. Kühn.	Yes	**(Aouali et Douici-Khalfi , (2009)**
	Loose smut	Ustilago tritici (Pers.) Rostr.	Yes	
	Head blight , head scalp	Fusarium spp. Microdochium nivale	Yes Yes	

Chapter 2: Fusarium of durum Wheat

I. Fusarium

A variety of Fusarium spp. can attack different plant organs in wheat, barley, and other small-grain cereals. Telluric diseases, such as "Seedling Blight", 7 "Foot Rot", "Head Blight"can occur at any stage of cereal development, from sowing to harvest. **(Fernandez and Jefferson, 2004)**.

According to **Demeke et al., (2005)**, these diseases directly affect yields by reducing the quantity and quality of grains produced per ear and increasing the possibility that the grains contain mycotoxins.

The Fusarium diseases are caused by the Fusarium Head Blight (FHB) species complex, which includes more than 17 Fusarium species (**O'Donnell et al, 2004; Boutigny et al., 2012).** FHB in wheat is caused by a fungal complex composed of two genera: *Fusarium* and *Michrodochium* **(Simpson et al., 2001).** Fusariosis on wheat is commonly caused by the following species: *Fusarium graminearum, Fusarium culmorum, Fusarium avenaceum, Fusarium poae, and Microdochium nivale (previously Fusarium nivale*) **(Parry et al., 1995)**.

Fusarium graminearum is the primary causative agent and the fourth most significant fungal plant pathogen in the world **(Dean et al., 2012)**.

The first report of Fusarium head blight came from England in 1884. The disease was first discovered in Canada about 40 years later. Fusariosis has spread globally, with outbreaks recently documented in Asia, Canada, Europe, and America. Losses can approach 40% of crop **(Goswami and Kistler, 2004)**.

1. Economic impact

Fusarium Causes crop loss, contamination, reduced animal productivity and human health costs **(Matny, 2015;Bacon et al.,2007).**In the USA, the economic impact of Fusarium disease in wheat and barley during the 1990s was estimated to exceed US 3 billion in terms of yield and quality loss **(Windels,2000).** In Canada, the financial toll of the disease has been significant, with losses ranging from US$50 million to US$300 million per year since the early 1990s

2. Taxonomy

The name Fusarium has been accepted as a unique name for all species, including teleomorphs, since 2013, following changes to the International Code of Nomenclature for fungi. As a result, the names Gibberella and other names are no longer accepted to denote the sexual stage of these pathogens **(Geiser et al., 2013).** The taxonomy of the genera Fusarium and Microdochium (Figure 5) is displayed in the following scheme based on the **MycoBank database as of 2022 (IMA, 2022).**

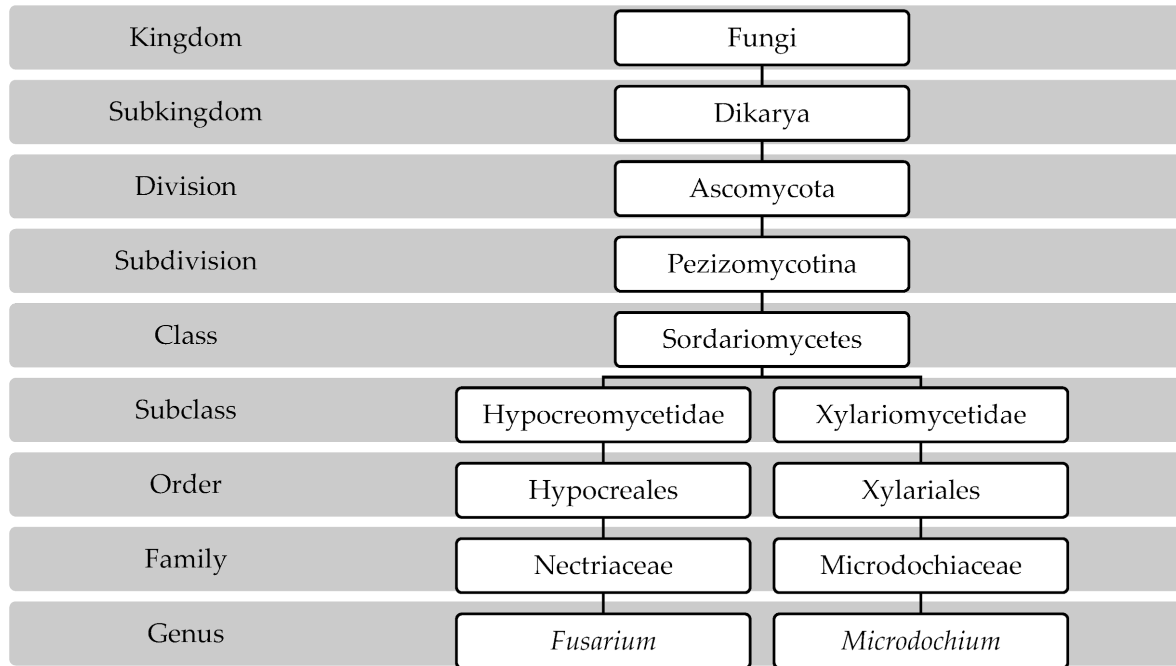

Figure 5: Taxonomical position of the genus *Fusarium* and *Microdochium* according to MycoBank database (2022).

3. Epidemiology

The disease-causing fungus survives and multiplies on the remains of infected plants, whether grains, grasses, or other cultivated or uncultivated plants in and around the field **(Zillinsky, 1983).** During anthesis, wheat plants are most vulnerable to infection, as anthers division releases pollen, allowing pathogens to enter (**Brown, 2011; Rittenouret al., 2010).** Ideal conditions for infection include prolonged periods of high humidity between 48 and 72 hours, moderately warm temperatures between 15 and 30 °C, and frequent rainfall, which generally occurs in spring **(Lenc, 2015; Muthomi et al., 2008; Lenc,. 2015)**. An increase in relative humidity leads to the

release of ascospores, while precipitation causes the walls of the ascus to rupture, promoting the dispersal of these spores **(Gilbert et al., 2013;Trail et al., 2002)**. These conditions, combined with abundant inoculum before, during and after anthesis, lead to yield and quality losses and the development of serious epidemics. The different species of the Fusarium wilt complex have varying climatic requirements and genetic and environmental adaptations that enable them to cause infections **(Shaner, 2003).**

4. Symptomatology

The initial indications are the presence of water-soaked lesions on infected spikelets, which subsequently evolve into necrosis and, in cases of significant severity, bleaching of the spikelets **(McMullen et al., 2012; McMullen et al.,1997)**. The bleaching process subsequently spreads throughout the spike, resulting in the formation of premature whiteheads. In warm, humid conditions, pinkish-red mycelium is observed on diseased tissue **(Trail, 2009; Deba et al., 2017)**. The kernels of infected ears are referred to as tombstones due to their light, shriveled, discolored (pinkish or chalky), and poor quality appearance **(Scherm et al., 2013)**. Infections from less virulent pathogens like *Fusarium poae*, can induce infection and significant mycotoxin contamination in infected kernels without manifesting visible symptoms on the spikelets or spikes **(Stenglein, 2009; Stenglien et al., 2014).**

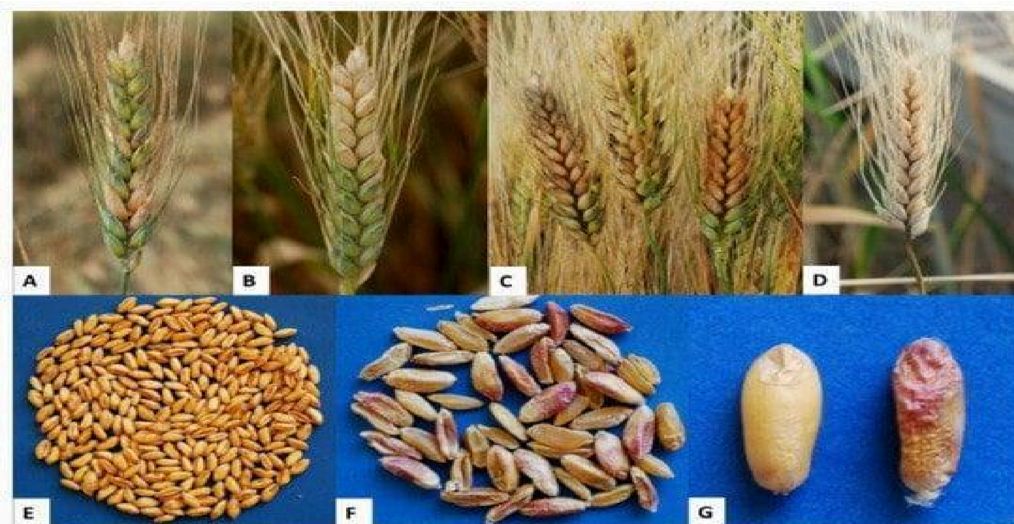

Figure 07: Symptoms of Fusarium head blight on durum wheat spikes (A-D) and kernels affected by fusariosis (F,G) compared to healthy kernels (E).

5. Morphology

Fusarium is distinguished by its fusiform, septate macroconidia and a fast-growing, variably pigmented thallus. The conidiophores can be highly branched, forming cushions on the thallus and carrying spores that appear to have a greasy appearance. The phialides are elongated and produce two types of conidia: fusiform macroconidia with a basal cell and small microconidia that are typically septate and

pyriform, fusiform, or ovoid. The presence of chlamydospores is variable and can be differentiated by either mycelium or conidia. These morphological features of Fusarium are described in detail by various sources, including **(Tabuc, 2007;Jeunot ,2005;Botton et al.,1990)**.

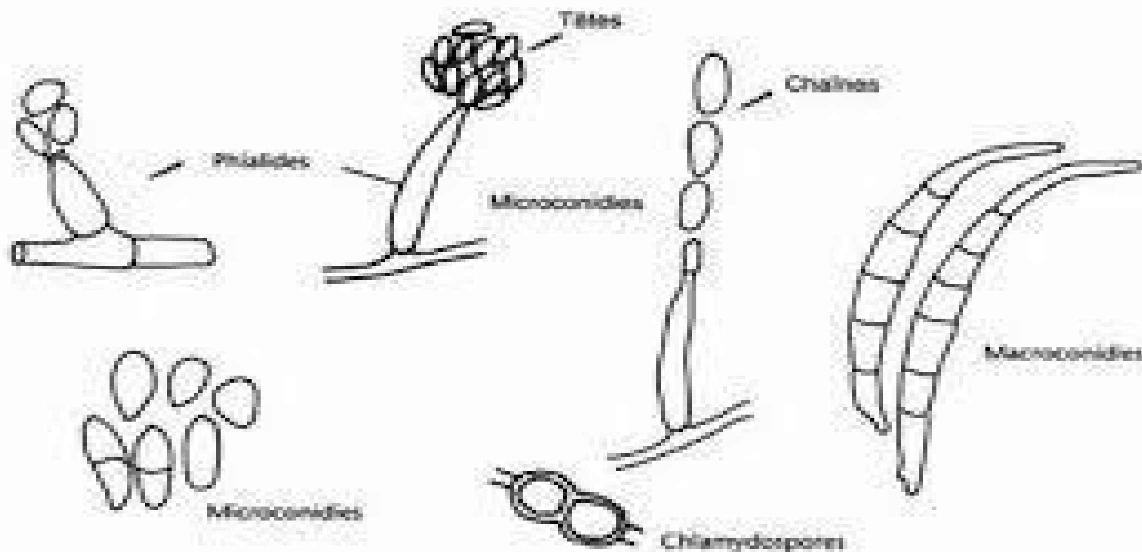

Figure 08: Morphological Characterization of Fusarium (Tabuc, 2007).

6. Life cycle

Fusarium is a monocyclic disease that lives in prior crop detritus as macroconidia or ascospores in sexual structures known as perithecia. These spores are the primary cause of infection. Grasses and weeds are Fusarium hosts as well as inoculum sources. When conditions are favorable during wheat anthesis, wind or rain spreads inoculum over open spikelets. The spores germinate, forming germination tubes on the spikelet tissue. The fungal hyphae propagate throughout the ovary, palea, and lemma, producing mycotoxins without reaching spikelet tissue **(McMullen et al., 2012;Tores at al., 2019;Trail, 2009; Mourelos et al., 2014; Surproniene et al., 2019; Dong et al., 2020)**. Eventually, the pathogen enters the host tissue, resulting in a biotrophic infection and intercellular development within the spikelet. This infection progresses to the necrotrophic stage, which includes lateral and vertical inter-and intracellular growth within the spikelet **(Brown et al., 2010; Divon et al., 2019) (figure 9).**

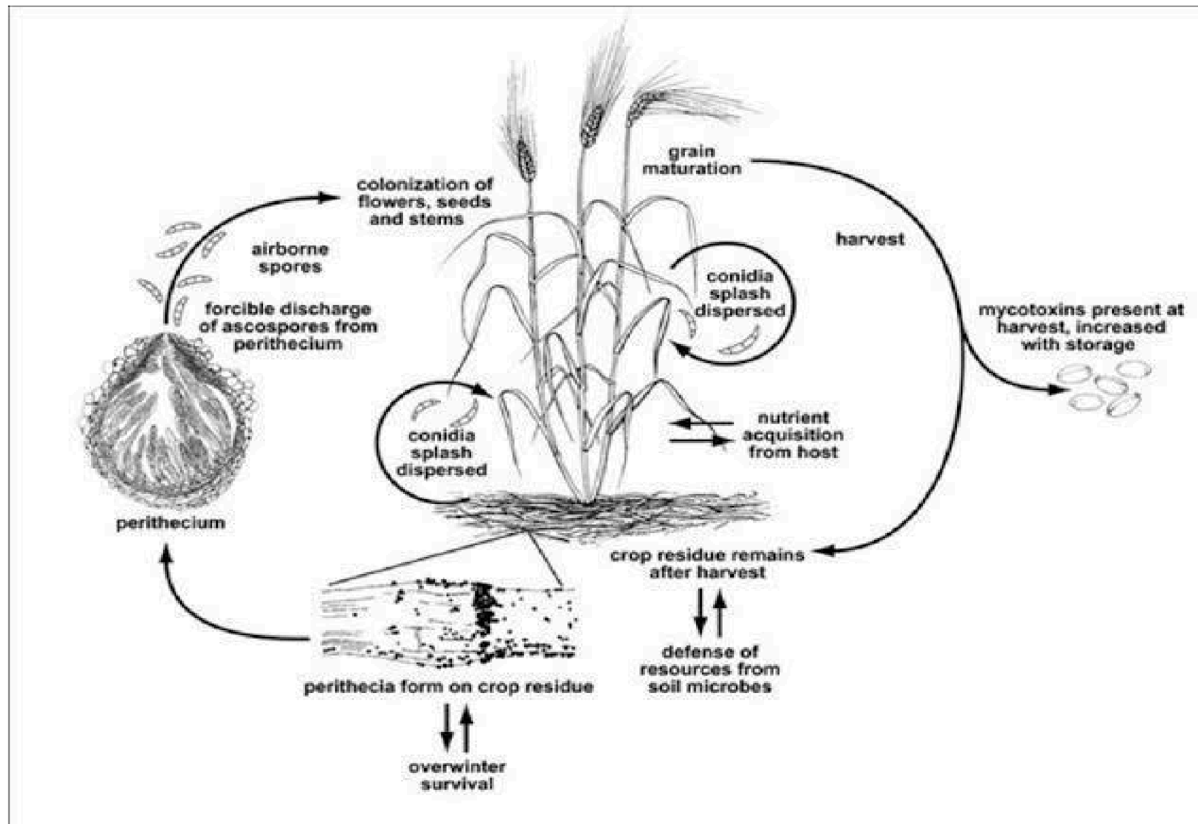

Figure 09:The life cycle of *Fusarium graminearum,* the causal pathogen of Fusarium head blight disease of wheat (Trail,. 2009).

II. Control methods

1. Cultural control

This control aims to limit the increase in soil inoculum and includes the rational use of nitrogen fertilizers. Elimination of contaminated crop residues by burning or deep burial. The use of solarization, which can reduce pathogen populations and disease incidence, and the use of healthy seed.

2. Genetic control

The development of resistant cultivars appears to be the most cost-effective and environmentally friendly approach **(West et al., 2011)**. Crop breeding and improvement programs aim to enhance genetic resistance to pathogens. Understanding the genetic and biochemical basis of the pathogen-host plant interaction is key to the success of this improvement. Wheat has multiple mechanisms of resistance, as the fungus needs open flowers to penetrate them. There are some varieties that flower without open spikelets, and other varieties are not susceptible due to a short flowering period, not many flowers or open spikelets that aid in rapid drying. Thick skin can prevent fungal penetration, and a long stem can protect the ear from contamination by spores from the soil or leaves **(Hilton et al., 1996; Fowler et Lafond2013; Tadesse et al., 2019; Mago et al., 2015).**

3. Chemical control

Studies indicate that the use of fungicides from the demethylation inhibitor class reduced disease severity and mycotoxin contamination in grain by up to 77% and 89% respectively. Timely application of triazole-based fungicides also showed success in reducing disease severity, resulting in reduced yield losses and improved quality **(Wigulo et al., 2015; Haidukowski et al.,2004; McMullen et al., 2012)**. Furthermore, the application of triazole fungicides to infected wheat plants resulted in a 90% reduction in infection rate and a 14% increase in yield. Metconazole, prothioconazole + tebuconazole and prothioconazole were identified as the top three fungicide treatments that resulted in the highest increase in yield and weight **(Paul et al., 2010; Paul et al., 2008)**.

4. Biologic control

Several microorganisms have been proven to be beneficial in protecting wheat from Fusarium. Numerous bacterial, fungal, and yeast strains have been recognized for their effectiveness in mitigating the severity of (FHB). Specifically, strains such as *Bacillus spp.* **(khan et al., 2001)**, *Pseudomonas* spp*.* **(wang et al,2015)**, *Streptomyces* spp.**(Palazzini et al., 2017)** and *Lactobacillus plantarum* **(Baffoni et al., 2015)** have been assessed for their antagonistic properties against *Fusarium graminearum*. These bacteria, isolated from a variety of environments, have been applied to anthers and/or plant residues, employing various biological control strategies, including antibiosis, competition, and mycoparasitism **(Legrand et al., 2017)**.

Chapter 3:
Biologic control

1. Definition

Biocontrol is the use of living organisms or natural substances to protect crops, and it has four main categories: macroorganisms, microorganisms, chemical mediators, and natural substances. Adopting biocontrol products in agriculture can contribute to sustainable practices and restore the natural balance in soil ecosystems animals **(Daguerre et al., 2014; Junaid et al., 2013).** Maintaining a balanced soil microbial life is increasingly recognized as important, and organic products can help achieve this. Available biocontrol products include plant extracts and soil microorganisms, which can combat pathogens by competing for space or releasing toxic compounds **(Pellan et al., 2021)**. Biocontrol methods offer a promising solution for environmentally friendly crop protection.

2. Historical review

Though the phrase itself is relatively new, biocontrol techniques do not date from today's world. As a defense against fungal pathogens, Field horsetail and garlic have been used as a defense against fungal pathogens **(Bach et al., 2016; García-Núñez et al., 2017; Shang et al., 2019)**.

Prior to the 1960s, biological control was only considered in experimental settings with little consideration for real-world applications. The remarkable advancement in chemical fungicide research during and soon following World War II had a lasting impact on this kind of study, preventing the development of these biological approaches, which remained in the background **(Sekhri et al., 2006)**.

3. The agents and the benefits of biocontrol

Biological control agents, often known as biopesticides, are plant protection treatments that use a living organism or its derived products as the active ingredient. They can consist of creatures (plants, insects, nematodes) or microorganisms (bacteria, yeasts, etc.).

Microorganisms (bacteria, yeasts, fungi, viruses) defend plants from phytopathogenic chemicals, including natural compounds like plant extracts and pheromones **(Thakore, 2006)**

Biopesticides can supplement chemical treatment, but they can also be utilized when no synthetic control option is available **(Saravanakumar et al., 2007).** According to **Fravel (2005)**, these biological products are designed to manage a variety of diseases. Research indicates that specific endophytic microbes and/or rhizobacteria that promote plant growth can bestow resistance against plant diseases and tolerance to abiotic conditions like salinity and drought on specific crops **(Wang et al., 2012)**. According to **(Weeden et al., 2007)**, beneficial organisms utilized in biological management need to be specialized, have a high rate of reproduction, be adaptable, and have a life cycle that coincides with the pest or parasite organisms.

Endophytes can be employed as biocontrol agents to reduce pathogen inoculum or inhibit pathogen activity **(Martins et al., 2013)**.

4. Antagonism mechanisms in biological control

Biological control agents have diverse mechanisms of action that impact the pest. **(Elad and others, 2016).**

4.1. Competition

"The direct detrimental effect of one organism on another, or directly, by the removal of certain resources from the environment," is how **Clark (1968)** described competition. When multiple micro-organisms consume the same substance at once, competition for nutrition arises. An antagonistic agent has to be able to quickly and effectively use nutrients that are present in low quantities on plant organs in order to be a competitive agent **(Jijakli, 2003)**. Some species, such the yeast Aureobasidium pullulans, compete with Botrytis cinerea for nutrients and space, which stops it from proliferating on the plant **(Elad et al., 2016).**

4.2. Antibiosis

Biological control agents employ antibiosis as a mechanism of action to inhibit the growth of pathogens. It entails the antagonistic agent producing antibiotics that are efficient against the infection **(Corbaz,1990)**. Some bacteria,including Pseudomonas, create antifungal substances like pyrrolnitrin that prevent pathogens from growing mycelium. The biological control agents' mode of action that has been investigated the most is antibiosis **(jijakli, 2003)**. The antibiotic tropolone, produced by a Pseudomonas strain, exhibited antagonistic characteristics against a variety of fungi, demonstrating the antibiosis exerted by fluorescent Pseudomonas spp. in vitro. Alternaria, Cladosporium, Rhizoctonia, Fusarium, Helminthosporium, Pyricularia, Pythium, and Diplodia are some of these fungi. Understanding how different microorganisms create antibiotics might help in the creation of efficient biocontrol. Antibiosis is a key mechanism in biological control **(Howell et Stipanovic, 1979, 1980).**

4.3. Mycoparasitism

This control system known as hyperparasitism, involves two microorganisms interacting directly, with one's live tissue serving as a source of nutrients for the other **(Helluy and Holmes, 2005)**. The parasite may or may not kill the pathogen to feed on its cells **(Latz et al., 2018).**

"*Talaromyces pinophilus*" targets "**Botrytis cinerea*", the pathogen causing wilt and umbel blight in onion seeds, through mycoparasitism. It wraps around the pathogen's hyphae, leading to cell wall breakdown and cytoplasmic disruption. The fungus produces enzymes such as chitinase, lipase, and protease that facilitate this process **(Abdel-Rahim and Abo-Elyousr, 2018)**.

Table 05: list of some microorganisms known or potentially effective against soil pathogens, along with their status (European Commission, 2023).

Microorganisms	Target category	Status under Regulation (EC) N°1107/2009	Date of approval
Bacillus amyloliquefaciens MBI 600	Fungi	Pending	
Bacillus amyloliquefaciens strain FZB24	Fungi	Pending	
Bacillus amyloliquefaciens subsp. plantarum D747	Fungi	Approved	01/04/2015
Bacillus firmus I-1582	Nematodes	Approved	01/10/2013
Bacillus pumilus QST 2808	Fungi	Approved	01/09/2014
Bacillus subtilis str. QST 713	Fungi , Bacteria	Approved	01/02/2007
Coniothyrium minitans	Fungi	Approved	01/01/2004
Gliocladium catenulatum strain J1446	Fungi	Approved	01/04/2005
Purpureocilium lilacinum strain	Nematodes	Approved	01/08/2008
Phlebiopsis gigantea (several strains)	Fungi	Approved	01/05/2009
Pseudomonas chlororaphis strain MA342	Fungi	Approved	01/10/2004
Pseudomonas sp. Strain DSMZ 13134			
Pythium oligandrum M1	Fungi	Approved	01/02/2009
Streptomyces K61	Fungi	Approved	01/05/2009
Streptomyces lydicus WYEC 108	Fungi	Approved	01/05/2009
Trichoderma asperellum strains ICC012, T25, and TV1	Fungi, Bacteria	Approved	01/01/2015
Trichoderma asperellum ICC012 TV1	Fungi	Approved	01/05/2009
Trichoderma atroviride (formerly T. harzianum) strains IMI 206040 and T11			
Trichoderma atroviride strain I-1237	Fungi	Approved	01/06/2013
Trichoderma atroviride strain SC1	Fungi	Approved	01/05/2009
Trichoderma gamsii (formerly T. viride) strain ICC080	Fungi	Approved	01/06/2013
Trichoderma harzianum strain T-22 and	Fungi	Pending	
ITEM 908	Fungi	Approved	01/05/2009
Trichoderma polysporum strain IMI 206039			
	Fungi	Approved	01/05/2009
	Fungi	approved	01/05/2009

4.4. Endophytes

Fungi that infiltrate plant stems and leaves without causing any clinical symptoms for the plant are referred to as "endophytes" (**Chanway., 1996).** Many plant species have endophytic microorganisms, which rarely cause disease symptoms **(Meenakchi et al., 2016)**.They might arise from native species or be introduced through

agricultural methods**(Shi et al., 2009).** Research has shown that specific endophytic microbes can significantly enhance plant resistance against various diseases. These microorganisms, which reside within plant tissues without causing harm, can promote plant health through various mechanisms, such as the production of antimicrobial compounds, competition with pathogenic microbes, and the enhancement of the plant's own defense responses. For instance, studies have demonstrated that certain endophytes can induce systemic resistance in plants, leading to increased tolerance against pathogens like Fusarium and Phytophthora species **(Ryan et al., 2009)**. Furthermore, the interaction between plants and their endophytic microbes can improve nutrient uptake and stress resilience, ultimately contributing to better crop yields and sustainability in agricultural systems **(Bacilio et al., 2006).**
The relationship between endophytes and plants was considered to be either:

- Symbiotic or mutualistic.
- Aggressive saprophyte.
- Opportunistic pathogen.

4.5. The endophyte’s colonization in the tissues of plants.

Endophytes have the ability to colonize the roots, stems, and leaves of plants, providing significant benefits. They offer protection against various pathogens and help improve the plants resistance to environmental stress. Moreover, some endophytes can even positively influence the growth and development of host plants, thereby enhancing their health and productivity. **(Strobel and Daisy, 2003).**
The colonization of internal plant tissues by endophytes may be confined to the root system. This is evident in the case of brown septate endophytes, or DSE, which are sterile Ascomycetes that inhabit the roots of a variety of higher plants **(Sieber, 2002).**
Penetration can occur either locally or systemically across different leaf parts. The fungus may directly invade the cell wall, as in the case of Rhabdocline parkeri, or it can enter through the plant's natural openings, such as stomata and substomatal chambers, as seen with Phaeosphaeria junicicola **(Schulz and Boyle, 2005).**

Experimental part

Chapter 4: Materials and methods

1. **Objective**

The objective of using seaweed endophytes to control Fusarium in durum wheat is to explore a biological control method for managing Fusarium infections, which are

known to cause significant crop losses and affect wheat quality. Here's a summary of the main goals:

- Disease Management: Fusarium species, such as Fusarium graminearum, can cause diseases like Fusarium head blight and root rot in durum wheat. Using seaweed endophytes aims to reduce the incidence and severity of these diseases.
- Sustainable Agriculture: This approach seeks to provide an environmentally friendly alternative to chemical pesticides, promoting sustainable agricultural practices by leveraging natural biological control agents.
- Enhanced Crop Health: Seaweed endophytes may enhance the overall health and resilience of durum wheat by competing with or inhibiting the growth of Fusarium pathogens.
- Improved Yield and Quality: By controlling Fusarium infections, the endophytes can potentially lead to higher yields and better quality of durum wheat, which is crucial for both economic and food security reasons.
- Mechanism Exploration: Understanding how seaweed endophytes interact with Fusarium pathogens helps in elucidating their mechanisms of action, which could be beneficial for developing more effective and targeted biological control strategies.
-

Overall, this method aims to offer a natural, effective, and sustainable solution to managing Fusarium diseases in durum wheat.

2. Vegetal material

In this experiment, three specific plant materials were used: grains, leaves, and fully germinated durum wheat. The grains utilized were from the Simito F1 variety, of Italian origin.

These grains were planted under standard conditions. The pots were filled with sand and compost (v/v). The resulting plants and their leaves were used for various experiments.

Other grains were not sown.They were used for the biopriming.

3. fungal material

3.1. Pathogenic Agent

During the agricultural season of 2023-2024, symptomatic wheat samples suspected of having Fusarium disease were collected by Mr. Mahiout from Al-Hajjaj, Al-Mnea Governorate . The following steps were taken to handle and prepare the pathogenic agents:

3.1.1. Sampling Infected stems, leaves and ears were collected from symptomatic wheat plants. The samples were transported to the laboratory and kept immediatly in the fridge at 4°C until their using.

3.1.2. Isolation

The collected plant parts were cut into small fragments of 5 mm^2 .

Seeds and fragments were disinfected by soaking in a 2% NaClO (sodium hypochlorite) solution for 20 seconds.
After disinfection, the vegetal material was rinsed in three successive baths of sterile distilled water for 1, 2, and 3 minutes, respectively.
The seeds and fragments were then dried on sterile absorbent paper.

3.1.3. Culturing and identification

The plant fragments and seeds were plated onto Potato Dextrose Agar (PDA) media in sterile Petri dishes, with five fragments per dish.
The plates were incubated at 26°C in the dark for 7 days to allow fungal growth.
Fungal colonies that were suspected to be Fusarium were transferred to new PDA media for further identification and analysis.
three different pathogenic strains of Fusarium with different morphological characteristics were then obtained. They were used for study their behavior when confronted with algal endophytes

3.2. Antagonistic agents

In this experiment, we used 15 endophytic fungal isolates that were previously identified and isolated by master students Fatima Talbi and Chelik Djamila. These strains were collected from marine algae in the Stidia region, approximately 17 km west of Mostaganem, during the previous year. They were maintained on PDA medium in storage at °4C.

3.2.1 Culturing and Maintenance

The identified fungal strains were transplanted into Potato Dextrose Agar (PDA) media to renew their cell cultures.
The objective was to obtain and use young, actively growing isolates for the current experiment.

3.2.2. Culture media

Choosing an appropriate culture medium is crucial for the optimal development of fungi. The PDA medium provides the best conditions for isolating and culturing both endophytes and pathogens, as well as for conducting antagonistic tests.
Composition of PDA medium for 1 L distilled water:

- Potatoes: 200g
- Glucose: 20g
- Agar-agar: 18g
- pH: 6.5

4. Methods

4.1. Direct Confrontation test

This study serves as a preliminary investigation focused on the selection and identification of endophytic fungi that are suspected to act as antagonistic agents against Fusarium. The isolates belong to different genera and species: Aspergillus niger (02 isolates), Aspergillus sp. (2 isolates), Gliocladium (1 isolate), Penicillium (1 isolate), Botrytis sp. (1 isolate) and Scopulariopsis (1 isolate). The remaining 7 isolates were not identified.
In Petri dishes with a diameter of 90 mm, containing 15 ml of PDA medium, two agar pellets (6 mm in diameter) are placed along a diametrical axis, one pellet carrying an endophytic strain and the other carrying one of the three pathogenic isolates each time. The pellets are positioned 4 cm apart one from each other (Figure 10). Incubation occurs at 22 °C for 10 days. Each treatment is replicated five times.

4.2. Evaluation of Mycelial Growth: Mycelial growth of *Fusarium* spp. was evaluated every 48 hours all by measuring the radius of the parasite on the side of the antagonist. After 10 days of incubation, measurements were made on the width of the zone of inhibition observed between the two colonies.

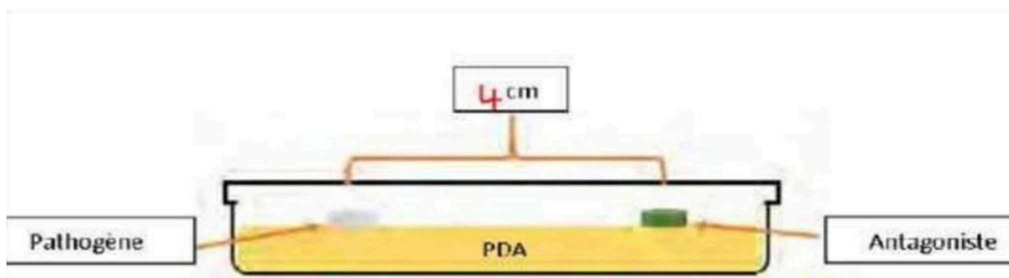

Figure 10: Device used for the direct confrontation test (Mahiout, 2007)

***In vivo* control trial using antagonists against Fusarium spp.**
Preparation of spore suspension of the antagonist and the pathogens
In this part we need to use the fungal colonies have produced sufficient spores. This typically takes several days to a week, depending on the fungus, to prepare the suspension we added 5 ml of sterilized water. We had to gently scrape the surface of the fungal culture with a sterile spatula or scalpel to dislodge the spores. We mixed the solution in a vortex mixer to separate the spores from the mycelial fragments.
In order to remove mycelial fragments and other debris, we filtered the spore suspension through chiffon. We adjusted the inoculum to 10^6 spores/ml using a Malassez cell.

***4.3. In vivo* effect of antagonist and pathogen on durum wheat**
In this part we used 3 different methods to evaluate the progress of the experiment's application on living tissue.

4.4. Application on seeds of durum wheat

Bio-priming is an advanced seed treatment technique that integrates both biological and physiological aspects for disease control. This method involves inoculating seeds with beneficial organisms to enhance their protection and hydrating the seeds to prepare them for stress resistance **(Reddy, 2012)**. Recently, bio-priming has emerged as an effective alternative for managing various seed and soil-borne pathogens. By using biocontrol agents, bio-priming serves as a standard strategy for introducing disease resistance. Compared to other methods, priming seeds with helpful microorganisms and biocontrol agents has been demonstrated to be more effective in managing diseases and pests **(Prabha et al., 2019)**.

II. Preparation

1.Preparation of Seeds

We choosed high-quality seeds that are free from visible damage or disease. To remove any debris or contaminants, we washed the seeds with tap water then they were sterilized for 30 seconds using a 2% NaClo. The seeds were rinsed in three baths of sterile distilled water and left to air dry on Joseph paper.

2.Initial Separation and Treatment

Grouping: Separate the plants into three distinct groups:

- Group 1: soaking the seeds in this group into sterilized distilled water (SW) for 10 hours.
- Group 2: soaking the seeds in this group into sterilized distilled water (SW) for 10 hours.
- Group 3: soaking the seeds in this group into an antagonistic spore suspension for 10 hours.

2. Seed exposure to pathogen spores

After the initial soaking, transfer the seeds from Group 2 and Group 3 into a pathogenic spore suspension (1x106 spores/ml), ensuring each group is exposed to the suspension of the three pathogens separately. We left the seeds in the pathogenic spore suspension for 10 hours.

We placed Group 1 seeds in sterilized distilled water for a second exposition period for 10 hours.

100 seeds of each treatment are distributed in four Petri dishes, 25 seeds per dish, containing filter paper moistened with sterile distilled water (Figure11).

Figure 11: distributed seeds in Petri dish

3. Calculation of germination rate

Germinated seeds are counted daily over a four-day period, during which the two seeds with the highest germination rates are selected for measurement each day.

We also calculated the germination index, and total average germination in the fourth day.

The germination index (GI) is a measure of seed viability and can be calculated using the following formula:

$GI=(NGS \times 100)/TPS$

4. Number of roots produced by the seed and measure of radicle length

We selected two germinated seeds which present the best morphological aspect from the total daily germination seed number.

The number of roots emerging from each seedling is counted on the fourth day. The selected seeds were replaced on another petri dish containing a humid filter paper until the fourth day, after that the best germinated seed were used for calculating the number of roots and the radicle length using a ruler.

Application on leaves of durum wheat

1. Preparation of leaf samples and Application of treatments

We cutted the edges of green young leaves into 10 cm lengths from plants of 6 weeks old. As for the test of seeds, we separated the leaf pieces into three distinct groups for 3 different treatments. The cut leaves pieces were immersed into 4 ml of the respective treatment's solution, ensuring that the leaves are fully submerged and reach the bottom of a 5 ml tube.

Each treatment should include:

- Normal Control: Leaves immersed in a solution of sterile distilled water (SW) without any antagonist or pathogen
for 2 days.

- Treatment with pathogen: Leaves were immersed in a solution of sterile distilled water (SW) then replaced in the second day into a sporal suspension (1x10^6 spores/ml) of the pathogen (PSS) (SW-FH1,SW-FH3,SW-FH4).

-Treatment with the antagonist and the pathogen: Leaves were immersed in the first day in a sporal suspension (1x10^6 spores/ml) of the antagonist (ASS) then replaced in the second day into a sporal suspension (1x10^6 spores/ml) of the pathogen (PSS) (AZ1-FH1,SW-FH3,SW-FH4) (Table 6).

Each treatment is repeated 3 times. The Leaves are left under laboratory conditions of temperature (25±2°C) and light. We recorded any changes or observations for each group, noting any differences in leaf symptoms.

Table 06:General steps for applying a spore suspension treatment

	Day 1: Solution 1	Day 2: Solution 2
Normal Witness	SW	SW
Pathogenic Witness	SW	PSS
Antagonist/Pathogen	ASS	PSS

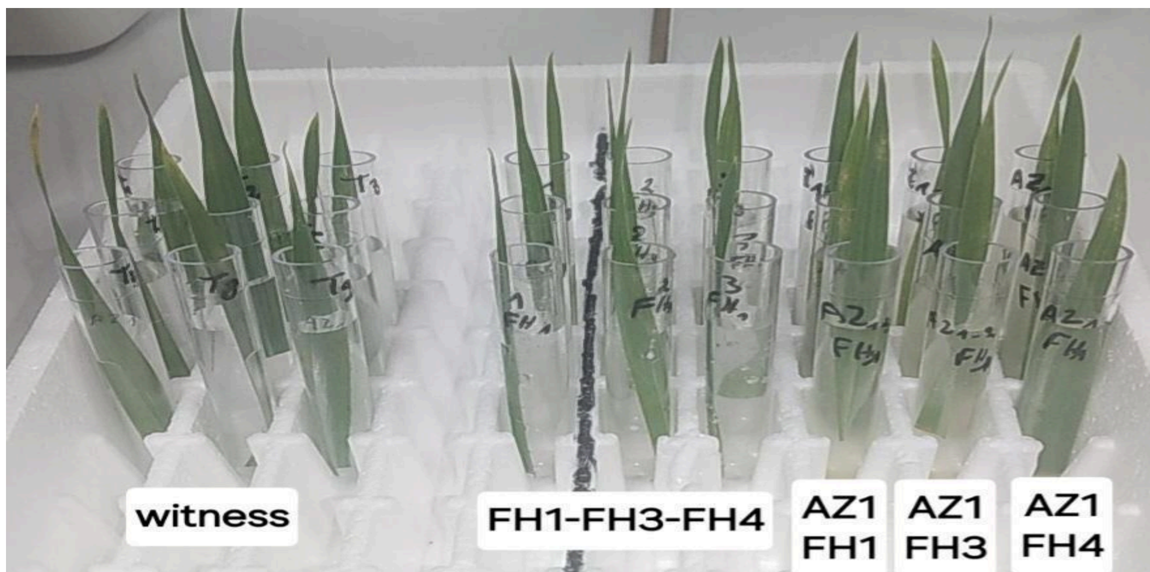

Figure 12: Experimental device of leaves inoculation.
FH1: Fusarium isolate 1; FH3: Fusarium isolate 3; FH4; Fusarium isolate 4
AZ1: Antagonist isolate.

Application on the entire plants of durum wheat

In this application we use all plants and we focus on the root system because the root system of a plant is vital for its stability, nutrient and water uptake, storage of energy, growth, and interaction with the environment. In this experiment we used durum wheat plants of 6 weeks old. These plants were previously planted in pots containing autoclaved soil (120°C, for 20 minutes).

1.Initial Separation and Treatment

We separated the plants into three distinct groups:

- Group 1: The roots were immersed into sterilized distilled water (SW) for 30 minutes, then they were immersed in sterilized distilled water (SW) for another 30 minutes.
- Group 2: The roots were immersed into sterilized distilled water for 30 minutes, then they were immersed into pathogenic sporal suspension of 10^6 spores /ml for 30 minutes (SW-FH1,SW-FH3,SW-FH4).
- Group 3: The roots were immersed into an antagonistic spore suspension for 30 minutes, then into pathogenic sporal suspension of 10^6 spores /ml for 30 minutes (AZ1-FH1,AZ1-FH3,AZ1-FH4).

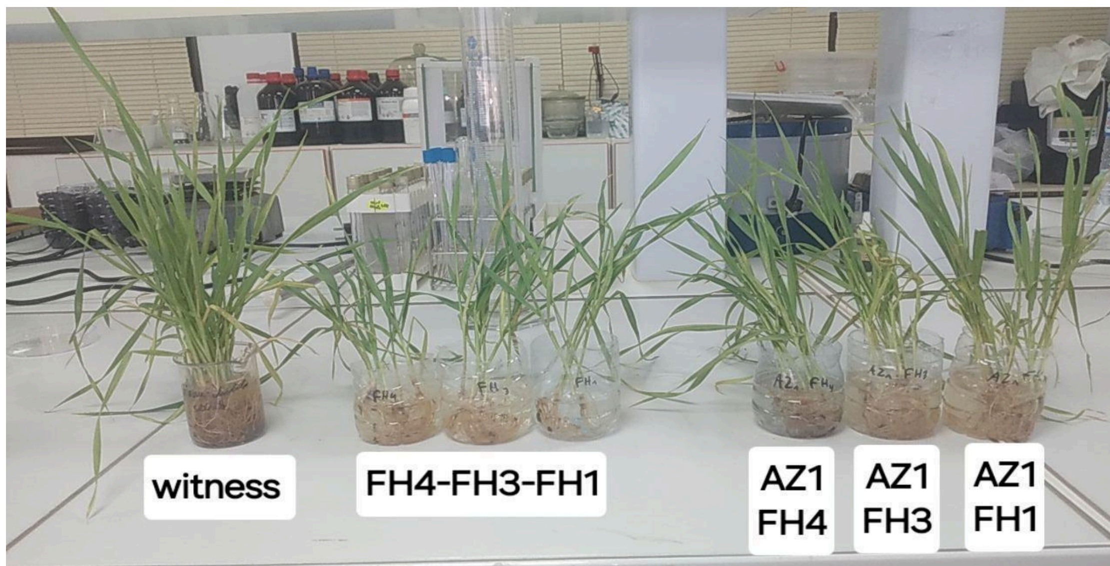

Figure 13: second immersion of DW planets in pathogenic spores.
FH1: Fusarium isolate 1; FH3: Fusarium isolate 3; FH4; Fusarium isolate 4
AZ1: Antagonist isolate.

3.Planting and Care

Plants from all groups are replanted in containers filled with 300 grams of horticultural compost, with 3 plants per pot. The plants are daily watered with distilled water to maintain soil moisture and support plant growth. The plants are left under laboratory conditions of temperature (25±2°C) and light. We recorded any changes or observations for each group, noting any differences in leaf symptoms.

Chapter 5 Results and discussion

RESULTS

Direct confrontation results

Preliminary selective experiments identified a single endophyte fungus among 15 of those tested that demonstrated significant effectiveness and yielded clear results compared to the others. The other endophytes exhibited negative or ambiguous

effects, including the covering of pathogenic isolates of Fusarium and the convergence or entanglement of their mycelium with that of the pathogens at various intervals before the conclusion of the 10-day period.

The results of the mycelial growth of pathogen strains against this antagonistic endophyte and the clear and the distance of zone of inhibition led us to select it as the foundation for our study on its impact against Fusarium.this antagonistic endophyte identified as Botrytis (AZ1).

Mycelial growth effect

The study revealed a highly significant effect on the mycelial growth of Fusarium spp. colonies when exposed to Botrytis isolate, its showed a reduction in growth compared to the witness**.** Indeed, Figure 14 shows a decrease in Fusarium spp. growth in the presence of the AZ1 endophyte, relative to unconfronted control colonies. The effect of the antagonist is apparent from 48 hours of confrontation, and increases with time. The average growth radius of FH4 colonies reached 1.76 cm after 240 hours of confrontation with AZ1, compared with 4.2 cm for non-confrontational control colonies. The average growth radius of FH3 colonies reached 2.36 cm after 240 hours of confrontation with AZ1, compared with 3.74 cm for non-confrontational control colonies .The average growth radius of FH1 colonies reached 2.28 cm after 240 hours of confrontation with AZ1, compared with 3.40 cm for non-confrontational control colonies.So FH4 seems to be the most sensitive to AZ1 antagonistic effect, then FH1 in position and FH3 in the third position.

The reduced growth of the parasite when confronted with the endophyte generated a zone of inhibition between the parasite colony and the antagonist colony. This zone of inhibition was measured after 10 days of incubation. Mycelial growth of Fusarium strains has been stopped, while mycelial growth of FH1, FH3 and FH4 colonies is 2.28 cm, 2.36 cm and 1.77 cm radius respectively, providing an inhibition zone of 1.12 cm, 0.82 cm and 1.18 cm respectively, while colonies of control explants continue their mycelial growth (Figure 14, Annexe 1). On the 10th day, the average radius of colonies of the parasite (FH1, FH3, FH4, faces the strain of AZ1 is significantly lower than control colonies.

The AZ1 strain demonstrated very slow mycelial growth. It exhibited a distinct antagonistic capability, specifically the ability to inhibit parasite development at a distance, resulting in the formation of an inhibition zone between the colonies, which varied with different Fusarium isolates.

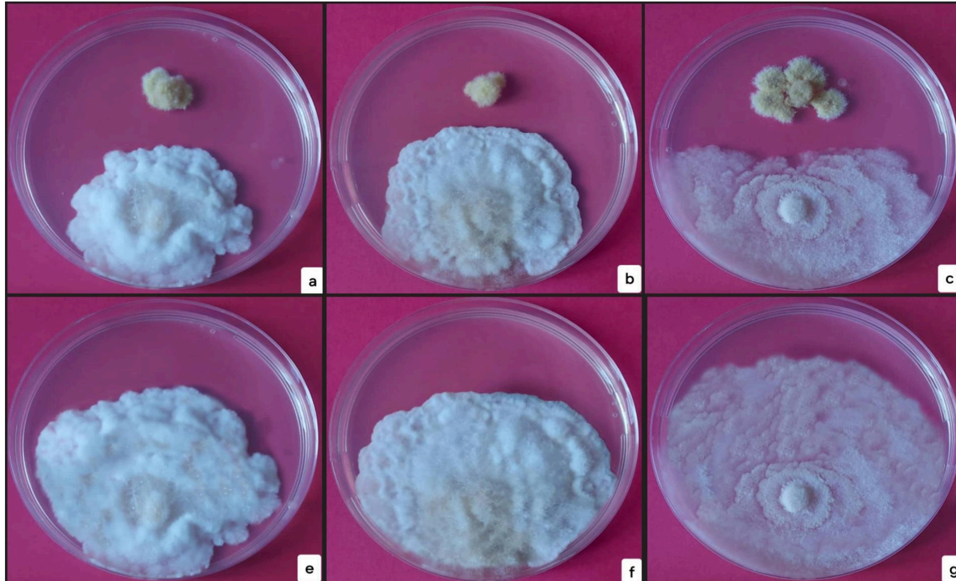

Figure 14:Evaluation of the mycelial growth of Fusarium spp. Confronted by Botrytis sp.a (AZ1-FH1),b (AZ1-FH3),c (AZ1-FH4), e(FH1) control, f(FH3) control, g(FH4) control.

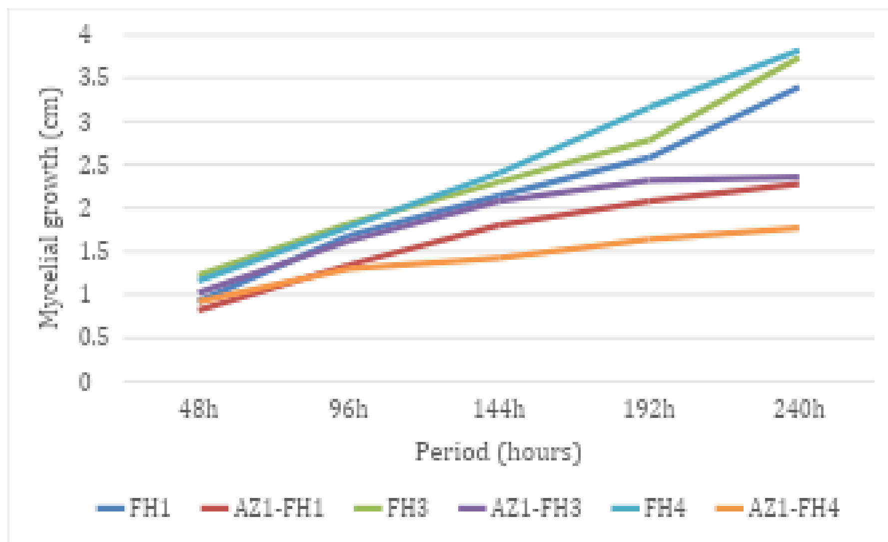

Figure 15: Germination results

Germination rate

The germination rate of the seeds was estimated after 4 days. It generally decreases in the presence of pathogens with and without endophyte treatment. This indicates that fusarium has a negative effect on germination rate. The germination rate decreased in seed lots treated with each of the Fusarium spp. spores, the decrease being even greater when the seeds were treated with the endophyte (*Botrytis*) and then the parasite (Fusarium) (Figure a,b and c; Annexe 2).

Germination rates were lower for seeds containing the endophytic fungus with pathogen than for those containing no endophyte. This indicates that the fungus did

not contribute to mitigating the negative effects of fusarium on germination; on the contrary, its effect was more negative compared to germination rate with the pathogen only.

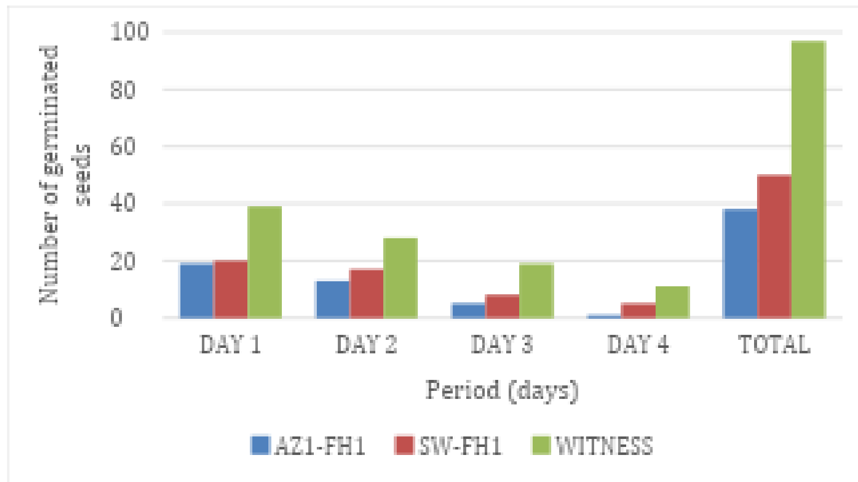

Figure 16*:* Number of germinated seeds treated with sterile water (control), treated with sterile water then pathogen (FH1); and treated with endophyte then pathogen (AZ1-FH1).

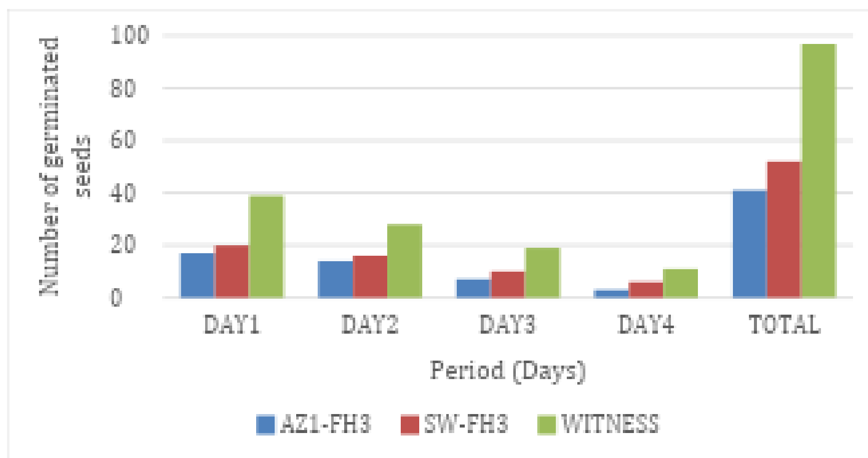

Figure 17:Number of germinated seeds treated with sterile water (control), treated with sterile water then pathogen (FH3); and treated with endophyte then pathogen (AZ1-FH3).

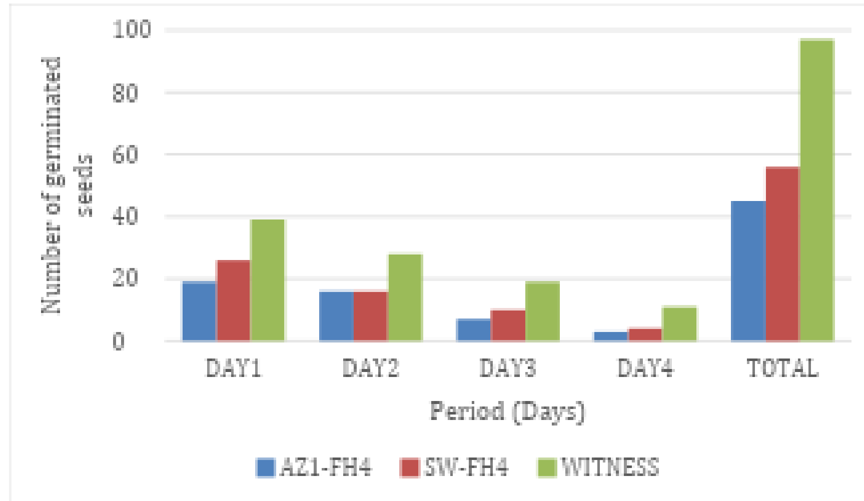

Figure 18: Number of germinated seeds treated with sterile water (control), treated with sterile water then pathogen (FH4); and treated with endophyte then pathogen (AZ1-FH4).

In the treatments without endophyte (SW-FH1; SW-FH3, SW-FH4 and the Control (SW)), the highest average germination rate calculated on day 4 was 24.25% in the Control, followed by 14% for SW-FH4, 13% for SW-FH3 and finally 12.5% for SW-FH1. the lowest rate recorded (9.5%) was obtained in the endophyte-pathogen treatment (AZ1-FH1), followed by AZ1/FH3 (10.25%) and finally AZ1/FH4 (11.25%).

Length of rootlet and number of roots emitted by the seed

Length of rootlet (radicle) emitted by the seed

Analysis of the results obtained after 4 days of germination of wheat seeds indicates that the different germination media applied have a significant effect on radicle length.

The average results show significant variations in length across the different fungus treatments. Application of the pathogenic strains resulted in a reduction in radicle length. This was 2.9 cm in the SW-FH1 treatment, 3.1 cm in the SW-FH3 treatment and 3.3 cm in the SW-FH4 treatment.The data show that the longest radicle is recorded in the control treatment (4.8 cm).

Comparison of means revealed a negative effect of endophytic fungi on radicle length.

There was a decrease in radicle length in seeds treated with the endophyte then the pathogen (AZ1-FH)compared to seeds treated with Fusarium ; this we recorded a difference in length of 0.9, 0.7 and 0.8 cm when comparing SW-FH1 and AZ1-FH1; SW-FH3 and AZ1-FH3 and finally between SW-FH4 and AZ1-FH4.

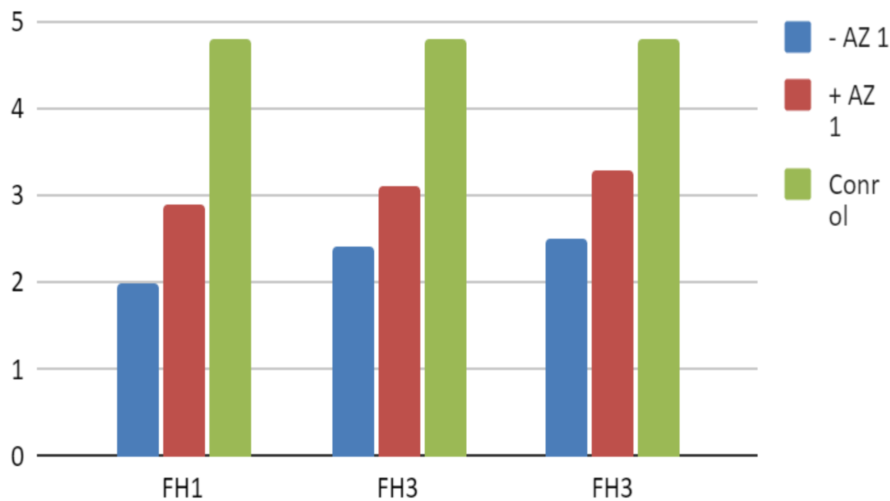

Figure 19: rootlet length in the three different treatment SW, SW-FH, AZ1-FH

Number of roots by the seed

The total number of roots formed was estimated after 4 days of seed germination.

The results obtained from the analysis show a significant difference between the action of fusarium strains. Indeed, in FH1, the number of roots elaborated is 3, and in FH3 and FH4 the number of roots elaborated is 4. The reduction in the number of roots remains dependent on the intensity of severity and aggressivity of the fusarium strains.

The control group recorded a lower number of 5 roots.

Similarly, the analysis of variance results indicate a significant difference in the action of the endophyte compared to the group treated solely with the pathogenic fungus. The interaction between the endophyte and Fusarium also demonstrates a significant negative effect. Furthermore, the average results reveal a difference in the total number of roots between the control group and those treated with Fusarium only, as well as those treated with both Fusarium and AZ1.

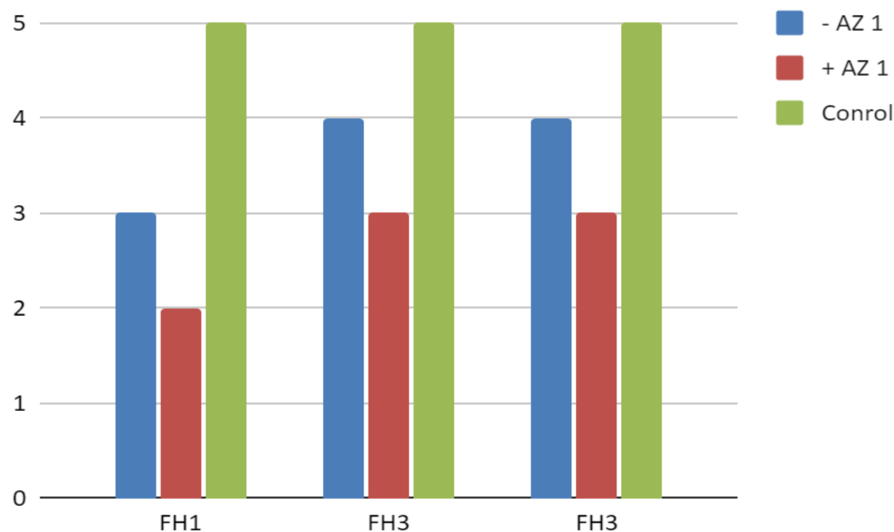

Figure 20: Roots number in the three different treatment SW, SW-FH, AZ1-FH

Results of treatment application on leaves of durum wheat

Analysis of the results obtained after 3 days of treatment on wheat leaves indicates that the different treatment media applied have a significant effect on wheat leaves. The symptoms generally increase in the presence of pathogens with and without endophyte treatment. This indicates that fusarium has a negative effect on physiological functions of leaves .The symptoms increased in leaves lots treated with each of the Fusarium spp. Spores, where FH1 demonstrated greater aggressiveness than FH3 and FH4. Symptoms included large, blackish-yellow spots on the leaves, which were also observed in FH3 and FH4, albeit with reduced severity. Notably, the increase being even greater when the leaves were treated with the endophyte (Botrytis) and then the parasite (Fusarium) the symptoms intensified; the spots turned blackish-brown, affecting nearly the entire leaf in AZ1-FH1 and AZ1-FH3, while approximately 90% of leaves in AZ1-FH4 exhibited similar changes. This indicates that the presence of the endophyte may exacerbate the pathogenic effects of these fungal strains, particularly in AZ1-FH1.

The pretreatment of wheat leaves with Botrytis did not confer resistance, as evidenced by the increased severity of disease observed three days post-infection with Fusarium. Notably, the leaves pre-treated with Botrytis displayed a marked increase in disease severity compared to untreated, pathogen-infected leaves.

Furthermore, the biocontrol efficacy of AZ1 against Fusarium isolates (FH1, FH3, FH4) was not demonstrated, with results indicating that pretreatment with AZ1 had a detrimental effect on durum wheat leaves, exacerbating disease symptoms rather than mitigating them. These findings suggest that AZ1 may not be effective in enhancing resistance or controlling disease progression in durum wheat under the conditions tested.

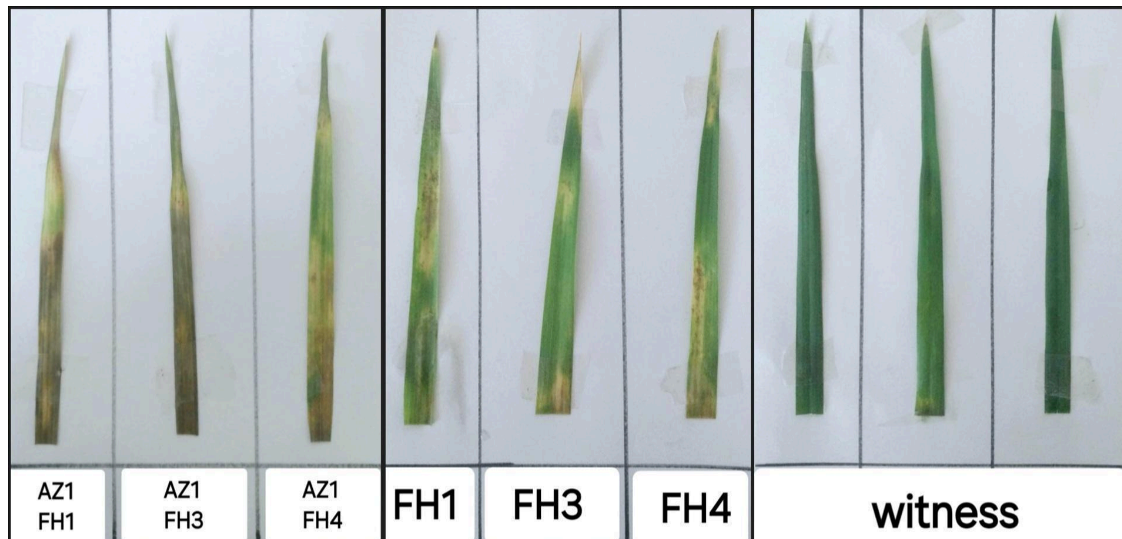

Figure 21: Effect of the three treatment on leaves SW, SW-FH, AZ1-FH

Results of treatment application on the plant of durum wheat
Analysis of the results obtained after 7 days of treatment on wheat plants indicates that the various treatment media used significantly impact the health and condition of the plants.The symptoms generally increase in the presence of pathogens with and without endophyte treatment.This indicates that fusarium has a negative effect on planets of durum wheat.The symptoms increased in planets lots treated with each of the Fusarium spp. Spores, where FH1 demonstrated greater aggressiveness than FH3 and FH4. Symptoms are characterized by the death of the plants after wilting and shrinking leaves and turning yellowish-brown, which were also observed in FH4 ,albeit with reduced severity, in FH3 Symptoms were severe wilting and total brownish yellowing of the leaves with some green tissue and leaves, which were minimal. the increase being even greater when the plants were treated with the endophyte (Botrytis) and then the parasite (Fusarium).
Notably, when wheat plants were cultivated in media containing the endophyte, the symptoms of infection intensified; the spots on the leaves became blackish-brown, affecting the entire leaf in FH1 and FH4, while approximately 90% of plants in FH3 exhibited similar changes. This observation suggests that the presence of the endophyte may exacerbate the pathogenic effects of these fungal strains, particularly in FH1 and FH4.
Additionally, pretreatment of wheat plants with Botrytis did not confer any resistance, as indicated by the increased severity of disease observed 7 days post-infection with Fusarium. Specifically, plants that were pre-treated with Botrytis displayed a significant increase in disease severity compared to untreated, pathogen-infected plants.
The biocontrol efficacy of AZ1 against Fusarium isolates (FH1, FH3, FH4) was not established, as pretreatment with AZ1 worsened disease symptoms in durum wheat

plants instead of mitigating them. This suggests that AZ1 may not effectively enhance resistance or control disease progression under the tested conditions.

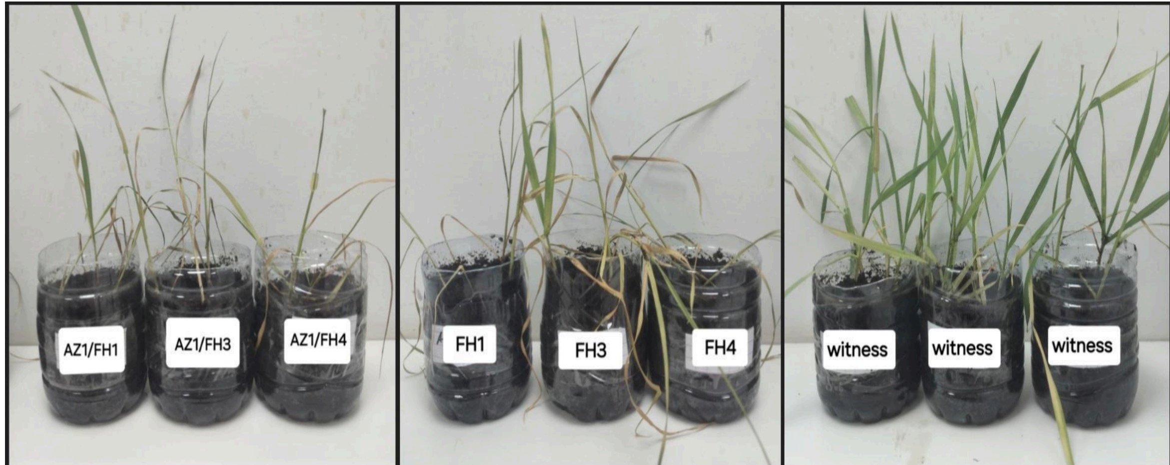

Figure 22 : Effect of the three treatment on plants of durum wheat SW, SW-FH, AZ1-FH

Discussion

Endophyte fungi are increasingly recognized for their potential in biocontrol, as they can enhance plant health and resistance to pathogens. Trichoderma species such as T. harzianum have shown significant antagonism against Fusarium spp., inhibiting mycelial growth. Studies indicate the production of antifungal metabolites plays a key role **(Benítez et al., 2004)**. Research demonstrates that certain Trichoderma isolates can reduce sporulation and mycelial growth of Fusarium by over 50% under laboratory conditions **(Sharma et al., 2015)**.However, our results show a significant antagonistic effect on mycelial growth in direct confrontation,presenting great inhibition zone which is varying depending on the isolate

We believe that Botrytis has antibiotic substances which inhibit the growth of Fusarium isolates of durum wheat.

Field trials showed that applying Trichoderma significantly reduced Fusarium-induced disease severity in durum wheat, leading to healthier plants **(Harman et al., 2004).**

Application of Trichoderma has been linked to increased yields due to enhanced resistance against Fusarium and improved nutrient uptake **(Singh et al., 2020)**.

Biopriming durum wheat seeds with *Trichoderma* spp. enhances seed performance and disease resistance against Fusarium pathogens. This method induces systemic resistance in seeds and significantly reduces Fusarium wilt **(Mishra et al., 2018).**Penicillium species have been reported to suppress Fusarium pathogens effectively. In vitro studies showed that certain Penicillium isolates inhibited Fusarium growth and promoted seed germination in wheat **(Asemani et al., 2020).**In tests involving biopriming seeds with Botrytis spp., a reduced germination rate was observed, alongside a lack of resistance to Fusarium and a decrease in disease severity.

The promotion of root architecture is another critical benefit of Trichoderma bio-priming. Studies have shown that Trichoderma-treated seeds develop more extensive root systems, which enhance nutrient and water uptake **(Hussain & Shaukat, 2021).** This improved root growth is essential for plant health, particularly under stress conditions associated with Fusarium.In the treatment with Botrytis sp, there was a decrease in radicle length and root growth in seeds treated with the endophyte followed by the pathogen, compared to seeds treated with Fusarium spp. Both treatments resulted in a reduction and inhibition of germination and the physiological performance of the seeds.

Certain Aspergillus species have been effective in reducing Fusarium colonization on wheat roots and seeds. These fungi can produce mycotoxins that inhibit pathogen growth **(Oladeji et al.,2018).**Field trials showed that applying Trichoderma significantly reduced Fusarium-induced disease severity in durum wheat, leading to healthier plants **(Harman et al., 2004).** Application of Trichoderma has been linked to

increased yields due to enhanced resistance against Fusarium and improved nutrient uptake **(Singh et al., 2020)**. Trichoderma applications resulted in improved root biomass and overall plant health, which contributed to increased resilience against Fusarium infections **(Vinale et al., 2008).**

in the test on leaves and plants of durum wheat using Botrytis did not confer any resistance, as indicated by the increased severity of the disease when the vegetal material was pretreated with Botrytis , more than treatment with the pathogen only .

Conclusion

Conclusion

The aim of the work presented in this dissertation was to develop a strategy for controlling against different isolates of Fusarium in durum wheat in vitro and in vivo, using the beneficial effect of seaweed endophytes.

The antagonistic activity of seaweed endophytes against pathogenic isolates of wheat Fusarium was studied using the direct confrontation method.

Direct confrontation test identified botrytis sp among 15 algal endophytes fungi as an antagonistic agent against wheat Fusarium isolates.Which inhibits the mycelial growth of Fusarium isolates and shows great inhibition zones varying from 1.12 cm ,0.82 cm and 1.18 cm depending the Fusarium isolates tested.

The biocontrol efficacy of Botrytis sp against Fusarium isolates was not demonstrated in the test in vivo on seeds, leaves and plants treated with pathogens and endophytic sporal suspension of 10^6 spores/ml with results presenting decrease in germination rate and reduce in root and rootlet growth in the biopriming test on seeds, and increase the disease's symptoms in leaves and plants which ended with their death, which leads us to conclude that Botrytis sp is exacerbating Fusarium severity rather than mitigating them. These findings suggest that Botrytis sp may be a pathogen for durum wheat or it may not be effective in enhancing resistance or controlling disease progression in durum wheat under the conditions tested which must be reconsidered.

Biocontrol won't be successful if we only identify pathogen-antagonistic agents in the laboratory ,but also on application plants to see their influences on living vegetal tissues, with accurate calculations and specific conditions to obtain accurate and conclusive results.

ANNEXE

Annexe 01: Evaluation of the mycelial growth of Fusarium spp. Confronted to Botrytis sp.

Confrontation test	Evaluation of Mycelial Growth of *Fusarium spp*					Inhibition zone
	48h	**96h**	**144h**	**192h**	**240h**	
AZ1/FH1	0.92 cm	1.34 cm	1.80 cm	2.08 cm	2.28 cm	1.12 cm
FH1 witness	0.92 cm	1.68 cm	2.14 cm	2.58 cm	3.40 cm	/
AZ1/FH3	1.02 cm	1.62 cm	2.08 cm	2.32 cm	2.36 cm	0.82 cm
FH3 witness	1.22 cm	1.82 cm	2.30 cm	2.78 cm	3.74 cm	/
AZ1/FH4	0.92 cm	1.30 cm	1.42 cm	1.64 cm	1.76 cm	1.18 cm
FH4 witness	1.16 cm	1.78 cm	2.40 cm	3.16 cm	4.20 cm	/

Annexe 02: number of germinated seeds in each treatment

	Day1	**Day2**	**Day3**	**Day4**	**Total**
AZ1/FH1	19	13	05	01	38
FH1	20	17	08	05	50
AZ1/FH3	17	14	07	03	41
FH3	20	16	10	06	52
AZ1/FH4	19	16	07	03	45
FH4	26	16	10	04	56
WITNESS	39	28	19	11	97

Annexe 03:calculation of germination rate, germination index and total average of germination.

	GR	GI	TAG
AZ1/FH1	38 %	38 %	09.50
FH1	50 %	50 %	12.50
AZ1/FH3	41 %	41 %	10.25
FH3	52 %	52 %	13.00
AZ1/FH4	45 %	45 %	11.25
FH4	56 %	56 %	14.00
witness	97 %	97 %	24.25

Annexe 04:rootlet length in each treatment

	-AZ1	+ AZ1
FH1	2.9	2.0
FH3	3.1	2.4
FH4	3.3	2.5
control	4.8	

Annexe 05: root number in each treatment

	-AZ1	+AZ1
FH1	3	2
FH3	4	3
FH4	4	3
witness	5	

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